15 April 2013
EMA/HMPC/892615/2011
Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Eucalyptus globulus* Labill., folium

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)	*Eucalyptus globulus* Labill., folium
Herbal preparation(s)	a) Comminuted herbal substance b) Tincture (1:5), extraction solvent: ethanol 68-80% (V/V)
Pharmaceutical form(s)	Comminuted herbal substance as herbal tea for oral use or for infusion preparation for inhalation. Herbal preparations in liquid dosage forms for oral use.
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1. Introduction

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

- Herbal substance(s)

Eucalypti folium consists of the dried leaves of *Eucalyptus globulus* Labill. (Myrtaceae).

Definition of the herbal substance:

European Pharmacopoeia 7th ed. 2011 (7.1), British Herbal Compendium 2006 [Bradley 2006], Real Farmacopea Espanola 2002 and according to the Deutsches Arzneibuch DAB 10 (German Pharmacopoeia): "Eucalyptus leaves are the whole or cut dried leaves of older branches of *Eucalyptus globulus* Labill. Content: minimum 20 ml/kg of essential oil for the whole drug (anhydrous drug) and minimum 15 ml/kg of essential oil for the cut drug (anhydrous drug)."

Pharmacopée française X (10ème édition): "Eucalyptus defined as dried leaves of *Eucalyptus globulus* Labill. It contains a minimum of 2% V/m essential oil."

Occurrence

Eucalyptus is indigenous to Tasmania and south-eastern Australia. It is cultivated in many parts of the world. Currently the main producer regions are the coasts of Spain, Black sea and the Caucasus geographic area [Blaschek *et al.* 2007].

Biology

Eucalyptus is an evergreen tree with leathery leaves. The leaves are ensiform or sword-shaped form, 6 to 12 inches (15 cm-30 cm up to 40 cm) long, 2 inches wide (5 cm), bluish-green in hue, which are alternate and vertical [Blaschek *et al.* 2007].

The leaves are studded with brown lenticels and colourless glands containing a fragrant volatile oil.

There are several varieties of Eucalyptus. *Eucalyptus globulus* is the most popular in terms of cultivation and medicinal use. The trees with the highest cineole content (80-95%) are *Eucalyptus kochii* and *Eucalyptus polybractea* [Clare 2010].

Powdered plant material is a greyish-green material consisting of fragments of chlorenchyma with numerous embedded, broken, yellow, schizogenous oil gland, calcium oxalate crystals in rosette aggregates or monoclyninic prisms, fragments of epidermis with polygonal cells having very thick cuticule, numerous anomocytic stomata of more than 80 mm in diameter, fragments of sclerenchyma fibres, fragments of cork, tracheids, vessels and fibres [WHO monographs 2002].

Constituents

The herbal substance (dried leaves) contains 1-3.5% volatile oil [Blaschek *et al.* 2007, Wichtl 2004]. The oil contains as a major constituent 1,8-cineole in an amount of 54-95% [WHO monographs 2002, Betts 2000]. The oil derived from fresh leaves consists of 45-75% 1,8-cineole. Other authors stated a 1,8-cineole content of 70-85% for the volatile oil [Wichtl 2004]. Beside 1,8-cineole, the oil contains monoterpenes such as cymene, α-pinen, β-pinen and small amounts of myrtenol, pinocarveol, aliphatic aldehyde, flavonoids such as rutin, hyperoside and quercitrin [Blaschek *et al.* 2007]. The concentration of α-terpineol was estimated to be 28% [WHO monographs 2002]. Takasaki *et al*. 1990 isolated 12 compounds with acylphloroglucinol-monoterpene or -sesquiterpene structures, euglobals from the leaves. The herbal substance also contains gallotannins and smaller amounts of procyanidines, triterpenoids (ursolic acid derivates) and flavonoids as well as

phloroglucinol derivates such as euglobals and macrocarpals [Wichtl 2004]. The leaves of *Eucalyptus globulus* have smaller amounts of tannins than many other Eucalyptus species [Duke 1985]. Tannin content can depend on the methods of drying leaves [Cork & Krockenberger 1991].

1,8-cineole is also known as eucalyptol. Some authors classified eucalyptol as the active ingredient in Eucalyptus oil. Aside from medicinal use, 1,8-cineole is used as a flavouring agent for lozenges, as a fragrance as well as in cosmetics [Clare 2010].

Recent investigations made by Daroui-Mokaddem *et al*. [2010] yielded an amount of 2.5% volatile oil derived from fresh leaves by hydrodistillation. The yellowish oil was analysed by GC/MS and among the twenty identified components the main components were 48.6% 1,8-cineole, 10.9% globulol, 10.7% trans-pinocarveol and 6.6% α-terpineol. In the dried aerial parts of *Eucalyptus globulus*, trans-pinocarveol was lower than 3.1%. Silvestre *et al*. 1997 had found that younger leaves tend to have higher oil content than adult ones, while 1,8-cineole showed a complex variation along the seasons, but adult leaves have higher contents of 1,8-cineole. It was not possible to establish a relationship between biochemistry of the plants and the season of the year or the geographic location from the data.

Non-volatile monoterpene glucosides, also conjugated with gallic acid, have also been described as components of the essential oils extracted from the leaves of the plant [Goodger *et al.* 2009, Hasegawa *et al*. 2008].

A further constituent of the leaves is the plant cuticle that consists of β-diketone-tritriacontan-16,18-dione and fatty acids [Blaschek *et al.* 2007].

- Herbal preparation(s)

The regular usage of the herbal tea in Germany led to the publication of a standard license in 1996 which is used by pharmacies and applicants. No further specific medicinal product was mentioned in the market overview with Eucalyptus leaf or preparation thereof as a single active substance.

There are many reports on herbal preparations in literature and monographs (see also section 2):

  - Dried leaf infusion; the hot infusion may also be used for inhalation [Bradley 2006]
  - Tincture (1:5)*, extraction solvent: ethanol 70% (V/V) [DAC 2004]
  - Tincture (1:5)*, extraction solvent: diluted wine spirit [Ergänzungsbuch zum Deutschen Arzneibuch EB6 1951]. “Diluted wine spirit” contains ethanol 68-69% (V/V) [DAB 1951].
  - Tincture (1:5)*, extraction solvent: ethanol 80% (V/V) [Pharmacopée Française IX 1976]
  - Eucalyptus tincture (1:5)*, extraction solvent ethanol 80% (V/V) [Pharmacopée Belge V 1962]
  - Tincture (1:5)*, extraction solvent: ethanol 45% [Bradley 2006]
  - Syrup 10%, without further specification [Pharmacopée Française IX 1976, Madaus 1938]
  - Powdered whole drug, herbal tea, aqueous alcoholic extracts of “low strength” prepared with ethyl alcohol of a strength with less than or equal to 30% (V/V), of “high strength” prepared with ethyl alcohol of more than 30% (V/V) [Cahier N°3 de l'Agence du Médicament 1998].

* ratio of herbal substance to extraction solvent

- 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.

Eucalyptus leaves are often marketed as a combination with other herbal substances/preparations for the treatment of cough associated with cold e.g. Peppermint oil, Juniper oil, Fennel oil, Turpentine oil, Pine oil, extract of pigmenta radicis curcumae, frangulaemodin, Calendula tincture, Abietis sibiricae oil, Hippophae oil, *Citrus sinensis*, Lagerstroemia, *Citrus limonum* or other active substances such as levomenthol, thymol, menthol, camphor, guaiazulene, benzalkonium chloride, methyl salicylate, magnesium salicylate and alpha tocopherol acetate.

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:	Combination
Belgium	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Bulgaria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Cyprus	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products
Czech Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Denmark	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products
Estonia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products
Finland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products
France	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products
Germany	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Standard licence for herbal tea
Greece	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products
Hungary	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
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:	Combination
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:	
Slovenia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Spain	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	3 products
Sweden	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products
United Kingdom	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Serbia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Food supplement

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

1.3. Search and assessment methodology

Data bases PubMed (April 2011) and DIMDI – DB (Deutsche Institut für medizinische Dokumentation und Information) were searched using the terms: "Eucalyptus folium, Eucalyptus leaves, *Eucalyptus globulus*, Cineole". Additional handbooks and textbooks as cited in the references list were used.

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

2. Historical data on medicinal use

2.1. Information on period of medicinal use in the Community

The usage of Eucalyptus leaf as herbal tea has a long history and the importance is underlined by the release of a standard license in Germany. Furthermore, a traditional use can be regarded as established because a lot of monographs on Eucalyptus folium are included in handbooks and the Pharmacopoeias of the European Union (EU) (see sections 2.2. and 2.3.).

Already in 1873, Köhler described the use in South Europe and the benefits on health of Eucalyptus preparations. As an example, he described effects on fever, neuralgic pain, asthma, lung tuberculosis, and antisepsis. In 1938, Madaus described the use of Eucalyptus leaves and its volatile oil as follows: "The oil stimulates the excretion of saliva and gastric juice. It enhances the appetite. Oral application of a syrup is described for whooping cough and external use of the leaves as an antiseptic agent. Additionally it is used as an inhalation for the treatment of catarrh and inflammatory diseases of respiratory tract and in cases of asthma. It is also used as a remedy for fever, catarrh of the urinary tract, influenza, rheumatism, neuralgias, malaria, fevered diarrhoea, gum bleeding and as an anthelmintic. Beside an oral application of Eucalyptus leaf, topical treatments as an antiseptic agent have been described."

A survey, filled in by parents of children and adolescents, was conducted in Germany in 2007. Its results showed that 43.9% of the children were given Eucalyptus preparations in the last 6 years [Hümer *et al*. 2010]. No further information about the applied products or their indication was given. However it confirmed the use and the tradition of Eucalyptus in the EU as a medicinal product.

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

In folk medicine, it is used internally for the treatment of asthma, fever, flu, whooping cough, loss of appetite, dyspeptic complaints, inflammatory and infectious diseases of kidneys and bladder, diabetes, rheumatic complaints. It is used externally for wounds, acne, poorly healing ulcers, stomatitis, bleeding gums, rheumatism, neuralgia and gonorrhoea. All these applications have not been confirmed scientifically [Blaschek *et al*. 2007].

Diepenbrock's Gehes Codes [1960] reports on the use of a distillate of the leaves as an inhalant for treatment of disorders of respiratory tract and bronchial asthma.

Penso [1980] listed Eucalyptus preparations for the treatment of respiratory diseases. No particular wording for indication is given.

The Pharmacopée Française monograph "Eucalyptus" [1976] listed the following indication: "antiseptic agent in the lung and broncho-pulmonary complications of influenza".

The Cahier N°3 de l'Agence du Médicament [1998] listed the following traditional indications:

- oral use: "Traditionally used during benign acute bronchial conditions. N.B. if the condition persists, consult a doctor".

- topical use: ”Traditionally used during benign acute bronchial conditions. N.B. if the condition persists, consult a doctor” and ”Traditional use in case of nasal congestion and common cold”.

Wichtl [1984] mentioned the following indication: “catarrhal disorders of the respiratory tract”.

The monograph of the Commission E approves Eucalyptus leaves as a medicinal product for the treatment of diseases of the respiratory tract [Blumenthal *et al*. 1998].

In Martindale [Sweetman 2007], it has been referred: “Eucalyptus leaf has been used in oral preparations for coughs and associated respiratory-tract disorders”.

The WHO monograph [2002] referred to the Commission E monograph and to the African pharmacopoeia 1985. According to the latter, *Eucalyptus* is used for the symptomatic treatment of asthma, fever and inflammation of the throat.

Traditional use of Eucalyptus leaf (powdered herbal substance and herbal substance) was reported by Spain (see sections 1.2. and 2.3.).

Traditional use outside the EU

In Australia, it is used for the treatment of headache and in South Africa for the treatment of stomach ache [Blaschek *et al.* 2007]. None of these indications are supported by scientific data.

An inquiry of 100 adults yielded that, in Oregon (USA), Eucalyptus and its preparations was used for the treatment of cough, colds, sore throat and sinusitis. Thirty-nine percent of the interviewed stated the use of Eucalyptus and 89.7% confirmed effectiveness [Brown & Marcy 1991].

A survey, conducted by Otoom *et al.* [2006], confirmed the use of Eucalyptus leaves as a traditional medicine used by diabetic patients in Jordan. No further information on posology or side effects was given.

Eucalyptus leaves have been listed as a Mexican traditional medicine used for treatment of common infections such as bronchial affections, cough, asthma, pharyngitis, cold, fever and wounds [Navarro *et al.* 1996].

As some reported indications require a supervision of a physician, they cannot be listed in the monograph as a traditional indication.

In response to the public call for scientific data, data on the traditional use of Eucalyptus leaf and its preparations in India have been submitted. Submitted data included the traditional use of the powdered leaves as a febrifuge, carminative, stimulant (no further information), expectorant, diaphoretic and antiseptic. As a single oral dose for adults 1-2 g leaves has been given. The traditional use of a tincture (not further specified) for the treatment of asthma, phthisis and chronic bronchitis and the use of a decoction that is used as an insect repellent have also been described. The posologies for the tincture and the decoction are missing. As an Ayurvedic treatment, an inhalant for the treatment of common colds, cough and asthma, an ointment applied on burns and a tincture used in asthma, phthisis and chronic bronchitis were described.

Summary:
Handbooks, Gehes Codes and Pharmacopée Française as well as Penso (1980) support the traditional use of Eucalyptus as a medicinal product in the EU for more than 30 years. Traditional use for the treatment of several medical conditions has been reported. Some of the cited therapeutic indications are insufficiently described and not supported by scientific data, thus it is recommended to list only the indications that are appropriate for traditional use without the supervision of a medical

practitioner for diagnostic purposes or for prescription or monitoring of treatment. Because bronchial asthma should be supervised by a medical practitioner, it is not included as a traditional indication.

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

The monograph on Eucalypti folium of the Commission E for herbal medicinal products [Blumenthal *et al*. 1998] gives a daily dose for oral use as follows: corresponding to 4-6 g herbal substance; for tincture is cited that a dose of 3-9 g per day is in accordance with the German Pharmacopoeia EB6. But this is not consistent with the posology given in EB6 for a single dose of 2.5 g tincture. Additionally a topical use for the same indication is listed, but information on the posology is missing.

For the treatment of diseases of respiratory tract, the traditional daily oral posology is 3 cups of about 1.8 g herbal substance in 150 ml boiling water, steeping time 10-15 minutes [published in Germany in the German Standardzulassung Eucalyptusblätter Nr. 9299.99.99 1996]. Blaschek *et al*. [2007] cited the posology as follows: oral or as an inhalant 4-6 g daily dose, as a tea 2-3 g drug substance with 150 ml boiling water 10 minutes steeping time.

The British Herbal Compendium gives the following posology: Hot infusion corresponding to 4-6 g daily, average single dose 2 g for oral use and as inhalant [Bradley 2006]. Tincture (1:5), extraction solvent: ethanol 45%, 5-20 ml daily [Bradley 2006].

According to “Médicaments traditionnels à base de plantes: Liste des indications acceptées pour une mise devant le comptoir” published by the Afssaps in July 2008 referring to the indications found in the Cahier N°3 de l'Agence du Médicament, the administration should be limited to one week and to adults and children aged 6 years and older. Concerning the posology, reference is made to the Pharmacopée Française monograph “Tisanes” [1989]. It recommends an oral dosage of 250-500 ml of herbal tea (10-20 g Eucalyptus leaves/l) a day, corresponding to 2.5 to 10 g leaves per day. The Pharmacopée Française monograph “Eucalyptus” [1976] recommended for broncho-pulmonary complications 100 to 200 ml a day of an infusion (100 g/l) or 1 to 10 g per day of a tincture (1:5) for adults only or 2 to 3 spoons per day of a 10% syrup for adults only. This corresponds to a daily dose of 10 to 20 g herbal substance for the infusion, 0.2 to 2 g herbal substance for the tincture and 3 to 4.5 g herbal substance for the syrup.

Penso [1980] described in the “*Piante medicinali nella terapia medica*” some oral antiseptics for treatment of respiratory diseases, including 0.5 g powdered Eucalyptus leaves 6 times a day, 200 ml herbal tea (prepared with 10 g leaves/1,000 ml water) every 3 h, 100 drops of tincture (prepared with 200 g leaves macerated 10 days with 1,000 g ethanol 80%) in a cup of warm honeyish water every 4 hours, or 2 to 5 table spoons of a syrup (prepared with 100 g powdered leaves and 1,500 g water, and subsequent added sugar 180 g per 100 ml infusion) per day. This corresponds to 0.2 to 8 g herbal substance as a daily dose.

Schmid *et al.* [1963] summarised the common posologies for Eucalyptus preparations. For Eucalyptus leaves they gave a common single dose of 1.5 g and for Eucalyptus tincture a single dose of 2.5 g. A later edition of their book gave as a single dose for Eucalyptus leaves 2 g and for Eucalyptus tincture 2.5 g. For Eucalyptus leaves, a daily dose is 2-3 times a cup of infusion, prepared with 2 g leaves in 150 ml hot water, 10 minutes steeping time. For the tincture, a daily dose of 1-3 times 2.5 g is given [Braun *et al.* 2011]. That corresponds with 4-6 g herbal substance a day for the infusion, and with 0.5-1.5 g herbal substance a day for the tincture. The information on the tincture is in accordance with the EB6 and the information on the infusion is in accordance with the Commission E.

Spain reported the traditional use of Eucalyptus leaf as herbal tea/vapour for inhalation and as hard capsules for the treatment of common cold and cough. Reported posology ranged from 230 to 690 mg powdered herbal substance as single dose and 1.8 to 2 g as daily dose, and 3 to 9 g daily as herbal tea (3 g/250 ml water)/inhalant (3 g in boiling water).

Summary:

In conclusion, the use of Eucalyptus leaf and its preparations for the treatment of disorders of upper respiratory tract and colds has been known for a long time. Based on the listed indications of European monographs and the documented usual dosage, only the following indication is proposed for a traditional use without the supervision of a medical practitioner for diagnostic purposes or for prescription or monitoring of treatment:

Oral use and as an inhalant: "Traditional herbal medicinal product used for relief of cough associated with cold."

This indication is not supported by sufficient pharmacological and clinical data (see section 4.) to consider a well-established use as defined in the European pharmaceutical legislation.

Reported dosages vary in wide range and are inconsistent.

For infusions for oral use, single dosages range from 1.5 to 3 g herbal substance and daily doses range from 2.5 to 20 g herbal substance. The most common posology is about 4 to 9 g herbal substance per day. The posology given in the Pharmacopée Française i.e. a daily dose of: up to 4 times a day a cup of herbal tea prepared with 1.5 to 3 g Eucalyptus leaves in 150 ml boiling water, steeping time 10 to 15 minutes, is appropriate for the Community herbal monograph.

The hot infusion may also be used as an inhalation. For the inhalant, the posology ranges from 2 to 3 g single dose up to 3 times a day. Bradley 2006 and Spain cite that the infusion preparation can also be used as an inhalant, with a single dose of 3 g herbal substance in boiling water, without further information.

For tinctures for oral use, the posology varies from 1 to 10 g a day. But the most cited dosage is 2.5 g tincture 1 to 4 times a day that corresponds to 0.5 g herbal substance as a single dose and 0.5 to 2 g herbal substance a day.

The posologies given in the monograph reflect the wide ranges found in the literature or tradition. A footnote in section 4.2. of the monograph points out that the posology applied for an individual medicinal product has to be specified in a tighter range and should be consistent concerning the single and daily dosages.

The posology of the syrup as well as that for the topical use are not sufficiently and comprehensively described. Therefore, these traditional uses cannot be regarded as sufficiently supported by the literature and are not listed in the monograph.

3. Non-Clinical Data

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

Pharmacological information on Eucalyptus leaves and/or their preparations is limited. Most publications deal with the effects of Eucalyptus oil. As Eucalyptus oil is extracted from fresh leaves of *Eucalyptus globulus* Labill., *Eucalyptus fructicetorum* F.V. Mueller (syn. *Eucalyptus polybractea*) and *Eucalyptus smithii* R.T. Baker, the data cannot be transferred to dried leaves.

Antibacterial, antifungal and antiviral effects

Many studies deal with the antibacterial effects of plants or plant extracts. For *Eucalyptus globulus* leaves extracts a high inhibition activity on bacteria and fungi growth has been described. Although Cimanga *et al.* [2002] found no correlation between the amount of 1,8-cineole content and the antibacterial activity, it seems to be the main active compound in the leaves.

The following comments refer mainly to the extracts, with less emphasis on the purified compounds.

Eucalyptus globulus leaves and preparations

Brantner & Grein [1994] tested an aqueous extract (50 ml boiling water to 5 g air dried, powdered drug and heated under reflux for 5 minutes) for its antibacterial potential by using hole-plate diffusion method and microdilution broth method (minimal inhibitory concentration MIC was calculated in mg/ml). The extracts were tested on gram positive cocci and *Bacillus subtilis, Staphylococcus aureus, S. aureus Oxford, Enterococcus faecalis* and *Escherichia coli* (applied on the surface of the agar medium). *Eucalyptus globulus* leaves extract (only the sterile aqueous extract with 16.7% dry residue was tested) showed an antibacterial activity on all tested bacterial species. Different concentrations have not been tested.

Osawa *et al.* [1995] showed that eucalyptone, an isolated compound of an ethanolic extract of Eucalyptus leaves (50% ethanolic extract from dried leaves), inhibited the enzyme glucosyltransferase and has an antibacterial activity against cariogenic bacteria (different *Streptococcus* species).

Navarro *et al*. [1996] investigated the antimicrobial activity against *S. aureus, E. coli, Pseudomonas aeruginosa* and *Candida albicans*. *Eucalyptus globulus* dry extracts (100 g dried material macerated with 1500 ml methanol, distilled to dry extracts and subsequent dilution in 10% Tween 80 to different concentrations) showed a general antimicrobial effect against all the microorganisms tested (10^8 CFU/ml) at concentrations of 10 mg/ml or below.

Salari *et al*. [2006] investigated the antimicrobial activity of *Eucalyptus globulus* leaf extracts (methanolic extract dissolved in Mueller-Hinton broth). Tested microorganisms were *S. aureus, Streptococcus pyogenes, Streptococcus pneumoniae* and *Haemophilus influenzae* all obtained from patients with respiratory tract disorders. Different concentrations of the *Eucalyptus globulus* extract were used. Standard inoculum of each microorganism (10^6 CFU/ml) were prepared. After incubation for 18-24 hours at 37°C the lowest concentration of *Eucalyptus globulus* extract that inhibits growth and the lowest concentration that allowed <0.1% of the original inoculum to survive (MIC), was given. The results showed a clear antibacterial activity of the *Eucalyptus globulus* leaf extract (MIC_{50} = 16 to 64 mg/l). The authors concluded that the results suggest that further studies to clarify the possible role of *Eucalyptus globulus* leaf extracts in the treatment of respiratory tract infections are warranted.

Caceres *et al*. [1987] found that an ethanolic maceration of 10 g powdered dried leaves with 100 ml 50% ethanol showed only an unclear inhibition on *C. albicans* and *E. coli.*

The same author found that an aqueous extract of leaves (40 g powdered plant material boiled 5 minutes with 200 ml water, then concentrated on 1 g/ml of dry material) inhibits the growth of *Epidermophyton floccosum*. Six dermatophyte strains were tested. An inhibition effect of *Eucalyptus* extract on the other strains was not found [Caceres *et al*. 1991].

In order to compare the antibacterial activity with six antibiotics, Al-Saimary *et al*. [2002] investigated the antibacterial activity of various concentrations of aqueous extract of *Eucalyptus globulus* leaves and *Myrtus communis* leaves (50, 100, 250, 500, 750, 1,000 µg/ml). The antibacterial activity was evaluated by growth inhibition and determination of MIC. *Pseudomonas*

aeruginosa was the predominant pathogen followed by *S. aureus* which was isolated from 92 cases of burn infections. The Eucalyptus extracts gave comparable effects on bacterial growth of *P. aeruginosa* to penicillin G (10 units), kannamycin (30 mg), cephalexin (30 mg), tetracycline (30 µg), neomycin (30 µg) and gentamicin (10 mg).

May & Willuhn [1978] examined aqueous extracts (1 part substance to 10 parts water infusion) from 178 plants, for their virustatic activity. Results were given in mm zone of inhibition. For Eucalyptus infusions, a 15-30 mm zone for Herpes Hominis HVP75, influenza virus A_2 and vaccine were detected. The authors indicated that the tannins content may be responsible for the virustatic effect.

Takasaki *et al*. [1990, 1995] examined the inhibitory effects on Epstein-Barr virus of euglobals, isolated compounds from *Eucalyptus globulus* leaves with acylphloroglucinol-monoterpene or -sesquiterpene structure. Some of the isolated euglobals showed *in-vitro* (Epstein-Barr virus early antigen test) inhibitory effects.

Amakura *et al*. [2002] isolated and identified constituents of Eucalyptus leaves extracts. Phloroglucinol-coupled compounds such as eucalyptone and macrocarpals were found. Macrocarpals and their antimicrobial activities on biological activities against oral bacteria have been investigated by Nagata *et al*. 2006. Purified macrocarpals were isolated from an ethanolic extract of the dried leaves (ethanol 60%, 80°C, 3 hours, lyophilised and dissolved in ethyl acetate). Eucalyptus leaf extracts were investigated for their inhibitory effects on the biological activity of *Porphyromonas gingivalis* and other periodontopathic bacteria. The results demonstrated an antibacterial activity against periodontopathic bacteria. MIC are 0.5-100 µg/ml. The authors concluded that Eucalyptus leaf extracts may be useful as a potent preventative of periodontal diseases.

In-vivo activities

Bokaeian *et al.* [2010] investigated Eucalyptus and its effect on systemic infection with *Candida albicans* in diabetic rats. Sixty normoglycaemic male Wistar rats were selected and divided into 6 groups. In three groups (each n=10) diabetes was induced by injection of streptozotocin. Four groups were inoculated with *C. albicans* 15 days after diabetes induction. Eucalyptus (dried and powdered leaves) was added to the diet (62.5 g/kg, pellets made from powdered leaves mixed with distilled water and subsequently drying at 45°C) and to the drinking water (2.5 g/l, 2.5 g powdered leaves mixed with 100 ml distilled water, brought to boil, then removed after 15 minutes, filtered and diluted with tap water) for 4 weeks. Eucalyptus lowered *C. albicans* concentration in liver and kidney homogenates in both diabetic group and control group. Additionally, Eucalyptus administration improved the hyperglycaemia and it compensated weight loss of diabetic rats.

Effects on bronchitis, asthma, cold and cough

Eucalyptus leaves

Ikawati *et al*. [2001] studied ethanolic and hexane plant extracts of several Indonesian medicinal plants, including *Eucalyptus globulus* leaves. The dried powdered plant material was extracted with n-hexane (500 g with 2 times 250 ml n-hexane) followed by ethanol extraction to give a dried n-hexane extract and a dried ethanol extract. Extracts were tested for their activity in inhibiting histamine release from RBL-2H3-cells (rat basophilic leukaemia cell line). Cells were incubated with 200 µl PIPES buffer or the dissolved dried extracts (0.5 mg/ml) at 37°C for 10 minutes. Both *Eucalyptus globulus* leaves extracts showed an inhibitory activity of about 84-85% on histamine release activity from RBL-2H3 cells either in the presence or absence of DNP-BSA (dinitrophenylated bovine serum albumin) as antigen to stimulate histamine release. As a standard for inhibitory effect, quercetin was used.

Cineole (*in-vivo* studies)

Laude *et al.* [1994] studied the antitussive effects of cineole in conscious guinea-pigs. Following the administration of cineole (0.8, 2.7 and 8 mg/l) using a vaporising apparatus that provided a constant airflow of 1 ml/min, cough was induced by citric acid (initial dose 300 mM/24 hours). Cineole had no significant effect on cough frequency or cough latency.

Zänker *et al*. [1980] investigated the effect of vapours of 1,8-cineole and Eucalyptus oil on synthetic and pulmonary surfactant layers. Under their experimental conditions, 1,8-cineole and Eucalyptus oil exhibited surfactant-like effects, namely a decrease in surface tension between water and air and thus improved lung compliance values *in-vivo*. During inhalation of 300 µmol 1,8-cineole, the lung compliance of 1,8-cineole treated anaesthetised rabbits was improved by a factor of 0.3. An increase of 1,8-cineole posology resulted in a decrease of lung compliance compared to starting values. No remarkable morphological damage of epithelium was observed.

Anti-inflammatory activities

Eucalyptus leaves

Eucalyptus globulus has been reported as a therapeutic plant in airway inflammatory diseases. Considering that reactive oxygen species and reactive nitrogen species may be implicated in the pathogenesis of airway inflammatory diseases such as asthma and chronic obstructive pulmonary diseases (COPD), Almeida *et al*. [2009] investigated an effective scavenging activity against these reactive species of *Eucalyptus globulus* leaf extract (2 g dried leaves were extracted 10 minutes with 5 times 50 ml de-ionized water, and subsequently the extract was lyophilized). Total phenolic content of the extract was 311±20 mg gallic acid equivalents/g of lyophilised extract. Four phenolic compounds were identified: chlorogenic acid, ellagic acid, rutin and quercitrin. Scavenging effects were measured by six different scavenging assays. The Eucalyptus leaf water extract presented a capacity to scavenge all the tested reactive species. These findings correspond to that in the *in-vitro* studies by Vigo *et al*. [2004]. They showed a significant NO-scavenging activity of Eucalyptus and *Thymus vulgaris* extracts.

1,8-cineole

Juergens *et al*. investigated the anti-inflammatory effect of 1,8-cineole by conducting *in-vitro* and *ex-vivo* studies in isolated blood monocytes of patients with bronchial asthma.
A study [1998a] showed that 1,8-cineole significantly inhibits leukotriene (LTB_4) and PGE_2, both pathways of arachidonic acid metabolism. The results of another study [1998b] suggested that 1,8-cineole is an inhibitor of cytokines by a highly significant and dose-dependent inhibition of production of TNF-α, interleukin-1β, LTB_4 and thromboxane-B2.
A study in 2001 yielded an increased lung function due to an inhibition of cytokine production. 600 µg 1,8-cineole per day decreased the necessary steroid therapy by about 36%. The same authors found that 1,8-cineole inhibited cytokine production *in-vitro*. Cell cultures (lymphocytes and monocytes from 9 volunteers, who donated their venous blood) were treated with 1,8-cineole (10^{-9} to 10^{-10} M) [Juergens *et al*. 2004]. In summary, the studies of Juergens *et al*. confirmed the inhibition of cytokine production by 1,8-cineole.

Santos & Rao [2000] demonstrated that 1,8-cineole in a dose of 100 to 400 mg/kg oral administration to rats and mice produces anti-inflammatory effects (clear effect for 400 mg/kg). Furthermore the observed antinociception in both acetic and formalin tests pointed to a likely peripheral analgesic action of 1,8-cineole that justifies the use for treatment of local painful conditions. A clear mechanism could not be provided by the presented data. The antinociceptive properties of 1,8-cineole, obtained by steam distillation of *Eucalyptus camaldulensis* leaves have been confirmed by Liapi *et al.* [2007]. A single dose of 1,8-cineole intraperitoneally (i.p.) 0.3 mg /kg

showed an antinociceptive activity comparable to that of morphine in male Wistar rats and male Swiss mice. It showed a significant effect at 30 minutes and 60 minutes in the Hot-plate- and the Tail-flick-test.

Antioxidant activities

Eucalyptus leaves

Antioxidant activities of Eucalyptus leaves extracts and its compounds were evaluated by the linoleic acid peroxidation assay, the superoxide anion radical generation assay and DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Isolated gallic acid and ellagic acid showed the majority of the antioxidant activity of Eucalyptus extract. But 1,8-cineole activity as an antioxidant was negligible [Amakura *et al*. 2002].

Antidiabetic effects

Already in 1902, Faulds described the traditional use of Eucalyptus for the treatment of diabetes. In order to investigate a possible antidiabetic effect, several *in-vivo* and *in-vitro* studies have been performed.

Eucalyptus leaves

Studies in streptozotocin (STZ)-induced diabetic mice:

Eucalyptus (6.25% by weight of diet) and a decoction of Eucalyptus leaves in the drinking water (1 g substance/400 ml water) lowered plasma glucose concentrations significantly in STZ-induced diabetic mice. Body weight loss in the diabetic mice was reduced [Swanston-Flatt *et al*. 1990]. Gray & Flatt [1998] also found that a Eucalyptus diet (62.5 g/kg) 5 days before and after STZ administration reduced the weight loss, polydipsia and the plasma glucose concentration.

Additionally *in-vitro* studies concerning glucose transport and glucose metabolism were conducted by preparing abdominal muscles. An Eucalyptus solution (0.5 g/l) enhanced 2-deoxy-glucose transport, glucose oxidation and incorporation of glucose into glycogen. Insulin secretion was investigated on isolated rat pancreatic beta cells. The studies conducted with an aqueous Eucalyptus extract showed an enhancement of insulin secretion. These results indicated that *Eucalyptus globulus* represents an effective antihyperglycaemic dietary adjunct for the treatment of diabetes.

Recently, Mahmoudzadeh-Sagheb *et al*. [2010] investigated the effects of Eucalyptus leaves (dried and fine powdered, mixed with distilled water and then dried and pelleted) on STZ-induced damage in pancreatic islets and pancreatic beta cells by stereological methods (estimation of volume density and total volume of islets and beta cells, mass of pancreas, and total number of islets by using microscope and so-called physical fractionation method). Effects of Eucalyptus diet (20 g/kg and 62.5 g/kg) on physical status, blood glucose level and islet histology of 50 Wistar rats were measured. Eucalyptus significantly decreased the weight loss and water and food intake in STZ-treated rats. Treatment of diabetic rats with Eucalyptus leaves showed a significant hypoglycaemic effect in comparison to non-treated diabetic rats. Eucalyptus treatment partially restored the STZ-caused atrophy of islets and degenerative changes of beta cells. The authors concluded that Eucalyptus leaves in dose dependent manner could compensate STZ-induced cell damage of pancreatic beta cells. Therefore it may be an effective antihyperglycaemic dietary supplement for the treatment of diabetes. But further comprehensive chemical and pharmacological investigations are necessary.

Studies in alloxan-induced diabetic animals:

Already in 1964, Lin *et al*. investigated the hypoglycaemic activity of medicinal herbs on alloxan-treated rabbits. But oral administration of 1 g/kg body weight (Eucalyptus dry residue of the ethanolic extract) showed no effect on the blood sugar levels.

In contrast to that by Lin *et al.*, other studies showed a significant effect. Perez *et al.* [1984] investigated the effect of different Mexican plants on mice with alloxan-induced diabetes. Eucalyptus extracts (25, 50, 75 and 100 g per 250 ml water) were administered orally and i.p. Hypoglycaemia activity was determined 5 hours after administration of the infusion, by the Nelson-Somogyi, the O-Toluidine and the Dextrostix tape methods on blood samples. Eucalyptus showed a lowering of blood sugar level by about 36% when administered orally and by about 25% when administered i.p. But other plants (e.g. *Permantiera edulis*, candle tree) showed a higher reduction.

A significant reduced blood glucose level in alloxan-induced diabetic rats was found after receiving a *Eucalyptus globulus* leaf extract (130 mg/kg body weight) by Ahlem *et al*. [2009]. Despite this reduction the liver glycogen level was not restored. Therefore, the authors suggested that the Eucalyptus extracts do not stimulate insulin secretion. Additionally, an antioxidative activity of the Eucalyptus extract could be demonstrated by measuring an increased activity of catalyse, superoxide-dismutase and glutathione-peroxidase in liver and kidney.

Two published studies by Sugimoto *et al.* [2005, 2010] deal with the effects of Eucalyptus aqueous extracts on sugar metabolism in Wistar rats. The results indicated that Eucalyptus extracts inhibit intestinal fructose absorption and can suppress adiposity.

Repellent effect

Although Eucalyptus oil and 1,8-cineole are well known for their use as repellents, studies with *Eucalyptus globulus* leaves on their insect-repellent effect have not been described until now.

Assessor's comment:
Studies on the effects on several bacterial strains, viruses and fungi may be seen to confirm an antimicrobial, antifungal and antiviral effect of Eucalyptus leaves preparations. But in most studies, the extract is not exactly defined. Therefore, it is difficult to give a defined concentration that may be effective against defined strains.

Studies on the effects on cough and cold have not been performed for Eucalyptus leaves preparations. One study dealt with an inhibition effect of histamine release that might be of importance for asthmatic patients. But asthma is not of interest in this context, because it cannot be regarded as a disease that should be treated with products on the basis of their long-standing and traditional use outside medical supervision. For 1,8-cineole, two in-vivo studies reported that it seems to cause improved lung compliance, but an antitussive effect could not be determined.

Studies on diabetic animals and the use as repellent are not supported by a traditional use; therefore these data are not relevant here.

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

No data are available for the herbal substance or its preparations. Limited data are available on Eucalyptus oil, but it has to be taken into account that it is made from fresh leaves of different species. The main component of the essential oil of Eucalyptus leaves is 1,8-cineole. Therefore, data found on the pharmacokinetic of 1,8-cineole are given in this section.

1,8-cineole is a lipophilic substance. It is rapidly absorbed through skin and mucosa. Oral and rectal administration and inhalation have been described [Saller *et al.* 1988].

There are only a few studies on the pharmacokinetics of 1,8-cineole and most of them have been performed in brushtail possum. Brushtail possums are herbivores that feed on Eucalyptus leaves [McLean *et al*. 2007, Pass *et al*. 2001, Southwell & Flynn 1980]. Their detoxification capacity is higher as shown by Pass *et al.* 2001, therefore the results of these studies are not relevant to humans and are not summarised in this assessment report. Pass *et al.* 2001 studied the metabolism of 1,8-cineole in brushtails possum, koala, rats and also in human (*ex-vivo*). After incubation of pooled human liver microsomes with 1,8-cineole three metabolites were detected: 2-hydroxycineole (72%), trace amounts of 9-hydroxycineole (8.9%) and one unknown hydroxycineole metabolite (19.2%). Miyazawa & Shindo [2001] investigated the biotransformation of 1,8-cineole in human liver microsomes. Only one metabolite, 2-exo-hydroxy-1,8-cineole, was found. Several metabolites such as 1,8-dihydroxy-10-carboxy-p-menthane, 2-hydroxycineole and 3-hydroxycineole have been identified in rat urine after oral administration [Madyastha & Chadha 1986, De Vincenzi *et al*. 2002]. In rabbit urine the same metabolites, 2- and 3-hydroxycineole have been identified [Miyazawa *et al.* 1989]. Hydroxycineole is excreted as a glucuronic acid [Opdyke 1975].

A maximum concentration of 1,8-cineole in rabbit plasma was found at 30 minutes and the concentration decreased slowly between 1 to 4 hours. For free metabolites (2-exo, 2-endo- and 3-exo-hydroxycineole), a maximum concentration occurred after 1 hour and decreased slowly after 2 hours. The conjugated metabolites showed a maximum concentration after 1.5 to 2 hours. Their concentration decreased 2 to 4 hours after the administration [Miyazawa *et al.* 1989].

Effects of 1,8-cineole on the liver and lung enzyme system

Cineole has been found to increase significantly the activity of the microsomal enzyme system [Jori *et al*. 1969]. Rats were treated by subcutaneous (s.c.) injection (cineole 500 mg/kg daily for 4 days) or aerosol inhalation (4 days, twice 15 minutes and twice 30 minutes; 50 mg/minutes). *In-vitro* microsomal activities of O-demethylation of p-nitroanisol, 4-N-demethylation of aminoantipyrene and p-hydroxylation of aniline were significantly increased after cineole administration. *In-vivo* effects were demonstrated on metabolism and pharmacological actions of pentobarbital (25-30 mg/kg i.p. 7, 18, 36 and 48 hours after administration of cineole). Administration of cineole, s.c. or by aerosol inhalation, showed a significant decrease in pentobarbital effects. The sleeping time and pentobarbital concentration in brain of treated rats were less than that in the control group. Effects were dose-dependent and disappeared after 48 hours (s.c.) and 72 hours (after last inhalation). A follow-up study confirmed these results, but showed that the liver concentration of the cytochrome P-450 was not modified by cineole administration [Jori *et al.* 1972].

The effect of 1,8-cineole on liver and lung microsomal cytochrome P-450 and b_5 systems of rats has been investigated by Madyastha & Chadha [1986]. They found that 1,8-cineole administered by inhalation induced liver microsomal cytochrome P-450 level after 3 to 9 days of treatment. The level of cytochrome b_5 showed only a slight increase. In contrast, the level in the lung microsomes was not increased. The levels of NADPH and NAD cytochrome c reductase from both lung and liver microsomes seemed to be unaffected.

An inhibitory effect on 3-hydroxy-3-methyl-glutaryl-Coenzyme A reductase (HMG CoA reductase) could be shown by administration of cineole to male Wistar rats (3 mmol/kg) [Clegg *et al*. 1980, 1982].

A dose-dependent increased activity of glucuronyl tranferase (GFA) in rats after administration of cineole (inhalation 150 mg/min for 5-8 days or s.c. 500 and 1,000 mg/kg for 4 days) was found by Hohenwallner & Klima [1971].

Saify *et al.* [2000] investigated the skin penetration enhancer effect of 1,8-cineole towards 5-fluorouracil (a model drug) into rats. 1,8-cineole was found to be very active, no lag time was observed, it caused an 83-fold increase in drug permeability. Due to their results, the authors assumed that enhanced penetration may not only be caused by an increased partition of the drug into stratum corneum, but also by modifying intercellular lipids. Disrupting their highly ordered structure, an increased diffusion of the drug through skin may occur.

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

Only scarce data are available on the herbal substance and preparations thereof. Therefore data on 1,8-cineole as the main constituent of the essential oil, are also cited.

Acute toxicity

Eucalyptus leaves

Aswal *et al.* [1984] cited a LD_{50} of 652 mg/kg i.p. on mice for the Eucalyptus tincture (1:5) that was produced by extraction with wine spirit. No further information on the study was given.

1,8-cineole

The LD_{50} oral dose found by Jenner *et al.* [1964] on Osborne-Mendel rats is 2,480 mg/kg body weight.

De Vincenzi *et al*. [2002] summarised the toxicity data of 1,8-cineole. Toxicity studies reported in rats and mice suggested that mice were less susceptible than rats to the toxicity of 1,8-cineole. After gavage of 150 to 2,400 mg/kg/day, a dose-related reduction in the body weight gain and histopathological damage of the liver in male rats was observed. The highest dose of 2,400 mg/kg/day showed 50% of mortality in both sexes. Cineole given in encapsulated form corresponding to 0, 381, 766, 1,740 and 3,342 mg/kg showed a dose-related histopathological alteration of liver, kidney and parotid gland at all dose levels only in male rats. Treatment of mice by gavage for 28 days, at dose of 0, 150, 300, 600, 1,200 mg/kg/day, did not result in any dose-related lesions in either sex. After the treatment by encapsulated cineole corresponding to 0, 600, 1,322, 2,448, 5,607 mg/kg/day, a minimal dose-related hypertrophy of centrilobular hepatocytes was observed. This supports the assumption that exposure to cineole over the whole day induces a stronger response in the tissue than a single short daily exposure.

Kristiansen & Madsen [1995] found that the treatment of Wistar rats with 1,8-cineole in feed at doses of 500 and 1,000 mg/kg body weight for 28 days can cause renal lesions. Body weight was decreased and relative liver and kidney weights were significantly increased in all groups, whereas the relative brain weight was increased only in the 1,000 mg/kg dosed group. Histopathological changes in the brain were not observed.

Mutagenicity

Eucalyptus leaves

Schimmer *et al*. [1994] evaluated Eucalyptus tincture (according to the German Pharmacopoeia 6th ed. supplement; manufactured with wine spirit), beside other plant extracts, in an AMES mutagenicity test. *Salmonella typhimurium* strains TA 98 and TA 100 were used with and without S9 mix (from induced rat liver microsomes) as indicator cells. At the maximum dose of 80 µl/plate, Eucalyptus tincture showed a toxicity that was reduced in the presence of S9 mix. Nevertheless, mutagenicity could not be detected. A possible effect of the ethanolic component of the extract on the results has not been discussed.

Cineole

Gomes-Carneiro *et al*. [1998] tested the mutagenicity of 1,8-cineole at the dose of 250 µg/plate by the *Salmonella*/microsome reverse mutation assay TA97a, TA98, TA100 and TA102 as tester strains. Positive and negative controls were included. No mutagenic effect was found. However, the bacterial strains used are not in accordance with those recommended in the OECD Guideline No. 471.

Sasaki *et al*. [1989] treated Chinese hamster ovary cells with 0.15 µm mitomycin C for 21 hours and post-treated them with cineole at concentrations of 0, 3.3, 10, 33.3, 100, 333 (toxic) µM to investigate the effects on sister-chromatid exchange (SCE). Treatment with cineole showed no influence on SCE induced by mitomycin C.

Carcinogenicity

No data on Eucalyptus leaf preparations are available.

Cineole

Stoner *et al*. [1973] examined the ability of 1,8-cineole to induce primary lung tumours. Mice received i.p. injections of 12 and 2.4 g/kg body weight for 8 weeks and were killed at 24 weeks after first injection. Four mice out of 15 (dosage 12 g/kg) and 3 mice out of 15 mice (dosage 2.4 g/kg) developed a lung tumour during the study. The authors stated that the pulmonary tumour response in mouse should not be used as a sole index of carcinogenic activity of an agent.

The treatment of male Wistar rats with 1,8-cineole at doses of 500-1,000 mg/kg body weight for 28 days can cause an accumulation of protein droplets containing alpha-2-microglobulin in the proximal tubular epithelial cells [Kristiansen & Madson 1995]. On this basis, the authors concluded that 1,8-cineole belongs to the so-called CIGA carcinogens (chemical inducing alpha-2-globulin accumulation). But it is important to recognise that alpha-2-microglobulins nephropathy is a phenomenon, which is exclusively found in adult male rats. Alpha-2-microglobulins nephropathy does not occur in humans. Therefore, a direct extrapolation of rat data cannot be made [Swenberg *et al.* 1989].

Assessor's comment:
A comparable concentration of 1,8-cineole cannot be reached by the administration of Eucalyptus leaves according to the traditional posology. Therefore, these findings are not relevant for a traditional use.

Reproductive toxicology

In order to investigate the possibility of stimulating drug metabolism in foetal and neonatal periods, pregnant and lactating rats were treated with cineole (500 mg/kg s.c. daily for 4 days between day 10 and 14 of pregnancy or during the last 4 days of pregnancy or between day 2 and 6 after delivery). Cineole increased liver microsomal enzyme activity of mothers (for all experiments) and foetuses, but not in suckling newborn rats. Nursing mother rats, treated with cineole, showed an increased liver enzymatic activity, too. The authors concluded that cineole cannot cross the blood-milk barrier, but it is able to penetrate the placenta tissue [Jori & Briatico 1973].

3.4. Overall conclusions on non-clinical data

Pharmacology

In summary, effects on a broad spectrum of bacteria, fungi and viruses reported by non-clinical data for Eucalyptus leaves and preparations thereof as well as for 1,8-cineole. There are some reports about anti-inflammatory and analgesic effects (*in-vitro*); however it is not proven that the relevant concentrations can be reached clinically.

According to Whitman & Ghazizadeh [1994], the therapeutic effects of Eucalyptus based medications, in settings of pharyngitis and pharyngeal inflammation, may be due to the presence of 1,8-cineole. On the other hand, Goldstein *et al.* [1976] could not demonstrate that a treatment of rats and mice with a cold preparation (containing a mixture of volatile oils: camphor, menthol, cineole and turpentine) decreases the ^{32}P-labelled *Staphylococcus aureus* load of animals lungs. No significant differences between treated and untreated animals were observed. Other constituents of *Eucalyptus globulus* such as macrocarpals, eucaglobine and eucalyptone may contribute to the effects of leaves preparations as well.

As shown in section 2., *Eucalyptus globulus* leaves and their preparations have a long tradition with benefits for diseases of the upper respiratory tract. Nevertheless, *in-vivo* studies were not performed to support the benefits. The antimicrobial and anti-inflammatory effects may represent a benefit on respiratory diseases. Inhibition of bacteria as well as an improvement of lung function (lung compliance) may contribute to a positive effect. The pharmaceutical forms are herbal tea and inhalant. Used as an inhalant, it humidifies the respiratory gas. Used internally as herbal tea, it increases also the daily fluid intake. Both methods of administration increase the dissolution of the respiratory mucus. Therefore, Haen [1989] classified Eucalyptus leaf preparations as an expectorant. According to Juergens *et al*. [2004], the reduction of cytokine production by Eucalyptus supports an anti-inflammatory effect and consequently supports the inhibition of cytokine-induced airways mucus hypersecretion rather than simple secretolytic activity.

Another benefit is that cineole causes a sensation of cold and this is accompanied with a facilitated respiration [Saller *et al.* 1988].

Pharmacokinetics

For the herbal substance or herbal preparations thereof, no sufficient data are available. Therefore, no statements on the pharmacokinetics of *Eucalyptus globulus* leaves preparations can be made.

Despite this fact the published data on cineole should be taken into account. 1,8-cineole as the main constituent of the volatile oil of Eucalyptus leaves is rapidly absorbed through skin and mucosa. Although no interaction studies were published, a number of *in-vitro* and *in-vivo* animal studies indicate that cineole affects the activity of liver enzymes. Therefore, an effect of Eucalyptus leaf preparations on drug metabolism is possible. Consequently, effects of other drugs may be increased or decreased following concomitant administration of cineole or cineole containing preparations.

Toxicology

No toxicological information on the herbal substance or herbal preparations thereof is available. Results for cineole are of limited value for the monograph on *Eucalyptus globulus*, folium. Therefore, a Community list entry cannot be recommended from a non-clinical point of view.

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

Clinical data on Eucalyptus leaves or preparations thereof are missing. But the effects of 1,8-cineole after inhalation have been evaluated. Study on 20 healthy humans showed that an inhalation of 100 µl 1,8-cineole led to an increased feeling of relaxation and a decreased reaction time [Ilmberger *et al*. 2001].

Assessor's comment:
The increased relaxation feeling, due to 1,8-cineole inhalation, may create a feeling of well-being in patients with coughs and colds.

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

Inhalation

Pharmacokinetic of 1,8-cineole was investigated by Jäger *et al.* [1996] on 4 healthy subjects. 1,8-cineole was administered by a closed breathing circuit with the air passing over 4 ml of 1,8-cineole for 20 minutes. Blood samples were drawn at 0, 5, 10, 20, 25, 30, 35, 45 and 60 minutes after application. The results showed that 1,8-cineole is well absorbed from breathing air, with a peak plasma concentration after about 18 minutes. The elimination from blood is biphasic, with a mean distribution half-life of 2-13 minutes and an elimination half-life of 31-281 minutes.

The uptake of 1,8-cineole via inhalation was studied in one healthy subject by Stimpfl *et al*. [1995]. During an inhalation period of 20 minutes (2 ml 1,8-cineole 99%) the 1,8-cineole concentration in blood serum was increasing in an almost linear way from 4-20 minutes to a maximum. When inhalation was stopped, the concentration of 1,8-cineole in serum dropped immediately. After further 40 minutes, a reduced value of about 10% of the maximum value was observed. Stimpf *et al.* obtained a half-life of about 10 minutes. These results are in accordance with the results of Jäger *et al*. 1996.

Inhalation and oral

Kohlert *et al.* [2000] reviewed the published data on bioavailability and pharmacokinetics of volatile terpenes. Concerning the above-summarised study of Jäger *et al.* 1996, he stated that there are substantial differences in the elimination half lives between the male and female subjects. Indeed elimination half lives were at least twice as long in female subjects, but tested subjects were only 4 (2 male and 2 female). Concerning another study of Zimmermann *et al*. [1995], it was deduced that the upper part of the gastrointestinal tract has no significant role with respect to the absorption of cineole. The study of Zimmermann was performed with capsules containing a mixture of limonene, cineole and α-pinene. Cineole was only measured as a marker. The results showed that an oral application of cineole led to a maximum cineole concentration in blood serum within 2.3 to 2.6 hours for the unchewed tablet and within 0.7 to 1.1 hours for the chewed tablet.

4.2. Clinical Efficacy

Eucalyptus leaves

Studies on efficacy of *Eucalyptus globulus* leaves and preparations thereof have only been performed for the treatment of dental and oral diseases or for the treatment of wound infections [Sato *et al*. 1998; Sherry *et al.* 2001]. All studies supported an antimicrobial and anti-inflammatory effect of Eucalyptus in accordance with the non-clinical studies cited in section 3.

Sato *et al.* [1998] studied the effect of chewing gum with Eucalyptus extract on plaque formation. Eucalyptus significantly reduced plaque compared to control gum. The authors attributed this effect to the antibacterial activity of macrocarpals (constituents of Eucalyptus) against *S. mutans* and *P. gingivalis*. No information about the Eucalyptus extract or about the amounts in gum were given. Sherry *et al.* [2001] reported that a medicinal product called Polytoxinol®, which is a complex formulation of Eucalyptus plant extracts, was administered percutaneously to orthopaedic patient

with wound infections. Polytoxinol® was more effective against a broad range of aerobic bacteria (e.g. MRSA) than antibiotics such as flucloxacillin.

Assessor's comment:
Some (pilot) studies have been performed for different indications. The data available are not sufficient to assess efficacy. Moreover, well-established use is defined as well-documented use for a time frame of minimum 10 years, which is not fulfilled. Therefore, a well-established use for Eucalyptus leaf preparations cannot be supported.

1,8-cineole

Several studies on the efficacy of cineole have been published.

Acute rhinosinusitis

Kehrl *et al*. [2004] compared efficacy and safety of cineole capsules with placebo in 152 patients with acute rhinosinusitis. A dosage of 100 mg 3 times a day was administrated for 7 days (75 placebo, 75 cineole). Significant differences were seen from beginning to the end of treatment for amelioration of headache on bending, sensitivity of pressure points of trigeminal nerve, nasal obstruction, secretion quantity and viscosity and rhinological secretion as well as for redness of mucosa. After 7 days, the differences between both groups were statistically significant in the same parameters. No significant differences were found according to clinical findings of inflammation such as conjunctivitis, tracheitis, pharyngitis and bronchitis. No differences have been found for parameters of leukocyte count and erythrocyte sedimentation rate. The authors concluded that the early treatment of sinusitis with cineole can avoid antibiotic treatment. Mild side effects were observed in two patients as heartburn and exanthema.

In another study, 150 patients with acute and viral rhinosinusitis were treated with cineole or a herbal combination product (Gentianae radix, Primulae flos, Ramicis herba, Sambuci flos, Verbenae herba). Both treated groups showed an improvement in all relevant characteristics for rhinosinusitis within 7 days. A significant benefit after treatment with cineole could be detected for bronchitis but not for pharyngitis, tracheitis and conjunctivitis. Scores for headache on bending, frontal headache, sensitivity of pressure points of trigeminal nerve, nasal obstruction and rhino-secretion for 7-day treatment were significantly lower for the treatment with cineole than for the treatment with the herbal combination product [Tesche *et al.* 2008].

Assessor's comment:
Sinusitis and its symptoms are difficult to score because of the individual perception. Image studies (such as ultrasonic or computed tomography) have not been performed.

Pulmonary diseases

Worth *et al*. [2009] studied the effects of cineole (200 mg 3 times a day for 6 months as concomitant therapy) in comparison with placebo in a double-blind, placebo-controlled study in 242 patients with chronic obstructive pulmonary disease (COPD). Cineole reduced exacerbations as well as dyspnea and improved lung function (forced expiratory volume, forced vital capacity, vital capacity) significantly in comparison to placebo. Worth declared the therapy with cineole as a concomitant one, but he did not give any data on the other medications.

In order to compare the effects of oral therapy with cineole (3 times 200 mg/day) and ambroxol (3 times 30 mg/day) after treatment over one week, a randomised double-blind, cross-over trial in 29 patients with COPD was performed by Wittmann *et al*. [1998]. All parameters of lung-function, peak-flow and symptom-score showed better improvements under therapy with cineole, but failed to reach statistical significance in comparison to ambroxol.

A double-blind placebo controlled study on 51 patients with COPD was carried out for 8 weeks by Habich & Repges [1994]. Both groups were treated with β2-sympathomimetics, glucocorticosteroids and methylxanthins and at the same time the testing group was treated with 200 mg cineole 3 times a day. The objective lung function parameters, airway resistance and specific airway resistance, were reduced by 21% and 26%, which were both clinically relevant and statistically significant in comparison to the placebo group.

Another combination product was studied by Böhlau & Schildwächter [1977]. Aerosol administration of the combination of cineole, thymol, anis oil, azulene and propylenglycol showed a decrease of cough intensity and amount of sputum, but no improvement of pulmonary function could be detected.

Assessor's comment:
Several studies dealt with effects of cineole on pulmonary diseases, but none with Eucalyptus leaves preparations. Due to the small number of treated patients, results failed to be significant or cineole treatment was only a concomitant one. Therefore, these results are not clear enough to apply them to Eucalyptus leaves.

Anti-inflammatory effect

Since 1,8-cineole had been found to possess an inhibitory effect on inflammatory mediator production, Juergens *et al.* [2003] performed a randomised, placebo-controlled trial to determine the oral glucocorticosteroid-saving effect of long-term 1,8-cineole therapy in patients with severe asthma. All subjects received between 5 and 24 mg prednisolone, inhaled corticosteroids (expressed as equivalent doses to beclomethasone) and, additionally, long acting β-agonists and/or theophylline. Dosages were kept constant throughout the study, except for short-acting β-agonists. Daily prednisolone dosage and concomitant asthma medication did not differ in the two groups. Each participant was randomly assigned either to 1,8-cineole (small gut soluble capsules, 200 mg t.i.d. for 12 weeks) or placebo capsules. Lung function tests were performed and venous blood was taken to determine blood cells. Twelve patients out of 16 remained clinically stable despite a reduction of oral steroid dosage of 5 mg every third week. The mean reduction of steroid dosage was 36% equivalent to 3.8 mg/day in the verum group which remained clinically stable, in contrast to the placebo group (7% equivalent to 0.9 mg/day, 12 out of 16 patients were not able to tolerate a decrease of oral steroids). The authors concluded that this is a confirmation of the anti-inflammatory activity of 1,8-cineole in bronchial asthma.

4.2.1. Dose response studies

No dose response studies on Eucalyptus leaves or preparation thereof have been published.

4.2.2. Clinical studies (case studies and clinical trials)

In contrast to its widespread use, clinical studies on Eucalyptus leaves containing preparations are missing. Due to its characteristic taste and smell, it may almost be impossible to conduct a blinded study.

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

No studies on special populations such as children or elderly have been performed.

4.3. Overall conclusions on clinical pharmacology and efficacy

Despite the popularity of Eucalyptus and its long usage for treatment of diseases of upper respiratory tracts, there has been little clinical research on their effects on cough and cold.

Studies on cineole showed that it is well absorbed from inhalation and from oral administration. Studies on clinical efficacy of cineole showed that it has some effects in rhinosinusitis and obstructive pulmonary diseases.

The content of cineole may contribute to the effects of Eucalyptus leaves preparations. Dried Eucalyptus leaves contain 1 to 3.5% essential oil. The essential oil contains about 54 to 95% of the major constituent 1,8-cineole. Provided that all essential oil and cineole pass over into the herbal infusion, the cineole content of the infusion would range between 10.8 mg and 99.75 mg in a single dose and the daily dose would contain about 32 to 300 mg 1,8-cineole. For the tincture (1:5) according to the EB6, the single dose of 1,8-cineole is 2.7 to 17 mg and, according to the Pharmacopée Française, the daily dose is 1.1 to 19 mg 1,8-cineole.

A daily dose of 300 mg 1,8-cineole corresponds to 50% to 100% of dosages in published clinical studies. The daily posology in clinical studies was 300 to 600 mg. Therefore, a pharmacological effect for the herbal tea is plausible. However for the tincture (1:5), a single dose according to the posology in the EB6 (2.5 g tincture once daily) corresponds to only 2.7 to 17 mg 1,8-cineole. The posology according to the Pharmacopée Française 1976 (1 to 10 g tincture a day) corresponds to 1.1 to 19 mg 1,8-cineole. Thus, the traditionally reported posology is not supported by the clinical data on 1,8-cineole.

Additionally, it should be taken into account that the pass over rates from the herbal substance into the infusion or the tincture might not be 100%. For an infusion made from *Salvia officinalis*, for example, a transfer rate of 23-36% is reported for 1,8-cineole [Sur *et al.* 1991]. That would mean that the daily dosage of 1,8-cineole for the traditional use corresponds to approximately 70 to 100 mg i.e. a maximum of 33% of the dosages in the published clinical studies.

5. Clinical Safety/Pharmacovigilance

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

No relevant data on Eucalyptus leaves preparations are available.

5.2. Patient exposure

1,8-cineole

Mild side effects such as heartburn and exanthema were observed in 2 out of 75 patients treated with 1,8-cineole (3 times 100 mg) by Kehrl *et al.* [2004]. Worth *et al*. [2009] reported adverse events with heartburn in 9 out of 110 patients and nausea, diarrhoea and heartburn in 3 out of 110 patients treated with cineole (3 times 200 mg).

5.3. Adverse events and serious adverse events and deaths

Eucalyptus leaves

The Commission E monograph lists nausea, vomiting and diarrhoea as possible side effects. As contraindications, inflammatory diseases of the gastrointestinal tract, gall bladder disease or impaired liver function are given. Additionally, Eucalyptus preparations for external use should not be

applied to the face, especially the nose, of infants or young children. The oil may induce liver enzymes that are involved in drug metabolism. Therefore, the effects of other drugs may influence concomitant administrations [WHO monographs 2002, Blumenthal *et al*. 1998].

Saller *et al*. 1988 cited a lethal dose for adults of 4 to 5 ml Eucalyptus oil. For Eucalyptus leaves, they stated that poisoning by oral intake is very unlikely, because dried and comminuted Eucalyptus leaves contain only 1.5% essential oil (minimum according to European Pharmacopoeia 2011) - that corresponds to 267 to 333 g dried leaves. Reported maximum content is about 3.5%, that corresponds to 114 to 143 g dried comminuted leaves. The uptake of a single dose of more than 100 g dried leaves is almost not possible.

One case report by Vidal & Cabeza 1992 described a development of localized erythema and pruritus after contact with Eucalyptus leaves (55-year-old woman). Lesions reappeared after subsequent exposure. Prick and open test with a pollen extract of Eucalyptus (acetone) showed a positive reaction.

Eucalyptus oil

A number of intoxication reports are available in the literature following accidental exposure to high doses of Eucalyptus oil. De Vincenzi *et al*. [2002] summarised the case reports. The amounts ingested ranged from 1 ml to 220 ml. The described effects were gastrointestinal symptoms, followed by CNS depression, transient coma; one subject collapsed with rapid shallow respiration and a rapid and feeble pulse. Two patients survived after dialysis (21-220 ml ingestion) and 2 patients died (3.5-5 ml ingestion).

Cineole

Melis *et al.* [1990] reported the following symptoms of poisoning with 1,8-cineole described by parents: nose and gastric burning, nausea, vomiting, dizziness and muscular weakness, miosis, tachycardia and feeling of suffocation, in infants aged 1 month to 3 years and 9 months.

5.4. Laboratory findings

None.

5.5. Safety in special populations and situations

It has been described that the inhalation of essential oils, observed also for menthol and camphor, may lead to an irritation of the nasal mucosa, which can lead to a closure of glottis in infants (Kratschmer-Reflex) [Jorch 2009].

Due to the cooling effect and strong odour of Eucalyptus preparations it is recommended that ointments should not be applied to or near the face of babies and very young children because of the risk of reflex spasm of the glottis [ESCOP monographs 2003, Blumenthal *et al.* 1998]. Even apnoea may occur [Blaschek *et al.* 2007].

Since essential oils are steam volatile components, the use as an oral fluid or an inhalant could also generate the risk of reflex spasm for babies and very young children. Their use should therefore also be contraindicated in babies and very young children.

In order to investigate whether 1,8-cineole treatment affects plasma levels of other drugs, Jori *et al.* [1970] studied the plasma levels of 5 volunteers. During treatment with cineole (inhalant 10 minutes of a 0.4 g 1,8-cineole suspension) and aminopyrine (600 mg orally before and after treatment with 1,8-cineole) for 10 days, they observed that 1,8-cineole treatment increased the disappearance of plasma aminopyrine in 4 out of 5 subjects.

5.6. Overall conclusions on clinical safety

The monograph of the Commission E “Eucalyptus leaves” gives some side effects and contraindications. Considering the history of development of this monograph, it appears that this information is based on the content of essential oil and cineole. Separate studies for the leaves, case reports or reports of unexpected drug interactions with preparations containing the leaves have not been published. Reporting system in Germany listed the following side effects for Eucalyptus oil containing medicinal products for adults:

Oral use:
allergic skin reaction (1), shock (1), tremor (1), decreased blood pressure and increased pulse (1), cold sweat (1), dizziness (3), tiredness (1), coordination abnormal (1), ataxia (1), aphasia (1), syncope (1), vomiting (1), urticaria (1)

Topical use:
skin disorders such as pustular rash (2).

These were considered for possible inclusion in the HMPC monograph; however Eucalyptus oil is only a small fraction of Eucalyptus leaf preparations. Dried and comminuted Eucalyptus leaves contain 1.5% essential oil (minimum according to European Pharmacopoeia 2011), therefore the reported side effects of Eucalyptus oil were considered irrelevant for the specified traditional use of Eucalyptus leaf preparations.

From the above-mentioned clinical trials, mild side effects were observed only in a small number of treated patients.

The results of Jori *et al*. [1970] correspond to the non-clinical studies concerning the inducing effect of cineole on liver microsomal enzymes reported in section 3.2. The results indicate that the effect of other drugs may be influenced following concomitant administration. But the posology of cineole tested by Jori *et al*. corresponds to about 14 g Eucalyptus leaves. This does not conform to the traditional daily dose for the inhalant.

6. Overall conclusions

Pharmacology:
As summarised in section 3.1, the effects of *Eucalyptus globulus* leaves on the upper respiratory tract can be regarded as sufficiently supported by long-standing use and non-clinical data. Its content of volatile oil and cineole makes an antimicrobial effect plausible. Macrocarpals may also contribute to the antimicrobial effect. Additionally, Eucalyptus causes a sensation of cold that facilitate respiration. And, used as an inhalant, the respiratory tract is humidified, which also contributes to a facilitated respiration.

Clinical efficacy:
No clinical data on the efficacy of Eucalyptus leaves preparations are available, thus a well-established use is not supported. From the clinical data on cineole, an effect on upper respiratory diseases is anticipated. But the actual cineole content of an herbal infusion or a tincture prepared from *Eucalyptus globulus* leaves is not known. Due to the widespread and long-standing use, Eucalyptus leaves can be listed for traditional use for the relief of cough associated with cold. Reported indications for asthma and bronchitis are not listed, because their treatment should be supervised by a medical practitioner.

The preparations are herbal infusion (oral or as inhalant) and tincture (oral), in suitable pharmaceutical forms. Some authors and handbooks described a syrup and an ointment, but, for

both, no comprehensive posologies were reported. Therefore, a traditional use with a specified strength and posology for such syrup and ointment cannot be provided.

A posology of about 2 to 3 g herbal substance as a single dose and 6 to 9 g herbal substance as a daily dose is sufficiently supported by literature.

Relevant data on an application for children under 12 years of age are not available. Therefore, they are excluded from this therapy. The use in adolescents between 12 and 18 years of age could be accepted in accordance to the data available for Eucalyptus oil. However, having considered the amount of alcohol intake from the use of the tincture at the highest posology, the HMPC decided to restrict the use of the tincture to adults and elderly.

In line with the recommendation found in the Cahier N°3 de l'Agence du Médicament, the duration of use is limited to one week.

Based on the data on Eucalyptus oil, several contraindications were listed in the Commission E monograph. Due to an oil content of only 1.5 to 3.5%, they are irrelevant to the use of Eucalyptus leaf preparations as specified in the HMPC monograph. But since essential oils used as an oral fluid or an inhalant could generate the risk of reflex spasm for babies and very young children, the use in children under 2 years of age should be contraindicated. After discussion (meeting January 2013), the HMPC decided that the use must be contraindicated in children below 30 months.

For tinctures containing ethanol, the appropriate labelling for ethanol, taken from the 'Guideline on excipients in the label and package leaflet of medicinal products for human use', has to be included.

Drug interactions are possible, because a lot of non-clinical studies and a clinical study by Jori *et al*. [1970] indicate that liver enzymes that are involved in drug metabolism may be induced. The effect of other drugs may be influenced following concomitant administration. However, considering the posology as explained in section 5.6., this does not justify a statement in the monograph's section on interactions.

Safety during pregnancy and lactation has only been established on rats. The studies indicate that cineole can cross the placenta barrier, but not the blood-milk barrier. Due to the lack of adequate clinical data, the following wording is given in section 4.6. of the monograph: "Safety during pregnancy and lactation has not been established. In the absence of sufficient data, the use during pregnancy and lactation is not recommended."

No relevant toxicological information on the herbal substance or herbal preparations thereof is available.

Due to the lack of toxicity data, a Community list entry cannot be supported.

Annex

List of references