8 July 2020
EMA/HMPC/52980/2017
Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Thymus vulgaris* L., *Thymus zygis* L., aetheroleum

Final – Revision 1

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

Herbal substance(s) (binomial scientific name of the plant, including plant part)		Not applicable
Herbal preparation(s)		*Thymus vulgaris* L., *Thymus zygis* L., aetheroleum
Pharmaceutical form(s)		Herbal preparations in liquid dosage forms for oral use and in liquid or semi-solid dosage forms for cutaneous use and use as a bath additive
First assessment	Rapporteur(s)	R Länger
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1. Introduction

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

- Herbal substance(s)

Not applicable.

- Herbal preparation(s)

Thyme essential oil (= thyme oil; Latin: Thymi typo thymolo aetherolum).

Definition

Essential oil obtained by steam distillation from the fresh flowering aerial parts of *Thymus vulgaris* L., *T. zygis* L. or a mixture of both species (Ph. Eur. Monograph 01/2012:1374).

Appearance

Clear, yellow or very dark reddish-brown, mobile liquid. Odour reminiscent of thymol (Ph. Eur. Monograph 01/2012:1374).

Commercially the crude thyme oil is called "red thyme oil" because of its deep colour. After redistillation "white thyme oil", a light-yellow oil, which smells similarly but sweeter and less pungent is obtained (Böhme *et al.* 2008).

Composition

Essential oil: there are at least 6 chemotypes of *Thymus vulgaris* L. (Thompson *et al.* 2003) with different compositions of the essential oil; only the 'thymol'-type with thymol as predominant compound complies with the definition in the European Pharmacopoeia. The dried herbal substance contains up to 2.5% essential oil; the main components are thymol, p-cymene, carvacrol, γ -terpinene, linalool, β - myrcene, terpinen-4-ol. Some compounds occur partly as glycosides (e.g. p-cymene-9-ol) (Takeuchi *et al.* 2004, Kitajima *et al.* 2004, Stahl-Biskup 1991).

Composition and limits of the compounds according to Ph. Eur. Monograph

- α-Thujene: 0.2% to 1.5%,
- β-Myrcene: 1.0% to 3.0%,
- α-Terpinene: 0.9% to 2.6%,
- p-Cymene: 14.0% to 28.0%,
- γ-Terpinene: 4.0% to 12.0%,
- Linalool: 1.5% to 6.5%,
- Terpinen-4-ol: 0.1% to 2.5%,
- Carvacrol methyl ether: 0.05% to 1.5%,
- Thymol: 37.0% to 55.0%,
- Carvacrol: 0.5% to 5.5%.

- Combinations of herbal substance(s) and/or herbal preparation(s) including a description of vitamin(s) and/or mineral(s) as ingredients of traditional combination herbal medicinal products assessed, where applicable.

Not applicable

1.2. Search and assessment methodology

The call for scientific data for the systematic review of the monograph on Thymi aetheroleum started on 15 April 2016 and ended on 15 July 2016. During the period of public call for scientific data no data was received.

Most of the EU Member States respond to the call for exchange of information regarding the new data on medicinal products marketed or combination of medicinal products or other relevant products.

Search for the peer-review scientific articles and review articles was performed using PUBMED, TOXLINE and SCOPUS scientific bases during the September and November 2016 with key words: *thyme oil, thymus essential oil.*

Search engines used: GOOGLE SCHOLAR

Scientific databases: PUBMED, SCOPUS, MEDLINE

Toxicological databases: TOXLINE

Pharmacovigilance resources: WHO Global ICSR database (VigiBase), EudraVigilance database.

2. Data on medicinal use

2.1. Information about products on the market

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

Information on medicinal products marketed in the EU/EEA

The overview of data obtained from marketed medicinal products in EU/EEA is presented in Table 1.

Table 1: Overview of data obtained from marketed medicinal products

Active substance	**Indication**	**Pharmaceutical form** **Strength** **Posology** **Duration of use**	**Regulatory Status[2]**
Thymi aetheroleum	Traditional herbal medicinal product for the relief of symptoms in coughs and colds with viscous mucus.	bath additive 8.44 g thyme oil in 100 ml (= 105.5 g) bath additive children≥2 years and adults: 20 ml bath additive/100 l water for 10-20 minutes at 35-38°C if necessary 1 time daily	At least since 1976, Germany
Thymi aetheroleum	Traditional herbal medicinal product for the relief of symptoms	bath additive 5.0 g thyme oil in 100 g	At least since 1990, Germany

[2] Date, Member State, Type of Marketing authorisation/registration where possible: e.g. full MA, WEU or bibliographical, TU

Active substance	Indication	Pharmaceutical form Strength Posology Duration of use	Regulatory Status[2]
	in coughs and colds.	bath additive children ≥2 years and <6 years: 5 ml bath additive/40–50 l water children 6-12 years: 10 ml bath additive/50 – 60 l water children ≥12 years and adults: 20 ml bath additive/100 l water for 10-20 minutes at 35-38°C 3-4 times weekly	
Thymi aetheroleum	Traditional herbal medicinal product for the relief of symptoms in coughs and colds with viscous mucus.	bath additive 6.0 g thyme oil in 100 g (= 95.2 ml) bath additive children ≥2 years and adults: 20 g bath additive/100 l water for 15 minutes at 35-38°C maximum 1 time daily	At least since 1976, Germany
Thymi aetheroleum	Traditional herbal medicinal product for the relief of symptoms in coughs and colds with viscous mucus.	bath additive 8.0 g thyme oil in 100 g (=95 ml) bath additive children ≥6 month and <2 years: 2.5 ml bath additive/30 l water children ≥2 years and <6 years: 5 ml bath additive/40 – 50 l water children 6-12 years: 10 ml bath additive/50-60 l water children≥12 years and adults: 20-30 ml bath additive/100 l water for 10-20 minutes at	At least since 1976, Germany

Active substance	Indication	Pharmaceutical form Strength Posology Duration of use	Regulatory Status[2]
		35-38°C 3-4 times weekly	

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

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

Not applicable

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

No information

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

No information

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

The medicinal use of thyme oil is documented at least since 1589 (Dispensatorium Noricum, cited in Gildemeister *et al.* 1961). The essential oil of *Thymus vulgaris* L. is published in pharmacopoeias and standard textbooks of phototherapy since many decades (e.g. Tschirch 1917, Stahl 1962, Martindale 1972).

The overview of historical data of use of thyme oil is presented in Table 2.

Table 2: Overview of historical data

Herbal preparation	Documented Use / Traditional Use	Pharmaceutical form Strength Posology Duration of use	Reference
Thyme oil	Oral use: Antihelmintic (use of thymol)	Complete posology not available 0.1-2 g	Tschirch 1917 Stahl 1962 Madaus 1938
Thyme oil	Oromucosal use: antiseptic	Mouthwash, tooth pastes No data on the strength available; a concentration of 0.005% thymol is proposed (equivalent to 0.009–0.014% essential oil)	Gildemeister *et al.* 1961 Haffner *et al.* 1984

Herbal preparation	Documented Use / Traditional Use	Pharmaceutical form Strength Posology Duration of use	Reference
Thyme oil	Oral use: Catarrh of the upper respiratory tract, bronchial catarrh, symptoms of bronchitis, cough with spasms	Posology: Oral use: 4-5 drops (for example on a piece of sugar or mixed with honey) 3-5 times daily	Martindale 1972 Böhme et al. 2008 Poletti *et al.* 1990 cited in Blaschek *et al.* 2008
Thyme oil	Oral use: Acute bronchitis, whooping cough and laryngitis	Internally: 3 to 5 times a day 4 to 5 drops on a piece of sugar cubes or mixed with honey	Leung (1980) cited in Blaschek *et al.* 2008
Thyme oil	Externally: in baths for the treatment of pruritus in dermatoses	Bath additive: with at least 0.004 g of thyme oil per liter of water	Pratzel and Schnizer, 1992 cited in German commission B, 1990
Thyme oil	Externally: Bruises, sprains	No posology available	Leung 1980
Thyme oil	Externally: in baths for the supportive treatment of acute and chronic diseases of the airways	Bath additive: with at least 0.004 g of thyme oil per liter of water	Pratzel and Schnizer, 1992 cited in German commission B, 1990
Thyme oil	Inhalation: (thyme oil as bath additive) Supportive treatment of acute and chronic diseases of the airways	For bath additives: full bath with at least 0.004 g of thyme oil per liter of water	German commission B, 1990
Thyme oil	Externally**:** Rubefacient, counter-irritation	No posology available	Martindale 1972 Stahl 1962
Thyme oil	Externally: in the form of rubs for treatment of inflammatory lesions and to relieve itching, bruising	Pharmaceutical form: in the form of rubs 10% Complete posology not available.	Leung (1980) cited in Blaschek *et al.* 2008 Pratzel and Schnizer, 1992 cited in German

Herbal preparation	Documented Use / Traditional Use	Pharmaceutical form Strength Posology Duration of use	Reference
	and dislocation		commission B, 1990 Hänsel *et al.* 1994

2.3. Overall conclusions on medicinal use

According to the data collected from traditional use of thyme oil, the evidence of use on period of medicinal use together with a posology and indication in traditional use is presented in Table 3.

Table 3: Overview of evidence on period of medicinal use

Pharmaceutical form, **Herbal preparation**	**Indication**	**Posology** Strength	**Period of medicinal use**
Oral use			
Thyme oil	Oral use: Catarrh of the upper respiratory tract, bronchial catarrh, symptoms of bronchitis, cough with spasms	Posology: Oral use: 4-5 drops (for example on a piece of sugar or mixed with honey) 3-5 times daily	Martindale 1972 Böhme *et al.* 2008 Leung (1980) cited in Blaschek *et al.* 2008
Bath additive			
Thymi aetheroleum	Traditional herbal medical product for the relief of symptoms in coughs and colds with viscous mucus.	bath additive 8.44 g thyme oil in 100 ml (=105.5 g) bath additive children≥2 years and adults: 20 ml bath additive/100 l water for 10-20 minutes at 35-38°C if necessary 1 time daily	At least since 1976, Germany
Thymi aetheroleum	Traditional herbal medical product for the relief of symptoms in coughs and colds.	bath additive 5.0 g thyme oil in 100 g bath additive children≥2 years and <6 years: 5 ml bath additive/40-50 l water children 6-12 years: 10 ml	At least since 1990, Germany

Pharmaceutical form, **Herbal preparation**	**Indication**	**Posology** Strength	**Period of medicinal use**
		bath additive/50-60 l water children≥12 years and adults: 20 ml bath additive/100 l water for 10-20 minutes at 35-38°C 3-4 times weekly	
Thymi aetheroleum	Traditional herbal medical product for the relief of symptoms in coughs and colds with viscous mucus.	bath additive 6.0 g thyme oil in 100 g (=95.2 ml) bath additive children≥2 years and adults: 20 g bath additive/100 l water for 15 minutes at 35-38°C maximum 1 times daily	At least since 1976, Germany
Thymi aetheroleum	Traditional herbal medical product for the relief of symptoms in coughs and colds with viscous mucus.	bath additive 8.0 g thyme oil in 100 g (=95 ml) bath additive children≥6 month and <2 years: 2.5 ml bath additive/30 l water children≥2 years and <6 years: 5 ml bath additive/40-50 l water children 6-12 years: 10 ml bath additive/50–60 l water children≥12 years and adults: 20-30 ml bath additive/100 l water for 10-20 minutes at 35-38°C 3-4 times weekly	At least since 1976, Germany

In Germany bath additives containing thyme oil as the only active ingredient are on the market at least since 1976 for the relief of symptoms in coughs and colds. Therefore, for thyme essential oil, a period of at least 30 years in medical use, as required by Directive 2004/24/EC for qualification as a traditional herbal medicinal product, is fulfilled. In the first version of the monograph, published in 2010, it was also concluded that the traditional use for cutaneous use of thyme oil in liquid and semi-solid dosage forms in concentrations up to 10% for the relief of symptoms in coughs and colds was considered fulfilled. This conclusion on indication and posology (including the dosage frequency, i.e. up to 3 times daily) from the first version of the monograph is retained.

According to the market overview and literature thyme oil fulfils the criteria of medicinal use throughout a period of at least 30 years, including at least 15 years within the EU/EEA, i.e. traditional medicinal use according to Directive 2004/24/EC in the following indications and posologies:

Indication 1)

Traditional herbal medicinal product used as an expectorant in cough associated with cold.

Indication 2)

Traditional herbal medicinal product for the relief of symptoms in coughs and colds.

The product is a traditional herbal medicinal product for use in specified indications exclusively based upon long-standing use.

Expectorants may be interpreted for oral use by the layman. Therefore, a different wording for the indication for cutaneous use and use as bath additive is proposed.

Posology

Indication 1)

Adults and elderly

Oral use: 4-5 drops corresponding to 0.2 to 0.25 ml, 3-5 times daily

If the symptoms persist longer than 1 week, a doctor or a qualified health care practitioner should be consulted.

Indication 2)

Adults and elderly

Cutaneous use: in liquid and semi-solid dosage forms in concentrations up to 10%; apply up to 3 times daily. Apply on the chest and the back.

If the symptoms persist longer than 1 week, a doctor or a qualified health care practitioner should be consulted.

Use as bath additive: 0.007-0.025 g per litre.

Adolescents

Use as bath additive: 0.007-0.025 g per litre.

Children 6-12 years

Use as bath additive: 0.0035-0.017 g per litre.

Children 3-6 years

Use as bath additive: 0.0017-0.0082 g per litre.

Duration of a bath: 10-20 minutes. One bath every day or every second day. Recommended temperature of bath: 35-38 °C.

Indications related to the cutaneous use in skin disease and skin injury is considered not appropriate due to the risk of skin irritation.

3. Non-Clinical Data

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

3.1.1. Primary pharmacodynamics

Secretomotoric activity

Only historic reports are available from experiments on the secretomotoric activity. Gordonoff *et al*. (1931, 1932, and 1933) and Vollmer (1932) demonstrated secretomotoric and secretolytic properties of thymol and thyme preparations. A stimulation of the ciliary movement in the pharynx mucosa of frogs treated with diluted solutions of thyme oil, thymol or carvacrol has been reported by Freytag (1933, cited in Blaschek *et al*. 2008).

Spasmolytic activity

Thyme oil shows a spasmolytic effect on the smooth muscle and a contracture involving a direct action on skeletal muscle by an unknown mechanism. The essential oils were diluted in methanol to give a final bath concentration of $5x10^{-5}$ and $2x10^{-4}$ g/ml for rat diaphragm *in vitro* with muscle stimulated directly or via phrenic nerve and $4x10^{-6}$-$8x10^{-5}$ g/ml for field-stimulated guinea-pig ileum studies (Lis-Balchin *et al.* 1997).

Thymol has *in vitro* an agonistic effect on α1-, α2- and β-adrenoreceptors; the spasmolytic activity is detectable in concentrations $>10^{-6}$ M. In a concentration of 10^{-4} M, thymol suppresses the spontaneous contractile activity of the non-striated muscles of the stomach of the guinea pig. In higher concentrations thymol exhibits a spasmolytic activity in the ratio of 1:10 compared to papaverine (Beer *et al.* 2007).

Thyme oil inhibits the phasic contractions of the ileal myenteric plexus-longitudinal muscle preparation of the guinea pig. The ED_{50} was found for thyme oil at 6.9 mg/L compared to papaverine, which is 5 times more effective, and isoprenalin (ED_{50} 0.0044 mg/L). On the tracheal guinea-pig preparation papaverine was 700-times more effective than thyme oil (Brandt 1988, Reiter & Brandt 1985).

3.1.2. Secondary pharmacodynamics

Antibacterial activity

The essential oil exerts a strong antibacterial activity on Gram-positive and Gram-negative bacteria. The activity is mainly attributed to thymol and carvacrol (numerous articles published, e.g. Simeon de Buochberg *et al.* 1976, Janssen *et al.* 1986, Menghini *et al.* 1987, Patakova *et al.* 1974, Allegrini *et al.* 1972, Janssen 1989, Farag *et al.* 1989, Lens-Lisbonne *et al.* 1987, Vampa *et al.* 1988, Dorman *et al.* 2000, Hersch-Martinez *et al.* 2005).

Oils with higher percentage of phenolic compounds are reported to show higher inhibitory activity (Penalver *et al.* 2005). Correlations between concentrations of thymol and MIC and minimal bactericidal concentration suggest that the formation of membrane perforations is the principal mode of action of thymol against oral bacteria (Shapiro *et al.* 1995).

Thyme essential oil had the lowest minimum inhibitory concentration (0.03% V/V) against *Escherichia coli* and *Candida* spp. among 20 essential oils tested (Hammer *et al.* 1999).

The antibacterial activity of 14 essential oils and their major components was evaluated by agar-plate dilution assay under sealed conditions, with agar used as a stabilizer for homogeneous dispersion by

Inouye *et al.* (2001). Of the selected strains of four major bacteria causing respiratory tract infection, *Haemophilus influenzae* was most susceptible to the essential oils, followed by *Streptococcus pneumoniae* and *Streptococcus pyogenes*. *Staphylococcus aureus* was less susceptible. No cross-resistance was observed between penicillin-sensitive and penicillin-resistant *S. pneumoniae*. *Escherichia coli*, used as a control bacterium, showed the lowest susceptibility. Essential oils containing aldehyde or phenol as a major component showed the highest antibacterial activity, followed by the essential oils containing terpene alcohols.

Studies on the antibacterial activity of thyme oil, thymol or carvacrol published during the revision period (2011-2016) is presented in Table 4. However, there are also several reports on the antimicrobial activity of thyme oil against foodborne microbes (*Clostridium perfringens*, *Listeria monocytogenes*, *Salmonella* spp.) (Radaelli *et al*. 2016; Reyes-Jurado *et al*. 2016; Miladi *et al*. 2016; Melo *et al*. 2015; Chaftar *et al*. 2015; Mancini *et al*. 2015), and against microbes which cause food-spoilage (Dunn *et al*. 2016).

Table 4: Overview of antibacterial activity of thyme essential oil during revision period (2011-2016)

Substance	**Microbial species tested**	**Source of microbial species**	**Outcome of antimicrobial testing**	**Reference**
Thyme oil (GC-MS analysis: thymol 56.6%, p-cymene 12.3%, carvacrol 8.7%, …)	*Escherichia coli*, *Staphylococcus aureus*, *Enterococcus* sp., *Proteus mirabilis*	ATCC collection, Clinical isolates	MIC 2.8-45.4 µL/mL (enterococci and *Proteus* sp. were resistant)	Bogavac *et al.* 2015
Thyme oil (no data of composition)	*S. aureus*, *Enterococcus faecalis*, *E. coli*	Oral pathogens from ATCC cell culture collection	MIC 2-32 µL/mL	Thosar *et al.* 2013
Thyme oil (GC showed abundant content of thymol, p-cymene and linalool without percentage)	*Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* (multi-drug resistant isolates)	Clinical isolates	MIC 0.25-4% V/V	Sakkas *et al.* 2016
Thyme oil (5 different collection areas; GC-MS analysis found abundant content of thymol up to 67.5% followed by carvacrol up to 7.3%)	*E. coli*, *K. pneumoniae*, *P. vulgaris*, *P. aeruginosa*	ATCC cell culture collection	MIC (depending on the thyme oil tested) from 6.25 to 100 µg/mg	Mancini *et al.* 2015

Abbreviation: MIC - minimal inhibitory concentration; MBC - minimal bactericidal concentration; ATCC - American Type Culture Collection, USA

Antifungal activity

The essential oil is highly antifungal, when tested on various fungi and yeasts, e.g. *Candida albicans*. This activity is mainly attributed to phenol compounds thymol and carvacrol. Thymol interferes with the formation and viability of hyphae and induces morphological alterations in the envelope of *C. albicans* (Braga *et al.* 2007, 2007a). Thyme oil inhibits the mycelial growth of *Aspergillus flavus* and *A. niger* (Paster *et al.* 1990) and at concentrations of <=500 ppm completely inhibits dose-dependently fungal growth and mycotoxin production of *A. flavus*, *A. parasiticus*, *A. ochraceus* and *Fusarium moniliforme* (Soliman & Badeaa 2002).

Studies on the antifungal activity of thyme oil, thymol and carvacrol published during the revision period (2011-2016) confirm earlier antifungal activity results of thyme oil or thymol and carvacrol *in vitro*. The thyme oil exhibited antifungal activity against moulds from environmental or human sources such as *Fusarium* spp. (Homa *et al.* 2015; Kumar *et al.* 2016), *Aspergillus fumigatus*, *A. terreus*, *Fusarium solani*, *Lichtheimia corymbifera*, *Rhisopus microspores* (Horváth *et al.* 2016), *Aspergillus niger*, *A. fumigatus*, *A. flavus*, *A. ochraceus*, *Penicillium citrinum*, *P. chrysogenum* (Sharifzadeh *et al.* 2016) and *Candida* spp. isolates from food (Rajkowska *et al.* 2017). The thyme oil showed also antifungal activity against clinical isolates and laboratory strains of yeasts (*Candida* spp., *Cryptococcus* spp.) with MIC ranged from 0.11 to >3200 µg/mL, depending on strain tested (Bogavac *et al.* 2015; Soares *et al.* 2015; Chaftar *et al.* 2015; Horváth *et al.* 2016; Szweda *et al.* 2015). Furthermore, thymol and carvacrol showed potent antifungal activity against *Candida* spp. with MIC 0.10-0.31 mg/mL, and 0.17-0.62 mg/mL, respectively (Chaftar *et al.* 2015).

Anti-dermatophytal activity of thyme oil or/and thymol

The therapeutic efficacy of a 1% solution of thyme oil and thymol against *Trichophyton mentagrophytes*, *T. rubrum* and *T. tonsurans* was examined on 2-months old Wistar rats. During the 37-day observation period the oil - treated rats were cured (Sokovic *et al.* 2008). In dilution assays thyme oil showed much higher antifungal potency than the commercial fungicide bifonazole (Sokovic *et al.* 2008).

Thyme oil was antagonistic by vapour contact against an experimental tinea pedis in guinea pigs infected with *Trichophyton mentagrophytes*. Thyme oil killed the conidia, inhibited germination and hyphal elongation at concentrations of 1-4 µg/mL air (Inouye *et al.* 2001a).

Inouye *et al.* (2007) investigated *in vitro* the ability to treat tinea pedis with a combination of essential oils, heat and salt in a foot bath. Agar blocks implanted with *Trichophyton mentagrophytes* were immersed in 0.1% aqueous agar containing two-fold dilutions of essential oils with or without sodium chloride at 27°C, 37°C and 42°C for 10 and 20 minutes. The fungicidal activity of essential oils was markedly enhanced by treating at 42°C for 20 minutes as compared with that at 27°C, showing 1/4 - 1/32-fold reduction of minimum fungicidal concentration (MFC to kill 99.99%). Thyme essential oil rich in thymol showed a conspicuous activity. MFCs were further reduced to 1/2 - 1/8 by the addition of 10% sodium chloride.

The *in vitro* activity of some essential oils (EO) (thyme red oil, fennel, clove, pine, sage, lemon balm and lavender) against clinical and environmental fungal strains was investigated. The minimal inhibitory concentrations were determined by a microdilution method in RPMI 1640 and by a vapour contact assay. The inhibiting effects of EO in vapour phase were generally higher than those in liquid state. According to both methods thyme red oil and clove were found to be the oils with the widest spectrum of activity against all fungi tested (Tullio *et al.* 2007).

The *in vitro* antifungal activity of thyme oil (composition of the oil according to GC-MS analysis: thymol 44.71%, γ-terpinene 26.01%, α-cymene 21.22%) was performed *in vitro* using measurement of mycelial biomass production and inhibition of mycelial radial growth of dermatophyte *Trichophyton rubrum* IOA9 and mould *Aspergillus fumigatus* MTCC 2550 and *A. niger*. Furthermore, thyme oil and thymol showed synergistic interaction with fluconazole against *A. fumigatus* and *T. rubrum* with FICI (Fractional Inhibitory Concentration obtained by chequerboard assay) values 0.187 and 0.156, respectively. Thymol also exhibited anti-elastase activity with reduction of 95.56% in comparison with untreated cells, while thyme oil also inhabited elastase activity with reduction of 90.75% in comparison to control. However, the study also reports on the lower anti-keratinase activity of thymol (13.9%) and thyme oil (15.96%), respectively (Khan *et al*. 2014).

In a study of Chaftar *et al*. (2015) (thyme oil GC-MS analysis: thymol 33.63%, p-cymene 33.14%, carvacrol 20.71%) MIC values of thyme oil using microdilution assay was in range 0.02 to 0.25 mg/mL, while thymol and carvacrol were inactive against two clinical isolates of dermatophyte species tested *Trichophyton mentagrophytes* and *T. rubrum*.

Antibiofilm activity* in vitro *of thyme oil or thymol

The linear correlation between percentage of inhibition of biofilm formation and thyme oil used *in vitro* was observed against *Candida albicans* (Bogavac *et al.* 2015). The study also demonstrates inhibition of germination of *C. albicans* blastopores, as one of the most important virulence factors in concentration of thyme oil at 0.25% V/V (Bogavac *et al.* 2015).

Thyme oil (GC-MS analysis revealed the main compounds thymol 41.33%, p-cymene 18.08%, γ-terpinene 13.12%, p-cymene-3-ol 5.24% etc.) was assessed *in vitro* for antibiofilm activity using *Salmonella typhimurium* ATCC 1408 and foodborne isolates (N=11). The thyme oil possessed antibiofilm activity determined by XTT/formazan-reduction assay in concentration range from 0.106 to 0.725 mg/mL with inhibition of 50% of biofilm formation. Biofilm inhibition concentration of 90% was found to be between 3.12 and 7.25 mg/mL. From the other side, thyme oil also inhibited biofilm formation on microscopic slide covers at concentrations of a half of MIC values (0.78 to 1.56 mg/mL) (Miladi *et al.* 2016).

In a study Vázquez-Sánchez *et al.* (2015), thyme oil showed the most potent anti-biofilm activity amongst essential oils against *Staphylococcus aureus* St1.01. strain using stainless steel coupons model for *in vitro* bacterial biofilm production. The effectiveness of thyme oil against planktonic cells was strongest among tested oils with MIC vale 0.04%. Using the model of 48-h-old biofilms of *S. aureus* St1.01. thyme oil showed strongest antimicrobial efficacy against biofilm formed with logarithmic reduction of 4.3 log CFU/cm^2. After exposing the thyme oil to *S. aureus* St1.01 in different concentrations ranged from 0.02% to 0.04%, thus in sub-lethal doses for *S. aureus* cells, in the long-term exposure to the thyme oil the *S. aureus* cells showed adaptation. The study showed anti-biofilm formation of thyme oil of biofilm formed onto stainless steel with *S. aureus* cells but with not completely eradication of biofilm (Vázquez-Sánchez *et al.* 2015).

Thyme oil (GC-MS analysis showed dominated compounds in oi: 1.8-cineole 53.46%, camphor 7.15%, terpinen-4-ol 5.46%) also reduces and eliminate biofilm formation of *Aeromonas hydrophila* on stainless steel surfaces by 3.84 log CFU/cm^2 at 25°C (Millezi *et al.* 2013).

In study of Kavanaugh and Ribbeck (2012), thyme oil showed more potent anti-biofilm formation *in vitro* that antibacterial activity on planktonic cells of *Pseudomonas aeruginosa* PA01 and *Pseudomonas putida* KT2440.

Antioxidant activity

Antioxidative effects of thyme oil have been determined in various test systems *in vitro* (e.g. Youdim *et al.* 1999a, Dorman *et al.* 2000, Kulisic *et al.* 2005). Essential oils with high proportions of the phenolic components thymol and/or carvacrol showed the highest antioxidant activities (Jukic *et al.* 2005, Chizzola *et al.* 2008). The antioxidant activity of p-cymene-2.3-diol, a minor component of the essential oil, is considered as more potent than thymol or carvacrol, which could be due to its dihydroxy structure (Schwarz *et al.* 1996).

Thyme essential oil exhibited a dose-dependent protective effect on the copper-induced LDL oxidation. The protective effect of essential oils is assigned to the presence of phenolic monoterpenes, thymol and carvacrol, which are identified as the dominant compounds (Kulisic *et al.* 2007).

Youdim *et al.* (1999, 1999a, 2000, 2002) investigated the influence of thyme oil and of thymol on the phospholipid polyunsaturated fatty acid composition, antioxidant enzyme activity and the phospholipid fatty acid composition in several rat tissues. The rats were fed with a diet containing thyme oil or thymol in an amount of 42.5 mg/kg BW per day. Beneficial effects could be found in different experimental settings. Thymol alone was not more effective compared to the entire essential oil.

Pérez-Rosés *et al.* (2016) showed that thyme oil possess inhibitory activity in non-biological test using DPPH scavenging activity and in biological test on MPO and ROS production in PMA stimulated leukocytes, however with weak inhibitory activity on ROS production using leukocytes which were stimulated by H_20_2.

The low free radical scavenging activity of thyme oil using assay with DPPH was confirmed in comparison with ascorbic acid and after determination of IC_{50} after 60 minutes (Mancini *et al.* 2015). The thyme oil in study of Mancini *et al.* 2015 was rich in thymol (up to 67.5%).

Anti-inflammatory activity

Thyme oil inhibits prostaglandin biosynthesis, thymol was less active in the COX-inhibition test (Wagner *et al.* 1986).

Thymol was shown to inhibit dose-dependently the experimentally induced release of neutrophil elastase. The authors concluded that thymol may have a helpful effect in the control of inflammatory processes present in many infections (Braga *et al.* 2006).

Thyme oil (composition of most abundant compounds: carvacrol 45.5%, α-terpineol 22.9%, endo-borneol 14.3 determined by GC-MS) and its constituents thymol and carvacrol were studied *in vitro* for modulation of inflammatory response (Fachini-Queiroz *et al.* 2012). The study showed that thymol inhibited inflammatory oedema and thymol is an effective chemoattractant, but it did not reduce the oedema formation. From the other side, carvacrol reduced oedema formation, exerting possible topical anti-inflammatory effects (Fachini-Queiroz *et al.* 2012).

Carvacrol showed reduction of IL-1β and TNF-α at the protein and mRNA levels. Thymol also modulates inflammatory response of stimulated mouse macrophages with reduction of IL-1β expression (Gholijani *et al.* 2015).

Further activities

<u>Effects on the alimentary canal</u>

In the stomach thymol (<0.5 mM) suppressed the generation of action potential and slow potential changes without any marked change in membrane potential and membrane resistance. Increased concentrations of thymol (>0.5 mM) reduced the membrane potential and membrane resistance. In the ileum and rectum, thymol suppressed spike activity without any marked change in the membrane potential. Although the membrane was completely depolarized, thymol (>1 mM) suppressed the generation of phasic and tonic responses of the K-induced contracture evoked in the various regions of

the alimentary canal. Thymol (0.5 mM) suppressed spontaneous mechanical responses in the various regions of the alimentary canal (Ito *et al.* 1974).

Acetylcholinesterase inhibition

Jukic *et al.* (2007) examined *in vitro* the inhibitory activity exerted by the main constituents of essential oil obtained from *Thymus vulgaris* L. on acetylcholinesterase (AChE). The total essential oil and selected compounds, specifically linalool and thymol, carvacrol and their derivatives thymoquinone and thymohydroquinone, were tested for AChE inhibition. Thymohydroquinone exhibited the strongest AChE inhibitory effect over the range of concentrations. The AChE inhibitory potential decreased in the following order: thymohydroquinone>carvacrol>thymoquinone>essential oil>thymol>linalool.

Wound healing

After topical treatment of burned rats with thyme oil (1:1 diluted with olive oil) an increase in the formation of new tissue could be observed (Dursun *et al.* 2003).

Bone Metabolism

Thyme oil and thymol have been demonstrated to be efficient inhibitors of bone absorption in rats. Thymol is a direct inhibitor in the osteoclast absorption pit assay (Mühlbauer *et al.* 2003).

Cardiovascular system

Thymol in concentrations of 1-10 mg/kg BW inhibited calcium channels in rats and lowered blood pressure (Aftab *et al.* 1995).

Magyar *et al.* (2002) achieved a similar inhibition of calcium and potassium channels in canine and human ventricular cardiomyocytes.

Szentandrassy *et al.* (2004) concluded from experiments on the Langendorff-perfused guinea pig heart that the negative inotropic action of thymol can be explained by reduction in calcium content of the sarcoplasmic reticulum due to the combination of the thymol-induced calcium release and inhibition of the calcium pump. The calcium-sensitizer effect, observed at lower thymol concentrations, indicates that thymol is likely to interact with the contractile machinery also.

Skeletal muscles

Thymol suppresses both Ca^{2+} and K^{+} currents in enzymatically isolated rat skeletal muscle fibers in a concentration-dependent manner (Szentandrassy *et al.* 2003).

Thymol and carvacrol were able to evoke Ca^{2+} release with EC_{50} values of 158 +/- 16 and 211 +/- 55 µM respectively in heavy sarcoplasmatic reticulum vesicles isolated from skeletal muscle and actively loaded with calcium (Sarkozi *et al.* 2007).

Effects on the CNS

Lim *et al.* (2005) investigated the stimulating or sedative effects of inhaling thyme essential oil by using the forced swimming test (FST) with mice. The inhalation of thyme oil ($p<0.05$) resulted in 22.87% reduction of immobility. The same results were achieved when over-agitation was artificially induced in the mice by an intraperitoneal injection of caffeine.

Mohammadi *et al.* (2001) investigated several phenol derivatives with regard to their ability to activate directly the gamma-aminobutyric acid (GABA(A)) receptors in the absence of the natural agonist. This mechanism is supposed to contribute to its sedative-hypnotic actions. Only compounds with the phenolic hydroxyl attached directly to the benzene ring and with aliphatic substituents in ortho position

to the phenolic hydroxyl activated chloride currents in the absence of GABA. The concentrations required for half-maximum effect were 200 µM for thymol, and 23 µM for the positive control propofol.

Other activities

Insecticidal actions

Thyme oil is lethal against adult *Oryzaephilus surinamensis*, *Rhyzopertha dominica* and *Sitophilus oryzae* (Shaaya *et al.* 1991).

Good insecticidal activity (>90%) against larvae of *Lycoriella ingenua* was achieved with thyme oil at 30 x 10^{-3} mg/1 air. Carvacrol and thymol were effective at 10 x 10^{-3} mg/l (Park *et al.* 2005). The LD_{50} against *Tyrophagus putrescentiae*, a stored food mite, is 10.2 µg/cm^2 in an impregnated fabric disk assay (Jeong *et al.* 2008).

Mosquito control

Thymol and carvacrol are potent repellents in concentrations of about 0.05% in topical treatment (Choi *et al.* 2002, Park *et al.* 2005).

Anthelmintic actions

Thyme oil in solutions of 1:2000 caused the death of ascarides *in vitro*. Non-phenolic constituents demonstrated less activity (Akacic & Petricic 1956).

Antiparasitic actions

Thyme oil is effective against *Trypanosoma cruzi*. Thymol may be the main component responsible for the trypanocidal activity (Santoro *et al.* 2007).

In an *in vitro* growth inhibition assay with bloodstream forms of *Trypanosoma brucei* the ED50 of thyme essential oil was found to be 0.4 µg/ml (Mikus *et al.* 2000).

Thyme oil was active against Anisakis larvae, a parasite which can cause human disease after consumption of raw or almost raw seafood products. Larvicidal effects of thyme oil (5% and 10% in sunflower oil, respectively) were determined after 7 and 14 hours after treatment, respectively. The authors conclude that the results suggested the use of thyme oil for prevention and treatment of anisakiasis (Giarratana *et al.* 2014). The composition of thyme oil used in study of Giarratana *et al.* (2014) was: thymol (50%), linalool (7%), carvacrol (3%), alpha-and beta-pinene (6%).

The thyme essential oil was active (toxic) against human head louse, *Pediculus humanus capitis*, an obligatory ectoparasite using *in vitro* fumigation toxicity assay against adult form of louse. The thyme oil was found to be ovicidal and possess direct pediculocidal activity against adult lice (Gutiérrez *et al.* 2016). Thyme oil used in study of Gutiérrez *et al.* 2016 was thymol type (content of thymol 47.19%) and p-cymene 28.37% determined by GC-MS.

In study of Yones *et al.* (2016) thyme oil (GC-MS analysis showed the most abundant compounds were: thymol 33.8%, *o*-cymene 14.8%, γ-terpinene 11.8%, thymol methyl ether 6.4% and caryophyllene 5.15%) showed insecticidal activity *in vitro* against female *P. humanus capitis* using the filter paper contact during 3 hours at dosed 0.25 and 0.5 mg/cm^2, respectively. However, the insecticidal activity (pediculicidal activity) was not comparable to the malathion (at dose 0.5 mg/cm^2) using knockdown 50% time (KT_{50}). Mean death time and the percentage of morbidity of thyme oil was 49±2.12 minutes for concentration tested at 0.25 mg/cm^2, and 38±2.17 minutes which was significantly different in comparison to control with 95% of morbidity ($p<0.05$), however with lower pediculicidal activity in comparison with malathion (mean death time 15±0.22 minutes with 100% of morbidity). Study of Yones *et al.* (2016) showed that thyme oil was effective adulticide against *P.*

humanus capitis and effective inhibitor of hatching of *P. humanus capitis* eggs at dose 0.25 and 0.5 mg/cm^2, comparable to insecticide malathion.

Antiviral actions

The IC_{50} for thyme oil against herpes simplex virus type 2 was determined at 0.007% when the essential oil was added at different stages during the viral infection cycle (Koch *et al.* 2008).

3.1.3. Safety pharmacology

No data available.

3.1.4. Pharmacodynamic interactions

No data available.

3.1.5. Conclusions

Results from relevant experimental studies on thyme oil to support the proposed indications are limited. Numerous other pharmacological activities are reported for thyme oil or for the isolated compound thymol and/or carvacrol. Most experiments refer to the *in vitro* antimicrobial activity of essential oil chemo-types where thymol was the dominant compound. This effect is primarily used in food industry, where thyme oil is an effective preservative agent.

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

No non-clinical data on the herbal preparation are available.

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

3.3.1. Single dose toxicity

The LD_{50} of the essential oil *p.o.* in rats was 2.84 g/kg body weight (Von Skramlik 1959). The intraperitoneal LD_{50} of *Thymus zygis* oil in mice was 600 mg/kg body weight (Jimenez *et al.* 1993).

3.3.2. Repeat dose toxicity

No toxic effects were observed in rats after the addition of 1.0% of thymol to their diet for 19 weeks (Hagan *et al.* 1967).

3.3.3. Genotoxicity

Mutagenicity and genotoxicity

Essential oil

Thyme essential oil had no mutagenic or DNA-damaging activity in either the Ames test (strains TA1535, TA1537, TA98, TA100, with and without metabolic activation) and *Bacillus subtilis* rec-Assay (Zani *et al.* 1991). These results were confirmed using TA98 and TA100 strains with and without rat liver S9 fraction (De Martino *et al.* 2009) and TA100 strain with and without rat liver S9 fraction (Shoeibi *et al.* 2009). However, results of Ames tests by Zani *et al.* 1991, De Martino *et al.* 2009, and

Shoeibi *et al.* 2009 were not performed using thyme oil with a composition in compliance with The European Pharmacopoeia monograph for thymol-chemotype.

Thymol and other constituents

Thymol did not show mutagenicity in *Salmonella typhimurium* strains TA97, TA98 and TA100, with and without S9 metabolic activation and 20 minutes standard preincubation time (Azizan *et al.* 1995).

Stammati *et al.* (1999) determined relative cytotoxicity of thymol and carvacrol and assessed their potential genotoxicity in short-term assays. Both substances inhibited the colony-forming ability of Hep-2 cells in dose-dependent manner. The results of an AMES-test in strains TA100 and TA98 were ambiguous. Both substances were marginally more toxic to repair-deficient strain than to its repair-proficient counterpart. The substances produced elevated revertant numbers in the strain TA100, but not to a level generally considered significant. Effects in the SOS chromotest are interpreted as signs of toxicity rather than real SOS induction. The authors conclude that the genotoxic potential of thymol and carvacrol is very weak.

Concentrations of thymol and γ-terpinene above 0.1 mM significantly induced DNA damage in human lymphocytes, however, below this concentration thymol and carvacrol significantly reduced the oxidative DNA damage induced by H_2O_2 (Aydin *et al.* 2005a) or imidazolquinoline and mitomycin C (Aydin *et al.* 2005b).

Thymol and carvacrol reduced the level of DNA-lesions caused by H_2O_2 in HepG2 and colonic Caco-2 cells (Horvathova *et al.* 2006).

Thymol in concentrations up to 520 µM did not increase the frequencies of chromosome aberrations in Syrian hamster embryo cells compared to the control cells (Hikiba *et al.* 2005).

Azirak & Rencuzogullari (2008) investigated the *in vivo* genotoxic effects of carvacrol and thymol in bone marrow cells of rats. Both carvacrol (10, 30, 50, and 70 mg/kg b.w. intraperitoneally) and thymol (40, 60, 80, and 100 mg/kg bw intraperitoneally) significantly induced the structural and total chromosome abnormalities (CA) in bone marrow cells for all treatment periods (6, 12, and 24 h) when compared with control. Both carvacrol and thymol showed similar effects with the positive control urethane on induction of the percentage of structural and total CA at the highest concentrations except the effects of carvacrol for 6 hours treatment (70 mg/kg bw and 100 mg/kg bw, respectively). In addition, carvacrol induced the numerical CA at all concentrations when compared to control and at two highest concentrations (50 and 70 mg/kg bw) when compared to solvent control. Thymol also induced the numerical CA especially at the highest concentration (100 mg/kg bw) for all treatment periods.

Undeger *et al.* (2009) examined the genotoxicity of thymol and carvacrol using comet assay. V79 Chinese hamster lung fibroblast cells were treated with 1, 5, and 25 µM thymol and carvacrol. The results of this study indicate a lack of clastogenic activity for thymol and carvacrol at biologically relevant concentrations, and a moderate antioxidant activity *in vitro*.

Several articles were published during revision period (2011-2016) dealing with mutagenicity and/or genotoxicity of thyme oil or its constituents (thymol and carvacrol) in which different toxicological approaches were noted. Thymol, the most abundant compound of thymol-chemotype of thyme oil, up to 250 µM did not induce DNA strand-breaks (Llana-Ruiz-Cabello *et al.* 2014), and did not increase the frequency of binucleated cells presenting at least one micronucleus at concentrations up to 250 µM with or without S9 fraction which indicate that thymol is not genotoxic in mammalian cell using micronucleus assay (Maisanaba *et al.* 2015). Genotoxicity testing *in vitro* showed that thymol increased sister chromatid exchange, induce structural chromosome aberration without dose-depended manner (from 25-100 µg/mL). Thymol did not induce chromosome abnormalities (aneuploidity and

euploidy) (Buyukleyla and Rencuzogullari, 2009). An overview of data concerning genotoxicity or mutagenicity of thyme oil, thymol and/or carvacrol *in vitro* is presented in Table 5.

Table 5: Overview of genotoxic or mutagenic activity of thyme oil, thymol and/or carvacrol

Toxicological approach *in vitro*	**Method used**	**Outcome of an assay**	**Reference**
Mutagenicity testing of **thyme oil** (GC-MS analysis showed highest content of o-cymene 56.2%, carvacrol 24.44% and thymol 8.75%)	Ames test using *Salmonella typhimurium* TA98 and TA100 with and without rat liver S9 fraction	No increase of revertant was noticed with and without metabolic activation in concentration of thyme oil up to 463 μg/plate	De Martino *et al.* 2009
Mutagenicity testing of **thyme oil** (GC-MS analysis showed high content of p-cymene, thymol and carvacrol)	Ames test using *Salmonella typhimurium* TA100 with and without rat liver S9 fraction	No increase of revertant was noticed with and without metabolic activation in concentration of thyme up to 500 μg/mL	Shoeibi *et al.* 2009
Mutagenicity testing of thymol and carvacrol	Ames test using *Salmonella typhimurium* TA97A, TA98, TA100, TA102, TA104 according to the OECD guideline 471 (2002) with and without metabolic activation system with S9 fraction	No indication of mutagenic activity of thymol (up to 205 μM) with/without metabolic activity. Carvacrol did not show mutagenic activity on TA100, TA102, TA104 (conc. up to 460 μM), however mutagenic activity was noticed on TA97A in the absence of S9 (conc. 29 and 115 and μM) and on TA98 strain both in the presence (conc. 29-460 μM) and without S9 (conc. 115-230 μM).	Llana-Ruiz-Cabello *et al.* 2014
Genotoxicity testing of thymol and carvacrol	Comet assay on Caco-2 cells without and with post-treatment with Endo III and FGA proteins	Carvacrol (up to 460 μM) and thymol (up to 250 μM) did not induce DNA strand-breaks after 24 hours and 48 hours of exposure. Carvacrol induces DNA breaks after 48 hours of FGA post-exposure in highest conc. of 460 μM (greater than after 24 hours exposure) which indicate formation of 8-oxoGua, ring opened purines.	Llana-Ruiz-Cabello *et al.* 2014
Genotoxicity testing of	Micronucleus assay	Thymol did not increase of	Maisanaba *et al.*

thymol and carvacrol	(MA) on mouse lymphoma L5178Y/Tk$^{\pm}$ cells	frequency of binucleated cells presenting at least one micronucleus at concentrations up to 250 μM with or without S9 fraction which indicate that thymol is not genotoxic in mammalian cell using MA. Carvacrol increase binucleated cells at 700 μM without metabolic activation with S9 fraction.	2015
Genotoxicity testing of thymol and carvacrol	Mouse lymphoma thymidine-kinase assay (MLA) on mouse lymphoma L5178Y/Tk$^{\pm}$ cells	Thymol was not mutagenic after 4 hours exposure of conc. up to 250 μM. Exposure of cells to carvacrol at conc. higher than 500 μM decrease its relative total growth, but no mutagenic effects were noted even after exposure cell to carvacrol to 24 hours indicating no mutagenic activity of carvacrol in MLA assay at conc. tested	Maisanaba *et al.* 2015
Genotoxicity testing of carvacrol	Comet assay on rat neurons and cultured N2a rat neuroblastoma cells	Carvacrol was not genotoxic in conc. up to 400 mg/L *in vitro* in cultures of rat neurons and N2a rat neuroblastoma cells	Aydin *et al.* 2014
Genotoxicity testing of thymol	Sister chromatid exchange (SCE), chromosome aberration (CA), micronucleus tests (MN), rate index (RI), mitogenic index (MI), nuclear division index (NDI) on human peripheral lymphocytes (HPL) *ex situ*	Thymol increased SCE induce structural CA and MN without dose-depended manner (from 25-100 μg/mL). Thymol did not induce chromosome abnormalities (aneuploidity and euploidy). Cytotoxic effect of thymol was shown by decreasing RI, MI and NDI at higher concentrations	Buyukleyla and Rencuzogullari, 2009

3.3.4. Carcinogenicity

No data available.

3.3.5. Reproductive and developmental toxicity

Thyme essential oil consisting of 48% p-cymene and 24% thymol (0.25% essential oil in the feed over 2 weeks and during 4 days of pregnancy, n=15, number of embryos: 126) showed no influence on the growth and development of mouse embryos *in vivo* (Domaracky *et al.* 2007).

3.3.6. Local tolerance

***In vivo* acute dermal irritation/corrosion**

Thyme oil (0.5 mL undiluted oil on 6 cm^2 gauze) was evaluated on young albino rabbit shaved skin for possible irritation and/or corrosion with exposure time between 3 minutes, 1 hour and 4 hours of application and no adverse effects were noticed using scoring system according to OECD Guideline No. 404 (OECD, 2002). Animals were observed also 14 days after exposure to thyme oil for possible late appearance of irritable effects and no adverse effects were noticed (Gutiérrez *et al.* 2016). The thyme oil used in study of Gutiérrez *et al.* 2016 was a thymol type (content of thymol 47.19%) and p-cymene 28.37%; determined by GC-MS.

3.3.7. Other special studies

During antimicrobial activity assessment of high doses of thyme oil (0.05% compared to 0.01% and 0.06 mM diluted in ethanol) and thymol (24% content in thyme oil) simultaneously strong cytotoxic effect on Caco-2 cells was stated (Fabian *et al.* 2006). The extent of damage in the cell population caused by enteroinvasive *E. coli* was also widened.

3.3.8. Conclusions

Thyme oil had no mutagenic activity in the Ames test (strains TA1535, TA1537, TA98, TA100) with and without metabolic activation and in *Bacillus subtilis* rec-assay (Zani *et al.* 1991). Although the Ames test on thyme oil by Zani *et al.* (1991), lacked one of the currently used strains the negative result could be regarded reliable, because the paper contains adequate description of findings and because the Ames test is supplemented by the rec-assay.

Mutagenicity and genotoxicity of the main components of thyme oil, thymol and carvacrol, have been assessed in a number of studies on both prokaryotic and eukaryotic *in vitro* and *in vivo* experimental systems. The studies have many weaknesses, findings are often contradictory, and reporting does not always contain sufficient details, which make interpretation difficult and conclusion equivocal. Thymol is also an antioxidant and prevents DNA damage in certain experimental conditions. It seems that in some studies thyme oil and its main components give weak indications towards genotoxicity, but at the best these indications are weak and debatable. Adequately performed mammalian cell studies are needed for the assessment of genotoxicity for the oral use of thyme oil.

In summary, from a non-clinical point of view, the use of thyme oil for cutaneous use and as a bath additive can be regarded as safe when administered in the recommended posology and duration of use.

3.4. Overall conclusions on non-clinical data

Results from relevant experimental studies on thyme oil to support the proposed indications are limited. Numerous other pharmacological activities are reported for thyme oil or for the isolated compound thymol and/or carvacrol. Most experiments refer to the *in vitro* antimicrobial activity of essential oil chemo-types where thymol was dominant compound. This effect is primarily used in food industry, where thyme oil is an effective preservative agent.

Adequate tests on reproductive and developmental toxicity have not been performed. The use during pregnancy and lactation cannot be recommended. No fertility data is available.

Mutagenicity and genotoxicity of thyme essential oil and its main components, thymol and carvacrol, have been assessed in a number of studies on both prokaryotic and eukaryotic *in vitro* and *in vivo*

experimental systems. The studies have many weaknesses, findings are often contradictory, and reporting does not always contain sufficient details, which make interpretation difficult and conclusion equivocal. Thymol is also an antioxidant and prevents DNA damage in certain experimental conditions. It seems that in some studies thyme oil and its main components give weak indications towards genotoxicity, but at the best these indications are weak and debatable. Adequately performed mammalian cell studies are needed for the assessment of the genotoxicity for the oral use of thyme oil. It is important to consider that tests of genotoxicity and mutagenicity *in vitro* were not performed in most cases with herbal substance (thyme oil) which composition is in compliance with recent European Pharmacopoeia monograph limits of thymol-chemotype thyme oil.

Tests on carcinogenicity have not been performed.

The use of thyme oil for topical use and as a bath additive can be regarded as safe when administered in the recommended posology and duration of use.

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

No data available.

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

Thymol: After application of a single dose of thyme dry extract (corresponding to 1.08 mg thymol) only the sulphate-conjugate could be detected in the human plasma, but not the free thymol nor the glucuronide. The sulphate could be detected 20 minutes after application; maximum plasma levels were reached after about 2 hours. Thymol can be detected in the plasma up to 38 hours; renal elimination was completed within 24 hours. Elimination half-life was determined as 10.2 hours (Kohlert *et al.* 2002).

4.2. Clinical efficacy

No data available.

4.2.1. Dose response studies

No data available.

4.2.2. Clinical studies (case studies and clinical trials)

Indication: Cough associated with cold

No data available from studies using thyme oil as the only active substance. Studies including combinations with synthetic drugs are considered as not relevant for the monograph on thyme oil.

Indication: Stomatitis

A comparison of different mouth rinses (containing thymol, chlorhexidine, povidone + H_2O_2) showed no differences in the papillary bleeding score and in plaque index between the treatment with thymol and water (Maruniak *et al.* 1992).

The application of a combination of thymol, menthol, methyl salicylate and 1.8-cineol over 6 months did not show statistically significant differences between the vehicle and the essential oil group. Neither development of bacterial resistance nor emergence of opportunistic pathogens could be observed (Charles *et al.* 2000).

Many clinical trials are published which investigate the efficacy of combinations of chlorhexidine and thymol (e.g. Twetman *et al.* 1999). The contribution of thymol to the overall efficacy cannot be estimated.

Thyme oil (from *Tyhmus zygis* L.) in the combination with oils of *Cymbopogon flexuosus* (Nees ex Steud.) W. Watson and *Rosmarinus officinialis* L.) showed positive effect on reduction of subgingival microbial biofilm formation in a randomized clinical trial (N=46, patients with moderate chronic periodontitis) and measuring the probing depth, attachments level, bleeding and probing and modified sulcus bleeding index after 3 and 6 months (Azad *et al.* 2016).

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

No data available.

4.4. Overall conclusions on clinical pharmacology and efficacy

There are no data available for thyme oil to support a well-established use indication.

5. Clinical Safety/Pharmacovigilance

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

No data available.

5.2. Patient exposure

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

5.3. Adverse events, serious adverse events and deaths

Adverse events related to the essential oil

No irritation of the skin of 25 volunteers was noted in concentrations of 8% thyme oil in petrolatum after 48 hours of closed-patch test. No sensitization reactions were noted using maximisation test at a concentration of 8% of thyme oil on petrolatum (Opdyke 1974 cited in Blaschek *et al*. 2008; Kligman 1966 cited in Opdyke 1979; Opdyke 1974 cited in Plants in cosmetics – Potentially harmful components, 2006). The daily application in gargles, mouthwashes and toothpastes over a longer period (no exact data available) may cause allergic reactions (Blaschek *et al*. 2008).

Yürüktümen (2011) reported a case of acute hepatitis after ingestion of 25 ml of thyme essential oil (unknown composition) obtained from a local market in Turkey. The patient developed nausea, vomiting and diarrhoea, and was subsequently admitted to the emergency unit, with high transaminase levels. The patient was placed in an observation unit for two days and under treatment

with N-acetylcysteine 300 mg per hour (150 mg/kg, during 50 hours) and metoclopramide 10 mg intravenously for first two days. His elevated aminotransferase levels and symptoms gradually decreased during the observation period.

There were no phototoxic effects recorded for thyme oil (Urbach and Forbes 1973 cited in Opdyke 1979).

There are 73 recorded exposures with *Thymus vulgaris* as key word on VigiBase databases till November 2016. The use of *Thymus vulgaris* L. as s single medicine was confirmed in 35 cases and other recorded exposures were in combination of *Thymus vulgaris* L. with synthetic medicinal products and/or with a herbal medicinal products.

From the EudraVigilance database data, there are 47 newly reported side-effects and adverse reactions during revision period using thyme oil as mono-component medicinal products and as in combination with other medicinal products (synthetic or in combination with other essential oils) till November 2016.

Adverse events related to thymol:

In very rare cases allergic reactions may occur due to the content of thymol (Hänsel *et al.* 1994).

Assessor's comment:

There are no new safety concerns identified from case-reports in the VigiBase and EudraVigilance databases up to November 2016.

Yürüktümen (2011) reported a case of acute hepatitis after ingestion of 25 ml of thyme essential oil (unknown composition) obtained from a local market in Turkey. The case report is considered not relevant for the monograph due to the unknown quality of the product and the high dose.

In the literature, there is information on cases of skin irritation and allergic reactions. However, in older literature data no skin irritations and sensitizations of thyme oil up to 8% in petrolatum were observed in volunteers. There is no new information that justify a change in section 4.8 of the monograph:

Oral use: Hypersensitivity reactions have been observed. The frequency is not known.

Cutaneous use: Hypersensitivity reactions and skin irritation have been observed. The frequency is not known.

5.4. Laboratory findings

No data

5.5. Safety in special populations and situations

No data

5.5.1. Use in children and adolescents

Use as bath additive: The use in children under 3 years of age is not recommended because medical advice should be sought and due to lack of adequate data.

Cutaneous use and oral use: The use in children and adolescents under 18 years of age is not recommended due to lack of adequate data.

5.5.2. Contraindications

Hypersensitivity to the active substance.

Use as bath additive: Full baths are contraindicated in cases of open wounds, large skin injuries, acute skin diseases, high fever, severe infections, severe circulatory disturbances and cardiac failure.

5.5.3. Special Warnings and precautions for use

Use as bath additive:

When dyspnoea, fever or purulent sputum occurs, a doctor or a qualified health care practitioner should be consulted. The use in children under 3 years of age is not recommended because medical advice should be sought and due to lack of adequate data.

In cases of hypertension, a full bath should be used with caution.

Cutaneous use:

Thyme oil should not be applied to the face particularly in the nasal area of babies and infants under the age of two years because of the risk of a laryngospasm. The nasal mucosa is an autonomic reflexogen organ, which has a distance action to the heart, lungs and circulation and may lead to sudden apnoea and glottal constriction. The children less than 2 years old present particularly this reflex, so all the substances with a strong odour must be avoided (Dost & Leiber, 1967).

Cutaneous use and oral use:

When dyspnoea, fever or purulent sputum occurs, a doctor or a qualified health care practitioner should be consulted.

5.5.4. Drug interactions and other forms of interaction

No data available.

5.5.5. Fertility, pregnancy and lactation

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 fertility data available.

5.5.6. Overdose

No cases are reported for thyme oil.

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

No studies on the effect on the ability to drive and use machines have been performed.

5.5.8. Safety in other special situations

No data available.

5.6. Overall conclusions on clinical safety

Safety during pregnancy and lactation has not been established for thyme oil. In the absence of sufficient data, the use during pregnancy and lactation is not recommended.

Oral use: Thyme oil used as an expectorant in cough associated with cold has a long-standing medicinal use in the EU. From a clinical safety point of view, there are no safety concerns for adults and elderly when administrated in accordance with the monograph. The oral use in children and adolescents under 18 years of age is not recommended due to lack of adequate data.

Cutaneous use: Thyme oil for the relief of symptoms in coughs and colds has a long-standing medicinal use in the EU. From a clinical safety point of view, there are no safety concerns for adults and elderly when administrated in accordance with the monograph. The cutaneous use in children and adolescents under 18 years of age is not recommended due to lack of adequate data.

The use as a bath additive: Thyme oil for the relief of symptoms in coughs and colds has a long-standing medicinal use in the EU. From a clinical safety point of view, there are no safety concerns for adults, elderly, adolescents and children from 3 years of age, when administrated in accordance with the monograph. The use as a bath additive in children under 3 years of age is not recommended because medical advice should be sought and due to lack of adequate data. In cases of hypertension, a full bath should be used with caution.

6. Overall conclusions (benefit-risk assessment)

There are no clinical studies available for thyme oil to support a well-established use indication in accordance with Article 10a of Directive 2001/83/EC.

According to the market overview and literature, thyme oil for oral use, cutaneous use and as a bath additive, fulfils the criteria of medicinal use throughout a period of at least 30 years, including at least 15 years within the EU/EEA, i.e. traditional medicinal use according to Directive 2004/24/EC for the following indications:

Oral use

Traditional herbal medicinal product used as an expectorant in cough associated with cold.

The product is a traditional herbal medicinal product for use in specified indications exclusively based upon long-standing use.

Cutaneous use, use as bath additive

Traditional herbal medicinal product for the relief of symptoms in coughs and colds.

The product is a traditional herbal medicinal product for use in specified indications exclusively based upon long-standing use.

Mutagenicity and genotoxicity of thyme essential oil and its main components, thymol and carvacrol, have been assessed in a number of studies on both prokaryotic and eukaryotic *in vitro* and *in vivo* experimental systems. The studies have many weaknesses, findings are often contradictory, and reporting does not always contain sufficient details, which make interpretation difficult and conclusion equivocal. The herbal substance used in mutagenicity/genotoxicity studies was not in compliance with European Pharmacopoeia monograph limits. For the oral use of thyme oil, adequately performed mammalian cell studies are needed for the assessment of the genotoxicity. However, the use of thyme oil for cutaneous use and as a bath additive can be regarded as safe when administered in the recommended posology and duration of use.

Adequate tests on reproductive toxicity have not been performed. Tests on carcinogenicity have not been performed.

Safety during pregnancy and lactation has not been established. In the absence of sufficient data, the use during pregnancy and lactation is not recommended.

The data on safety are considered sufficient to support a European Union list entry for the cutaneous use and use as bath additive of thyme oil for the relief of symptoms in coughs and colds. There is no relevant change in the monograph during this first revision that triggers a revision of the list entry. However, it should be noted that the Ph. Eur. monograph reference has been changed.

No constituent with known therapeutic activity or active marker can be recognised by the HMPC.

Therapeutic area: Cough and cold

Annex

List of references