EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

12 November 2013
EMEA/HMPC/321181/2012
Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Pimpinella anisum* L., fructus and *Pimpinella anisum* L., aetheroleum

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

Final

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Pimpinella anisum L.,* fructus (aniseed)
Herbal preparation(s)	Dried aniseed, comminuted or crushed; Anisi aetheroleum (anise oil)
Pharmaceutical form (s)	Herbal substance or herbal preparations in solid or liquid dosage forms or as a herbal tea for oral use
Rapporteur	Marisa Delbò
Assessor(s)	Gioacchino Calapai, Marisa Delbò, Vittorio Silano

7 Westferry Circus ● Canary Wharf ● London E14 4HB ● United Kingdom
Telephone +44 (0)20 7418 8400 **Facsimile** +44 (0)20 7523 7051
E-mail info@ema.europa.eu **Website** www.ema.europa.eu
An agency of the European Union

Table of contents

1. Introduction

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

- Herbal substance(s)

Pimpinella anisum L. belongs to the *Apiaceae* (Umbelliferae) botanical family. The material of interest for medicinal use is the fruit (i.e. whole cremocarp), generally entire, having a reminiscent odour of anethole; a small fragment of the thin, rigid, slightly curved pedicel is frequently attached. This herbal substance is administered in liquid or, after crushing, in solid dosage forms.

Pimpinella anisum L., fructus (aniseed) is characterised by a content of essential oil (anise oil) not lower than 20 ml per kg anhydrous fruit (Ph. Eur. 7th Edition) and its medicinal properties are mainly attributed to its content of essential oil (see anise oil composition in section "Herbal preparation").

Other constituents include flavonol glycosides (El-Moghazi *et al.*, 1979; Kunzemann, & Herrmann, 1977), phenolic acid (Schulz, & Herrmann, 1980; El-Wakeil *et al.*, 1986), a phenolic glycoside (Dirks & Herrmann, 1984a; Dirks & Herrmann, 1984b), furocoumarins, mainly bergaptene (Ceska *et al.*, 1987), hydroxycoumarins, mainly umbelliferone (Hänsel *et al.*, 1994) and fixed oil and lipids, mainly constituted of petroselinic acid (Van Loon, 1973). Twelve new and 5 known glycosides of phenyl-propanoids, including 4 stereoisomers of anethole glycol 2'-O-beta-D-glucopyranoside and 4 stereoisomers of 1'-(4-hydroxyphenyl)propane-1',2'-diol 2'-O-beta-glucopyranoside were extracted from the water-soluble portion of the methanolic extract of aniseed together with anethole glycols and guaiacyl glycerol (Ishikawa *et al.*, 2002).

The isolation and characterisation of eight 2-C-methyl-D-erythritol glycosides and of twelve phenylpropanoid glycosides from the water-soluble portion of aniseed have been carried out by Kitajima *et al.,* 2003. Four aromatic glycosides, an alkyl glycoside and a glucide were isolated together with 24 known compounds by Fujimatu *et al.,* 2003.

Aniseed stored in different conditions was evaluated for deterioration in terms of trans-anethole, anisaldehyde and other compositional characteristics by Guneyli & Kacarcali ,2002; changes over 1 year were relatively minor and deterioration was observed only in seeds that were in contact with the air and with high relative humidity.

- Herbal preparation(s)

The essential oil obtained by steam distillation from the dry ripe fruits is also used.
Separate monographs are published in the European Pharmacopeia for aniseed and anise oil.
The essential oil is a clear, colourless or pale yellow liquid, obtained by steam distillation of dry ripe fruits (European Pharmacopeia 7th Ed); it varies between 1.5% and 6% v/w and contains mainly trans-anethole (80-95%) (Hänsel *et al.*, 1994; Schultze *et al.*, 1987; Shojaii & Mehri, 2012).

In contrast to the essential oil of fennel, anise oil does not contain appreciable amounts of fenchone and also contains much smaller amounts of estragole, cis-anethole, p-anisaldehyde and pseudoisoeugenyl-2-methylbutyrate (Hänsel *et al.*, 1994; Schultze *et al.*, 1987).

Anise oil contains sesquiterpene and monoterpene hydrocarbons (Kubeczka *et al.,* 1978; Schultze *et al.*, 1987; Burkhardt *et al.*, 1986) with a variety of other compounds including linalool and β-farnesene (for some examples see Table 1).

The quality of anise oil depends upon the absence of anethole oxidised forms such as anisaldehyde, anisalketone and anisic acid.

Yield and quality of the oil obtained by supercritical fluid extraction and steam distillation were compared by Ondarza & Sanchez, 1990 and Moyler, 1994. When extracted by means of supercritical fluid extraction using carbon dioxide at 30°C and pressure between 80 and 180 bar, the total amount of extractable substances varied from 3.13 to 10.67%. The major compounds identified were anethole (about 90%), γ-himachalene (2-4%), p-anisaldehyde (<1%), estragole (0.9-1.5%), cis-pseudoisoeugenyl 2-methylbutyrate and trans- pseudoisoeugenyl 2-methylbutyrate (Rodrigues *et al.*, 2003).

Composition of essential oil coming from aniseed of various regions significantly differ in composition (see example Table 1).

Table 1 Main compounds identified in essential oils obtained by steam distillation from anisi fructus

Compound	**(+)**	**(++)**	**(+++)**	**(++++)**
Trans-anethole	87-94%	76.7%	78.63-94.46%	75-96.0%
Estragole	0.5- 5.0%	6.1%	traces-2.61	1-4%
Anisaldehyde	0.1-3.5%	1.5%	traces-0.77%	0.5-0.9%
Linalol	0.1-1.5%		0.01-2.42%	
Alpha-terpineol	<1.2 %		n.d.-3.49%	
Cis-anethole	0.1-0.4 %		n.d.-0.17%	0.3-0.4%
Pseudoisoeugenyl 2-methylbutirate	0.3-2.0%			1%
Anisalacetone		7.1%		

(+) Monograph on anise fruit oil (European Pharmacopeia 5th Ed), (++) Kreydiyyeh *et al.*,2003; (+++) (Arslan *et al.,* 2004, (++++) EMEA, CVMP: Anisi aetheroleum, summary report (1998)

Changes in the content and chemical composition of *Pimpinella anisum* essential oil at various harvest times were studied by Omidbaigi (Omidbaigi *et al.,* 2003).

Problems related to adulteration of anise oil are very common in the real market. Therefore quality control is crucial for this product and an appropriate set of specifications capable to detect any substitution should be established.

According to the monograph of the European Pharmacopeia 7th Ed. the percentage contents of the main components of anise oil are within the following ranges:

Table 2 Main compounds in anise oil according to European Pharmacopeia 7th Ed.

Compound	**Aniseed**
Trans-anethole	87- 94.0%
Estragole	0.5-5.0%
Anisaldehyde	0.1- 1.4%
Linalol	<1.5%
Alpha-terpineol	<1.2%
Cis-anethole	0.1-0.4%
Pseudoisoeugenyl 2-methylbutirate	0.3-2.0%
Fenchone	max 0.01%

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

Not applicable

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

Regulatory status overview

Member State	Regulatory Status				Comments
Austria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No authorised/registered medicinal products
Belgium	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No authorised/registered medicinal products
Bulgaria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Cyprus	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Czech Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No authorised/registered medicinal products
Denmark	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Estonia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Finland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No authorised/registered medicinal products
France	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Herbal tea
Germany	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Herbal tea
Greece	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Hungary	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Iceland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Ireland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No authorised/registered medicinal products
Italy	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No authorised/registered products
Latvia	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Aniseed: combination products Anise oil: manufactured and used since 1969
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:	No authorised/registered medicinal products
Norway	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No authorised/registered medicinal products
Poland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Portugal	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No authorised/registered medicinal products
Romania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	

Member State	Regulatory Status				Comments
Slovak Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Slovenia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Spain	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No authorised/registered medicinal products
Sweden	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No authorised/registered medicinal products
United Kingdom	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Essential oil Water extract

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

This assessment report reviews the available scientific data for aniseed (*Pimpinella anisum* L., fructus) and anise oil.

In preparing this report, a number of data sources were reviewed. The main ones are as follows:

- The ESCOP monographs published in 2003.
- The results of a data search carried out in mid 2005 by the Italian National Institute of Health in several electronic archives (i.e. Napralert, Caplus, Dart, Toxcenter, Embase and Medline).
- The bibliographic references made available by the Association of the European Self-Medication Industry (AESGP) at the end of 2005.
- The European Pharmacopoeia (5^{th} and 7^{th} edition) monographs published on aniseed (Anisi fructus) and anise oil (Anisi aetheroleum)
- The results of a data search carried out at the end of 2005 on Thomson Micromedex (including Martindale, Drugdex, Posindex, Altmedex, Reprotox, Herbal Medicines: A Guide for Health-Care Professionals).
- The results of a data search carried out until the end of October mid 2012 on phytovigilance data banks available on internet (i.e. www/farmacovigilanza.org, www.epicentro.iss.it/focus/erbe/sorv_piante_officinali.htm).
- The results of a literature search carried out in mid 2012 in Pubmed.
- The result of the update in literature search carried out in Medline, Embase, Google scolar and other databases until the end of October 2012 by the Department of Clinical and Experimental Medicine of Messina University.

2. Historical data on medicinal use

2.1. Information on period of medicinal use in the Community

Aniseed has been used as a popular medicine to treat dyspeptic complaints as well as catarrh of the respiratory tract and as a mild expectorant (Bellakhdar *et al.*, 1991; Czygan, 1992; Hänsel *et al.*, 1994; European Pharmacopoeia, 1997; Weiss, 1997, British Herbal Pharmacopoeia, 1983; Steinegger & Hänsel 18; Czygan & Hiller, 2002; Sweetmann, 2002).

In the traditional system of Indian medicine, aniseed is used as antiseptic, stomachic, carminative, stimulant and to prevent flatulence and colic (Singh *et al.,* 2002).

A concoction of aniseed in hot water is also reported to be diuretic and digestive (Bellakhdar *et al.*, 1991) and as a folk remedy to insomnia and constipation as well as to neurologic disorders (Wichtl, 1994).

In traditional medicine, the drug is also reputed able to alleviate pain associated with the female cycle and to be galactagogue and aphrodisiac (Albert-Puleo, 1980; Czygan, 1992; Linares & Bye, 1987).

Use of aniseed products (tincture) as an expectorant in cough and cold is not supported by clinical data, however, it is well known and reported in books on traditional medicine (Weiss, 1985).

Herbal teas are authorised in Germany and France; a water extract (oral liquid) is authorised in UK. The oldest Marketing Authorisation (MA) is dated 1986 for the herbal tea (DE) and 27.04.1992 for the water extract.

Anise oil is authorised in UK (lozenges and syrup). The oldest MA is dated 01.10.1987

Various fixed combinations containing aniseed, aniseed preparations and anise oil are authorised/registered in different European countries.

Food supplements containing aniseed and aniseed preparations are on the market.

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

Crushed aniseeds are traditionally used as infusions for the treatment of a variety of symptoms including:

- Dyspeptic complaints, a broad range of adverse symptoms including spasmodic ailments involving altered functional motility of local smooth muscles induced by anomalous hormonal secretions, *Helicobacter* infections, stress and psychological disturbances and other idiopathic causes;
- Bloating and flatulence, symptoms associated with an altered composition of intestinal flora mainly caused by food born infections or physiological alterations causing a slowing down of the intestinal content transit;
- Catarrh, an excessive secretion of epithelial cells due to respiratory tract infections generally also inducing prostaglandin-mediated bronchoconstriction; this secretion, cleared by pneumocyte cilia, consists mainly of flaked away epithelial cells, micro-organisms and mononuclear cells.

These uses are substantiated mainly by empirical data deriving from investigations on the phytochemical constituents and their pharmacology, while no clinical data are available.

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

Specified strength and posology

Aniseed (*Pimpinella anisum* L. fructus)

Adult and children over 12 years:

A single dose of 1 g 3 times daily is recommended by the German Commission E (Blumenthal et al., 1998).

The single dose provided by the first ESCOP monograph consists of 1-5 g of crushed fruits in 150 ml of water as a herbal tea (ESCOP, 1996-99; Hänsel et al., 1994; Czygan, 1992). The revised monograph confirms the adult average daily dose of 3 g (ESCOP, 2003; Czygan & Hiller, 2002; British Herbal Pharmacopoeia, 1983); Valnet, 1990 recommends half coffee-spoon for 1 cup of tea, three times daily; Leclerc, 1983 reports 1 coffee-spoon for 1 cup of tea.

For the powder 0.2 to 2 g per day are recommended both by Valnet & Leclerc. Czygan, 1992 refers to the Commission E (1 g 3 times daily), but also to the Standardzulassung (standard authorisation): unless otherwise specified, as an expectorant, 1 cup of tea freshly prepared from one to two tea-spoons up to twice a day. One tea-spoon corresponds to 3.5 g.

Therefore the range of traditional posology is broad. The following posology may be considered as usual in the practice: 1 to 3.5 g of whole or (freshly[1]) comminuted or crushed aniseed in 150 ml of water as a herbal tea, three times daily.

Aniseed tincture

Posology for the tincture is not available. The posology of a mixture of anise tincture (120 ml) and anise oil (0.5 ml) is 0.5-1.5 ml three times daily (Weiss, 1985).

Anise oil

For the symptomatic treatment of mild, spasmodic gastro-intestinal complaints including bloating and flatulence and as expectorant in cough and cold, a posology of 0.05-0.2 ml of anise oil, three times daily is given in the British Herbal Pharmacopoeia (1983). The recommended daily dosage by the Commission E for anise oil is 0.3 g (0.4 ml) (Blumenthal et al., 1998). Due to the presence of compounds that do not have a clear toxicological profile (such as estragole and trans-anethole), as a precautionary approach, the lower dosage of BHP is preferable.

Because of the presence of estragole the use in sensitive groups, such as young children, pregnant and breastfeeding women should be minimised[2]. Therefore, the use of aniseed is not recommended in these groups of population.

No data are available on the use of essential oil in adolescents under 18 years of age.

Duration of use

Because of the lack of available safety data on long-term use of aniseed preparations, and due to the presence of compounds such as trans-anethole and estragole, a limit of two weeks is consistent with a self-medication indication, which is the case for traditional herbal medicinal products. This is also supported by EMA public statement on use of herbal medicinal products containing estragole.

[1] For commercial preparations of comminuted or crushed aniseed the applicant must carry out appropriate stability testing related to the content of essential oil components

[2] HMPC Public statement on the use of herbal medicinal products containing estragole (EMA/HMPC/137212/2005).

Suggesting that “Exposure to estragole resulting from consumption of herbal medicinal products (short time use in adults at recommended posology) does not pose a significant cancer risk.”

Method of administration

Oral use.

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

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

The medicinal use of aniseed is largely due to antispasmodic, secretolytic, secretomotor and antibacterial effects of its essential oil.

- *Spasmolytic effect on contracted smooth muscles*

Aniseed alcoholic extracts and oil exerted a relaxing effect on in vitro pre-contracted smooth muscles from different organs (tracheal and ileal) by antagonising several contraction-inducing agents.

The relaxant effect of water extract (DER 5:2), ethanol extract (DER 5:2) and anise oil on methacholine pre-contracted isolated tracheal chains of guinea pig was studied by Boskabady & Ramazani-Assari (2001). A statistically significant spasmolytic effect of the essential oil ($p<0.05$), water extract ($p <0.005$) and ethanol extract ($p<0.001$) was detected.

In the isolated tracheal smooth muscle from guinea pig, anise oil (200 mg/l) produced a complete relaxation of carbachol-induced contractions. In contrast, the oil increased the contraction force in electrically-stimulated guinea pig ileal smooth muscle (Reiter & Brandt, 1985).

Anise oil, at a dose of 0.3 ml/kg b.w., prevented the reduction of surfactant and increased pulmonary resistance in case of bronchopulmonary congestion in rats produced by injection of doses of 10 mg/kg b.w. of paraquat dichloride (Cambar & Aviado, 1970).

Anethole was reported to have a contractile effect on smooth muscle (Reiter & Brandt, 1985).

- *Secretolytic and expectorant effects*

Aniseed

An increase of about 12% in mucociliary transport velocity was observed 90 seconds after the application of 200 µl of an aniseed infusion (4.6 g per 100 ml of water) to isolated ciliated epithelium of frog oesophagus, (Müller-Limmroth & Fröhlich, 1980).

A water extract of a mixture of eight herbs including anise (2.5 g of each of powdered anise, fennel and caraway fruits, 7.5 g powdered chamomile flowers, 0.3 g each of powdered licorice roots and saffron flowers, 2.5 g and 1.3 g of freshly ground cardamom and Nigella sativa seeds), was tested for its inhibitory effect on histamine released from rat peritoneal mast cells stimulated either by compound 48/80 or by IgE/anti-IgE. The effect of the herbal extract was compared to that of the flavonoid quercetin. The herbal water-extract inhibited histamine released from chemically- and immunologically-induced cells by 81% and 85%, respectively; quercetin treated cells were inhibited by 95% and 97%, respectively (Haggag et al., 2003). The same preparation was tested in one open pilot study (see section 4.2).

A solution of essential oil in 12% ethanol, administered intra-gastrically to anaesthetised guinea pigs at 50 mg/kg b.w., induced a 3 to 6-fold increase in respiratory tract fluid during the first 2 hours after administration (Boyd & Pearson, 1946).

A similar experiment in anaesthetised rats, orally dosed with the essential oil at 0.0015 ml/kg, resulted in a 28% increase of respiratory tract fluid (Boyd, 1954). Similar results were also observed in cats (Boyd, 1946).

An emulsion of 2 drops of the essential oil, administered intragastrically to cats, caused hypersecretion of mucus, in the air passages and stimulated ciliary removal of mucus, previously inhibited by opium alkaloids (Van Dongen & Leusink, 1953).

The volume of respiratory secretion of anaesthetised rabbits was increased dose-dependently from 19% to 82% following administration of anise oil by inhalation (in steam) in doses of 0.7 to 6.5 g/kg b.w. via a vaporizer, but signs of tissue damage and a mortality rate of 20% was observed at the highest dose level (Boyd & Sheppard, 1968).

Anethole and fenchone

Anethole and fenchone vapours were given by inhalation via a steam vaporiser to rabbits anesthetised with urethane in doses from 1 to 243 mg/kg b.w. (the amount actually absorbed by the animals was considerably less, estimated as not more than 1% of that added to the vaporiser). Inhalation of anethole did not affect the volume but produced a dose-dependent (1-9 mg/kg) decrease in the specific gravity of respiratory tract fluid (Boyd & Sheppard, 1968).

- ***Antimicrobial effect***

Aniseed extracts

Antimicrobial activity of different concentrations of both water (25 g in 500 ml of boiling water for 15 min and then lyophilised) and ethanol extracts (25 g extracted with ethanol until exhaustion and then dried) of aniseed was tested against *Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Citrobacter koseri, Staphylococcus aureus, Streptococcus pneumoniae, Enterobacter aerogenes, Micrococcus luteus, Staphylococcus epidermidis and Candida albicans* (Gülçin *et al.*, 2003). Most micro-organisms were inhibited, but no activity of the aniseed water extract was detected against *Pseudomonas aeruginosa* and *Escherichia coli.*

A methanol dry extract (DER not specified) of aniseed reduced the resistance of *Pseudomonas aeruginosa* to a series of antibiotics. When both the extract and the antibiotics were tested using concentrations that individually would be unable to inhibit microbial growth, the aniseed extract, in combination with either chloramphenicol, gentamicin, cephalexin, tetracycline or nalixidic acid caused almost complete inhibition of growth of the standard strain of *P. aeruginosa* (Aburjai *et al.*, 2001).

An acetone extract of aniseed (20 g in 50 ml of acetone) inhibited the growth of a range of bacteria including *Escherichia coli* and *Staphylococcus aureus* and also exhibited antifungal activity against *Candida albicans* and other organisms (Maruzzella & Freundlich, 1959).

Lignin-carbohydrate complexes (LCs, LC1, LC2 and LC3) isolated from water extracts from aniseed showed antiviral activity against herpes simplex virus types 1 and 2 (HSV-1 and 2) human cytomegalovirus (HCMV) and measles virus. LCs were also found to increase nitric oxide production, to interfere with virus adsorption to the host cell surface and directly inactivate viruses (Lee *et al.,* 2011).

Anise oil

Anise oil exhibited *in vitro* strong inhibitory activities against the growth of a wide spectrum of bacteria and fungi known to be pathogenic for man and other species. Greatest inhibition of growth was found against

Yersinia enterocolitica, *Aspergillus niger* and *Geotrichum candidum*; moderate inhibition was showed *Lactobacillum plantarum*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhimurium* (Elgayyar *et al.*, 2001).

The essential oils of aniseed and other aromatic plants showed a toxic activity against several soil-borne plant disease-causing fungi including *Fusarium moniliforme*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *Phytophtora capsici*; this activity was attributed to the phenolic fraction of the essential oils (Müller-Riebau *et al.*, 1955).

Anise oil (0.2%) alone showed an *in vitro* activity against *Salmonella enteriditis*. It has synergistic activity against *Salmonella enteriditis* and, more weakly, against *Listeria monocytogenes* when mixed with methylparaben or benzoic acid (Fyfe *et al.*, 1998).

Anise oil inhibited the growth of *Escherichia coli* (minimal inhibitory concentration (MIC): 0.5% V/V), *Staphylococcus aureus* (MIC: 0.25%), *Salmonella typhimurium* (MIC: 2.0%) and *Candida albicans* (MIC: 0.5%) using the agar dilution method (Hammer *et al.*, 1999). An antimicrobial activity of the oil was also demonstrated in other studies (Ramadan *et al.*, 1972; Ibrahim & Ogumondede, 1991; Shukla & Tripathi, 1987; Sokmen *et al.*, 1999).

The essential oils of anise, fennel and other plants showed a dose-dependent inhibitory effect on the growth of tested fungi including *Aspergillus flavus, A. parasiticus, A. ochraceus* and *Fusarium moniliformis.* Anise oil at ≤ 500 ppm completely inhibited *A. flavus, A. parasiticus, A. ochraceus* and *F. moniliformis* (Farag *et al.*, 1989; Soliman & Badeaa, 2002). The higher concentration of anethole in anise oil might explain its greater fungicidal effect in respect to fennel and caraway. Anise oil also inhibited the production of aflatoxins, Ochratoxin A and Fumosin in inoculated wheat samples. (Soliman & Badeaa, 2002).

Bactericidal activities of a number of plant essential oils, including anise oil, and of their isolated constituents were tested against *Campylobacter jejuni, Escherichia coli*, *Listeria monocytogenes* and *Salmonella enterica.* Anise oil was shown to reduce bacterial activity of all tested bacteria (*C. jejuni* > *L. monocytogene*s > *S. enterica* = *E. coli*). For the isolated compounds estragole inhibited all the tested strains; limonene showed an inhibitory activity only on *C. jejuni* and *L. monocytogenes* and trans-anethole only inhibited *C. jejuni* (Friedman *et al.*, 2002*).*

The anise and fennel oils was found to have a high antibacterial activity against *Staphylococcus aureus* (responsible for bases, sepses and skin infections), *Streptococcus haemoliticus* (causing infection of the throat and nose)*, Bacillus subtilis* (infection in immune compromised patients)*, Pseudomonas aeruginosa* (causing hospital acquired infection)*, Escherichia coli* (responsible for urogenital tract infections and diarrhoea)*, Klebsiella species* and *Proteus vulgaris* (Singh *et al.*, 2002).

3.1.2 Secondary pharmacodynamics

- *Estrogenic and anti-estrogenic effects*

Aniseed water extract

Water extracts (DER 10:1) of aniseed, flowers of *Sideritis euboea* and *clandestina* and *Matricaria camomilla*, at a concentration range between 10-100 μg/ml, were investigated *in vitro*. The extracts were found to be active in stimulating the differentiation and mineralisation of osteoblastic cell culture and inducing, like antiestrogens, the insulin growth factor binding protein 3 (IGFBP3) in MCF-7 breast cancer cells. No effect was observed on the proliferation of cervical adenocarcinoma (HeLa) cells using the MTT assay (a laboratory test for measuring cellular proliferation) (Kassi *et al.*, 2004). The presence of estradiol inhibited the anti-estrogenic effect, thus suggesting an estrogen receptor-related mechanism.

Trans-anethole

Trans-anethole administered orally to immature female rats at 80 mg/kg b.w. for 3 days significantly increased uterine weight, to 2 g/kg compared to 0.5 g/kg in controls and 3 g/kg in animals given estradiol valerate subcutaneously at 0.1 µg/rat/day ($p<0.001$). The results confirmed that trans-anethole has estrogenic activity; other experiments showed that it has no anti-estrogenic, progestational, anti-progestational, androgenic or anti-androgenic activity (Dhar, 1995).

Estrogenic activity of trans-anethole at high concentrations was determined by a sensitive and specific bioassay using recombinant yeast cells expressing the human estrogen receptor (Howes *et al.*, 2002).

Estrogenic activity described for trans-anethole has not been studied for aniseed alcoholic extracts and it is not confirmed in humans on the basis of epidemiological data related to the common use of aniseed alcoholic beverages.

- *Anti-tumour activity of anethole*

In Swiss albino mice with Ehrlich ascites tumour (EAT) in the paw, anethole administered orally at 500 or 1000 mg/kg on alternate days for 60 days significantly and dose-dependently reduced tumour weight ($p<0.05$ at 500 mg/kg, $p<0.01$ at 1000 mg/kg), tumour volume ($p<0.01$ at 500 mg/kg, $p<0.001$ at 1,000 mg/kg) and b. w. ($p<0.05$ to 0.01) compared to EAT-bearing controls. Mean survival time increased from 54.6 days to 62.2 days (500 mg/ kg) or 71.2 days (1000 mg/kg). Histopathological changes were comparable to those after treatment with cyclophosphamide. These and other results demonstrated the anti-carcinogenic, cytotoxic and non-clastogenic nature of anethole (Al-Harbi *et al.*, 1995).

Anethole at a concentration below 1 mM has been shown to be *in vitro* a potent inhibitor of tumour necrosis factor (TNF)-induced cellular responses, such as activation of nuclear factor-kappa B (NF-kB) and other transcription factors, and also to block TNF-induced activation of the apoptotic pathway. This might explain the role of anethole in suppression of inflammation and carcinogenesis (Chainy *et al.*, 2000).

- *Antioxidant activity*

The antioxidant properties of water (25 g in 500 ml of boiling water for 15 min and then lyophilised) and ethanol (25 g extracted with ethanol until exhaustion and then dried) extracts of aniseed were investigated using different antioxidant tests, including reducing power, free radical scavenging, super oxide anion radical scavenging, hydrogen peroxide scavenging, and metal chelating activities. In general the water extract exhibited greater antioxidant activity than ethanol extract (Gülçin *et al.*, 2003).

Anise oil and many other essential oils were observed *in vitro* to inhibit copper-catalysed oxidation of human Low-Density Lipoproteins (LDL); such an activity correlated well with the total phenol content of the oil (Teissedre & Waterhouse, 2000).

- *Local anaesthetic activity of trans-anethole*

Trans-anethole concentration-dependently reduced electrically-evoked contractions of rat phrenic nerve-hemidiaphragm, by 10.3% at 10^{-3} µg/ml, by 43.9% at 10^{-2} µg/ml, by 79.7% at 10^{-1} µg/ml and by 100% at 1 µg/ml (Ghelardini *et al.*, 2001).

In the rabbit conjuctival reflex test, solutions of trans-anethole administered into the conjunctival sac increased concentration-dependently the number of stimuli required to evoke the conjuctival reflex ($p< 0.01$); the effect was comparable to that of procaine (Ghelardini *et al.*, 2001).

- *Sedative effect*

The pentobarbital-induced sleeping time of mice was increased by 93.5% ($p<0.01$) after simultaneous intra-peritoneal administration of essential oil at 50 mg/kg b.w.; trans-anethole gave similar results (Marcus & Lichtenstein, 1982).

- *Other effects*

Aniseed

A water extract of aniseed (100 mg/ml extracted at 40°C) exhibited a weak *in vitro* cytotoxic activity against melanoma cells (Sathiyamoorthy *et al.*, 1999).

An aniseed water extract (DER 1:10 extracted at 60°C) did not show any activity when tested *in vitro* on the activity of Na^+-K^+- ATPase from rat jejunum (Kreydiyyeh *et al.*, 2000).

A dry methanol extract of aniseed (DER not specified) diluted at a concentration of 500 µg/ml showed a weak antiaggregant effect on human platelets *in vitro* (Okazaki *et al.*, 1998).

Anise oil

Anise oil given intraperitoneally (i.p.) significantly ($p < 0.001$) and dose-dependently counteracted convulsant effects induced in male mice by injection of phenylenetetrazole or by electroshock. The ED50 of anise oil was 0.52 (0.35 to 0.76) ml/kg and its efficacy was less than i.p. ethosuximide and phenytoin (Pourgholami *et al.*, 1999).

In another study, whether anise oil (0.01% and 0.05%) affects the bioelectrical activity of snail neurons in control condition or after pentylenetetrazol (PTZ) induced epileptic activity has been determined. Anise oil changed the firing pattern to irregular and then to bursting in intact cells or resulted in the robustness of the burst firing and the steepness of the paroxysmal shift induced by PTZ treatment. It also significantly increased the firing frequency and decreased both the after-hyperpolarisation potential (AHP) following single action potential and the post-pulse AHP. Likely candidate cellular mechanisms underlying the hyperexcitability produced by anise oil include enhancement of Ca^{2+} channels activity or inhibition of voltage and/or Ca^{2+} dependent K^+ channels activity underlying AHPs. These finding indicates that a certain caution is needed when *Pimpinella anisum* is used for treating patients suffering from epilepsy (Janahmadi *et al.,* 2008).

Subcutaneous administration of the essential oil (100 mg/ kg b.w. per day) for 7 days to partially hepatectomised rats stimulated liver regeneration ($p<0.01$) (Gershbein, 1977).

Tunc *et al.*, 2000 studied the fumigant activity of the essential oils of *Pimpinella anisum* and other herbs against eggs of two storage products insect pests and found 100% mortality of the exposed eggs.

The effects of anise oil on the acquisition and expression of morphine-induced conditioned place preference in mice were studied by Sahraei *et al.,* 2002. The authors concluded that the anise oil may reduce the morphine-induced effect via a GABAergic mechanism.

Anise oil enhanced significantly in dose dependent manner glucose absorption from the rat perfused jejunum and increased the Na^+-K^+-ATPase activity in jejunal homogenate. The oil did not affect water absorption from the perfused colon or the activity of the Na^+-K^+- ATPase in the colon. When added for 24 h to drinking water, anise oil reduced the volume of urine produced in the rat and increased the activity of renal Na^+-K^+-ATPase even at very low concentrations (0.05%) (Kreydiyyeh *et al.*, 2003).

The effects of codeine, diazepam, midazolam, pentobarbital, imipramine and fluoxetine were tested in mice after 5 days of peroral pretreatment with human equivalent dose of anise oil (0.3 mg/kg). The intake of essential oil led to significant increase of analgesic effect of codeine and enhanced the motor impairment caused by midazolam. The application of diazepam decreased the number and percentage

of entries in open arm in elevated maze plus test in the group pretreated with essential oil indicating augmented effect of drug on motor activity. Essential oil pretreatment caused significant shortage of pentobarbital induced sleeping time when compared to control. The decrease in antidepressant effect of imipramine and fluoxetine was diminished by the pretreatment with anise oil. Results indicate that concomitant intake of anise oil preparations and drugs that act on CNS may cause herb–drug interactions. However, this finding needs further clinical confirmation (Samojlik et al., 2012).

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

No data available.

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

- *Single dose toxicity*

Anise oil

Oral LD_{50} values per kg b.w. were determined for the essential oil as 2.7 g in rats (Von Skramlik, 1959) and for trans-anethole as 1.8-5.0 g in mice; 2.1-3.2 g in rats; and 2.16 g in guinea pigs (Lin, 1991).

Probable oral lethal dose of anise oil had been reported for human beings to be in the range from 50 to 500 mg/kg b.w. (Oil of anise, Toxnet).

Trans-anethole

Intraperitoneal LD_{50} values for trans-anethole were determined as 0.65-1.41 g/kg in mice and 0.9-2.67 g/kg in rats (Lin, 1991).

- *Repeat dose toxicity*

Data are available only for **anethole** and **trans-anethole**.

In 90-day experiments in rats, 0.1% trans-anethole in the diet induced no toxic effects, whereas a dose-related oedema of the liver was reported at levels of 0.3, 1% and 3.0%, concentration which have no therapeutic relevance (Lin, 1991).

Male rats receiving 0.25% anethole in their diet for one year did not show any toxic effects, whereas those receiving 1% anethole for 15 weeks had slight oedematous changes in liver cells (Hagan et al., 1967).

Rats treated with 0.2%; 0.5%; 1.0% or 2% anethole of their diet for 12-22 months showed no effects on clinical chemistry, haematology, histopathology or mortality, but lower body weight and reduced fat storage were observed a 1.0% and 2.0% dose levels (Lin, 1991; Le Bourhis, 1973).

- *Mutagenicity and carcinogenicity*

Balachandran (1991) screened a number of commonly consumed foods and food components in south India for their genotoxic effects on Swiss mice. Spices like pyrolysed cumin and aniseeds showed genotoxic moderate effects (Hänsel et al., 1994).

Aniseed extracts

A water extract prepared by boiling aniseed in 100 ml of water for 10 min, followed by filtration through paper and centrifugation, did not show any mutagenic activity on *Salmonella typhimurium* strains TA 97a, TA98 (a frameshift mutation detector), TA 100 (a base-pair substitution mutation detector) and

TA102 (an oxidative mutation detector) (Al-Bataina *et al.*, 2003).

A concentrated ethanol aniseed extract (1 part of aniseed and 2 parts of ethanol) was mutagenic at high concentrations (5 mg/plate) to streptomycin-dependent strains (SD#510 and SD#4) of *Salmonella typhimurium* TA 98 (Shashikanth & Hosono, 1986).

An ethanol aniseed extract did not show any activity at the maximum non-toxic concentration of 0.1 mg/ml in chromosomal aberration tests using a Chinese hamster fibroblast cell line with no metabolic activation (Ishidate *et al.*, 1984).

Anise oil

The mutagenic effect of anise oil (90% trans-anethole), fennel oil (70% trans-anethole) and trans-anethole (isolated compound) was tested with two strains of *Salmonella typhimurium* (TA 100 and TA98) with and without microsomal activation in a plate test using dose ranges from zero (control) to the level of cell toxicity. All test materials increased mutagenic activities with TA100 tester strain with implementation of microsomal activation (S13) system. Peak mutagenic activity, 4 and 4.4 times that of the background rate, occurred with 2 mg anise oil/plate and 2.5 mg/fennel oil/plate respectively. Isolated trans-anethole was (also mutagenic, but the dose level and rate of mutagenicity were not stated. The compound was considered not to be mutagenic unless it was capable of inducing a mutation (reversion) rate at least 3 times that of the incident background (Marcus & Liechtenstein, 1982 in Lin, 1991). Gorelick, 1995 reviewed the results of this study were by and were not confirmed under standard protocol conditions.

Salmonella thyphimurium tester strains TA1535, TA1537, TA1538, TA98 and TA100 were used to study the mutagenic activity of anise oil and anethole in the Ames test with slight modification. In the absence of metabolic activation results were negative. In presence of S9 activation only anethole showed a linear dose-response against strain 100 up to the dose of 120 µg, but anethole was inactive in *a B. subtilis* Rec assay and was negative in an *E. coli* uvr reversion test (Sekizawa & Shibamoto, 1982).

Anethole

From a series of studies investigating the effect of anethole when added to female CD-1 mice diet or given orally or by i.p. injection to male pre-weaning B6C3F1 mice, Miller *et al.,* 1983 concluded that anethole was not a hepato-carcinogen; although these studies were not carried out for test animal lifetimes. Safrole and estragole were found to be highly active as liver carcinogens in both these tests.

In another bioassay carried out in Sprague–Dawley (SD) rats, 0.25, 0.5, or 1.0% anethole was administered in the diet for 121 weeks. Results showed the occurrence of a small, but statistically significant, incidence of hepatocellular carcinomas in female rats receiving 1% anethole (Truhaut *et al.*, 1989). These hepatocellular carcinomas were associated with other changes to the liver (increase in relative liver weight) similar to those observed after enzyme induction (Newberne *et al.*, 1989) and were considered not to be caused by a direct genotoxic effect of trans-anethole (Lin, 1991). Also Reed & Caldwell 1992 showed that i.p. administration of anethole to SD rats increased liver weight, microsomal protein and cytochrome P-450 content.

Tested in *Salmonella* mutagenesis assay and also in mouse lymphoma L5178Y $TK^{+/-}$cell mutagenesis assay, anethole was inactive in *Salmonella thyphimurium* tester strains TA1535, TA1537, TA15358, TA98 and TA 100 and was active in the mouse lymphoma assay only with Aroclor 1254-induced rat liver S9 activation (Heck *et al.*, 1989).

In the *Salmonella*/microsome mutagenicity assay with Aroclor 1254-induced rat liver S9 activation performed with *Salmonella thyphimurium* tester strains TA1535, TA1537, TA15358, TA98 and TA 100

showed that anethole may have a very weak activity for strain TA100; however, no obvious dose-related response can be found (Hsia *et al.*, 1979).

Mortelmans *et al.* 1986 reported negative results of mutagenicity testing of anethole performed in the *Salmonella* pre-incubation assay, which is a modification of the standard plate incorporation assay, using four *Salmonella* strains (TA1535, TA1537, TA98 and TA100) in the presence and absence of rat and hamster Aroclor 1254-induced liver S9 activation.

The mutagenic activities of anethole and its metabolite 3'-idroxyanethole were studied using three tester strains of *Salmonella thyphimurium (*TA1535, TA00, TA98). Addition of an NADPH-generating system and liver S13 fraction from Aroclor-treated rats (6.8 mg liver/protein plate) to the incubation mixture of TA100 tester strain increased mutagenic activities. Approximately 45 revertants were obtained per µmole of anethole. Under the same conditions, 3'-hydroxyanethole showed no significant mutagenic activity with less than 7 µmoles/plate. Above this concentration the S13-mediated mutagenicity increased linearly with increased doses up to 15 µmoles/plate (about 1000 revertants with 15 µmoles/plate) (Swanson *et al.*, 1979).

Five strains of *Salmonella thyphimurium* (TA1535, TA1537, TA1538, TA98 and TA100) with and without S9 fractions from Aroclor 1254-induced rats were used to study potential mutagenic effects of trans-anethole. The lowest overtly toxic concentration for trans-anethole was 1 mg/plate. No mutagenic activity was observed at concentration of up to 50 µg trans-anethole/plate with or without metabolic activation. However the addition of 3'phosphoadenosine- 5' phosphosulphate (PAPS) to the microsomal assay markedly increase the mutagenicity of trans-anethole in TA1535 tester stain, The mutation rate observed was approximately 4, 5, 10, 11, 9 and 3 times that of the background rate at trans—anethole concentrations off 0.05, 0.20, 1.0, 5.0, 15.0 and 50.0 µg/plate respectively (To *et al.*, 1982).

Gorelick,1995 reviewed nine previously conducted gene mutation studies (Heck *et al.*, 1989; Hsia *et al.*, 1979; Marcus & Liechtenstein, 1982; Mortelmans *et al.,* 1986; Nestmann *et al.*, 1980; Sekizawa & Shibamoto, 1982; Swanson *et al.*, 1979; To *et al.*, 1982) and repeated the *Salmonella* /microsome test as well as the L5178Y mouse lymphoma TK +/-assay to ascertain their reproducibility and relevance. In the nine studies reviewed, anethole was uniformly negative in the *Salmonella* tests to detect base-pair substitutions or frameshift mutations without metabolic activation and this was also the case in four studies with metabolic activation after careful consideration of all experimental conditions. The studies which suggested a weak mutagenic potential of anethole (Marcus & Liechtenstein, 1982; Swanson *et al.*, 1979; Mortelmans *et al.*, 1986; Sekizawa & Shibamoto, 1982) were the result of the use of non-standard protocols (using longer pre-incubation times, excessive quantities of S-9 protein and/or the addition of co-factors) and have also been found to be irreproducible (Gorelick, 1995).

Gorelik,1995 reports dose-dependent response of trans-anethole only in the mouse lymphoma assay with metabolic activation. Anethole was found to be mutagenic in the mouse lymphoma assay which is known for its extreme sensitivity and poor selectivity for genotoxicity also by other authors (Heck *et al.*, 1989; Caldwell, 1993).

Other results showing the absence of mutagenic potential of anethole include assays in *Escherichia coli* (Sekizawa & Shibamoto, 1982) and in *Saccharomyces cerevisiae* (Nestmann & Lee, 1983).

A mouse micronucleus assay was negative, with no micronuclei found at 6 and 30 hours after anethole i.p. administration to groups of 5 male and 5 female mice in two doses of 0.25 or 0.5 g/kg b.w. ((iLin, 1991). Similarly no significant increase in genotoxicity was observed in the mouse bone marrow micronucleus test after the oral pre-treatment of mice with trans-anethole at 40-400 mg/kg b.w. 2 and 20 hours before i.p. injection of genotoxins; a moderate, dose-dependent protective effects against

known genotoxins such as cyclophosphamide, pro-carbazine, N-methyl-N'-nitrosoguanidine, urethane and ethyl methane sulfonate was observed ($p<0.05$ to $p<0.01$ at various dose levels) (Abraham, 2001).

Very low levels of DNA adducts (<1.4 pmol/mg DNA) were observed after administration of anethole to mice, whereas 150 and 220 times as many adducts were detected following administration of safrole and estragole, respectively (Phillips *et al.*, 1984).

Unscheduled DNA synthesis (UDS) assays in rat hepatocytes did not indicate any mutagenic potential of anethole (Howes *et al.*, 1990; Müller *et al.*, 1994).

Anethole has three primary metabolites in the rat and the pathway of toxicological concern is that of epoxidation of the 1,2 double bond at the side chain; in fact, 3'-hydroxylation does not result in genotoxicity or marked cytotoxicity and O-demethylation is a detoxication reaction (Sangster *et al.*, 1984a and 1984b; Bounds & Caldwell, 1996). Cytotoxicity of anethole is enhanced when the cellular epoxide defence mechanisms of conjugation with reduced glutathione and hydration by cytosolic epoxide hydrolase are severely compromised. However, modulation of epoxide metabolism by the same mechanism in cultured cells failed to induce UDS by anethole nor was there a UDS response in hepatocytes of female rats dosed with anethole *in vivo* (Marshall & Caldwell, 1996). The synthetic epoxide of anethole was also tested and found to be cytotoxic, but not genotoxic. The lack of induction of UDS by anethole epoxide provided a further support to the hypothesis that marginal hepatocarcinogenesis observed in female rats given 1% anethole in the diet for 121 weeks was not initiated by a genotoxic event (Marshall & Caldwell, 1996).

In the 51st meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) a document on safety evaluation of trans-anethole was prepared; the conclusions were that trans-anethole and its metabolites are unlikely to be genotoxic *in vivo*; the cytotoxic metabolite, anethole epoxide, was suggested to be the possible causative agent of the hepatotoxic effect observed in pre-clinical studies in rats. The report of JECFA allocated the acceptable daily intake (ADI) at the dose of 0.2 mg/kg b.w. on the basis of scientific pre-clinical data published on trans-anethole (JECFA, 1999).

In 1999 the USA Expert Panel of FEMA (Flavour and Extract Manufacturers' Association) released a review of scientific data relevant to the safety evaluation of trans-anethole as a flavouring substance. The review concluded that trans-anethole does not represent a carcinogenic risk to humans and can be "generally recognised as safe" (GRAS) at low level of intake (54 μg/kg b.w./day) (Newberne *et al.*, 1999).

Estragole

Estragole, a minor constituent of anise oil, has shown its ability to produce genotoxic effects in bacteria, yeasts and mammalian cells, while no mutagenic activity was observed in *Salmonella typhimurium* probably because of the absence of the complex metabolism needed for bioactivation (Public statement on the use of herbal medicinal products containing estragole'(EMEA/HMPC/137212/2005).

It has been shown that estragole and its 1'-hydroxy metabolite caused significant increases in the incidences of hepatocellular carcinomas in male CD-1 mice that received the compounds by subcutaneous injection at 1-22 days of age (Drinkwater *et al.*, 1976).

Estragole or its metabolite, 1'-hydroxyestragole, administered to mice binds readily to DNA and several DNA adducts have been characterised. Several studies showed the carcinogenic effects of estragole in mice (mainly malignant liver tumours). 1'-hydroxyestragole and other metabolites and synthetic derivatives were shown to be potent carcinogens in mice (Wiseman *et al.*, 1987; `Public statement on the use of herbal medicinal products containing estragole'.EMEA/HMPC/137212/2005).

The Public statement EMEA/HMPC/137212/2005 states that the profiles of metabolism, metabolic activation and covalent binding of estragole are dose-dependent and tend markedly to decrease at low levels of exposure (less than linear decrease with respect to dose. According to this assessment, rodent tudies indicate that these events are probably minimal in the dose range 1-10 mg estragole/kg b.w., which is approximately 100-1,000 times the anticipated human exposure to this substance from traditional diet and as added flavouring substance. The major metabolic pathway of low doses of estragole as established in rats and mice is O-demethylation with carbon dioxide being the terminal metabolite, but as the dose increases the proportion of O-demethylation decreases and other pathways, notably 1'-hydroxylation, come into prominence.

- *Reproductive toxicity*

Trans-anethole exerted a dose-dependent, anti-implantation activity after oral administration to adult female rats on days 1-10 of pregnancy. When compared with control animals (all of which delivered normal offspring on completion of term), trans-anethole administered at 50, 70 and 80 mg/kg b.w. inhibited implantation by 33%, 66% and 100% respectively. Further experiments were conducted with the 80 mg/kg dose at different stages of pregnancy. When rats were administered trans-anethole on days 1-2 of pregnancy, normal implantation and delivery occurred; however rats administered anethole on days
3-5 of pregnancy, implantation was completely inhibited; and in those given trans-anethole on days 6-10 of pregnancy three out of five rats failed to deliver at term. No gross malformations of offspring were observed in any of the groups. The results demonstrated that trans-anethole has antifertility activity. From comparison with the days 1-2 group (lack of antizygotic activity), the lower level of delivery in the days 6-10 group was interpreted as a sign of early abortifacient activity (Dhar, 1995).

3.4. Overall conclusions on non-clinical data

The non-clinical data make plausible the traditional use of aniseed and anise oil in mild spasmodic gastro-intestinal complaints, including bloating and flatulence, and as an expectorant in cough associated with cold.

The medicinal use of aniseed is largely due to antispasmodic, secretolytic, secretomotor and antibacterial effects of its essential oil.

Significant relaxing effect of anise oil has been shown on tracheal and ileal smooth muscles contracted by several contraction-inducing agents (e.g. metacholine and carbachol). Moreover, a number of compounds detected in aniseed, very active in inhibiting growth of pathogenic bacteria and fungi, might concur in relieving bloating and flatulence.

These effects are also likely to play a beneficial role in the treatment of inflammation of mucous membranes of the upper respiratory tract. This indication is also made plausible by the secretolytic and expectorant effects exhibited by anethole, a main component of anise oil.

On the basis of non-clinical data and of the current state of knowledge, it can be concluded that short term use of aniseed-based medicinal products in human adults according to the traditional indications is unlikely to be linked to any significant risk for health.

Ethanolic aniseed extracts are mutagenic at high concentrations and results from studies carried out in laboratory animals showed a weak mutagenic potential of anethole. However, taking into consideration the more recent results of the *Salmonella* tests repeated with the updated protocols as well as the results from the other genotoxicity studies it is considered that the positive response of anethole observed in the mouse lymphoma assay is most likely to be via a non-DNA mechanism (Caldwell, 1993). Moreover, trans-anethole is reported as "generally recognised as safe" (GRAS) at the intake of 54

μg/kg b.w./day) and the acceptable daily intake is about 0.2 mg/kg b.w.. Studies on trans-anethole cytotoxicity do not clarify which is the real dimension of the risk occurring with preparations containing anethole. Both JECFA and FEMA reports concluded that this metabolite is safe as a flavouring agent. No evidence exists on safe level intake of trans-anethole contained in anise oil for medicinal use. Moreover, acceptable daily intake values, when not otherwise specified, are generally referred to adult 60 kg b.w. (European Commission, COM/2001/0542). Therefore, level intake indicated by JECFA report is useful for the evaluation in human adults, but specific values for children are missing.

Several studies have shown the carcinogenic effects of estragole and some of its metabolites in mice (mainly malignant liver tumours).The EMEA/HMPC/137212/2005 it is concluded that the profiles of metabolism, metabolic activation and covalent binding of estragole are dose-dependent and tend to markedly decrease at low levels of exposure. The genotoxic risk related to estragole is not considered to be relevant for adults in the recommended dosage due to the small amount present in anise oil but the risk cannot be calculated with high doses or prolonged use or in children.

An anti-tumour activity of anethole has also been reported (see section 3.1 Overview of available pharmacological data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof).

Considering the above-mentioned data and all the uses of aniseed, it is concluded that human exposure resulting from short term use of aniseed-based medicinal products, complying with the proposed specifications, is unlikely to pose any significant cancer risk.

However, because of the presence of estragole, the use in sensitive groups, such as young children, pregnant and breastfeeding women should be minimised (EMEA/HMPC/137212/2005). No data are available on the use of essential oil in adolescents under 18 years of age.

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

No data available for aniseed in human beings.

To date very little is known about the metabolism of trans-anethole by humans. Caldwell's research group published two articles on metabolism of trans-anethole in humans, both including essentially the same experiments (Sangster *et al.*, 1987; Caldwell & Sutton, 1988). The fundamental conclusion of the authors regarding these experiments is only that "the pattern of urinary metabolites of trans-anethole is unaffected by dose size". Any consideration on the risk influence is lacking. These Caldwell's experiments show essentially the difference in anethole metabolism between rodents and humans.

After oral administration of radioactively-labelled trans-anethole (as the methoxy-^{14}C compound) to 5 healthy volunteers at dose levels of 1, 50 and 250 mg on separate occasions, it was rapidly absorbed. 54-69% of the dose (detected as ^{14}C) was eliminated in the urine and 13-17% in exhaled carbon dioxide; it was not detected in the faeces. The bulk of elimination occurred within 8 hours and, irrespective of the dose level, the principal metabolite (more than 90% of urinary ^{14}C) was

4-methoxyhippuric acid (Caldwell & Sutton, 1988). Trans-anethole is metabolised in part to the inactive metabolite 4-methoxybenzoic acid (Schulz *et al.*, 1998). An earlier study with 2 healthy subjects taking 1 mg of trans-anethole gave similar results (Sangster *et al.,* 1987).

In mice and rats trans-anethole is reported to be metabolised by O-demethylation and by oxidative transformation of the C3-side chain. After low doses (0.05 and 5 mg/kg b.w.) O-demethylation occurs predominantly, whereas higher doses (up to 1500 mg/kg b.w.) give rise to higher yields of oxygenated metabolites (Sangster *et al.*, 1984a; Sangster *et al.*, 1984b).

4.2. Clinical Efficacy

Therapeutic use of anise alone is not substantiated with human clinical trials.

4.2.1. Dose response studies

There are no dose-finding studies available.

The recommended dosage for adults and children over 12 years is supported by clinical experience and expert opinions (British Herbal Pharmacopoeia, 1983; Blumenthal *et al.*, 1988; Czygan & Hiller, 2002; Dorsch *et al.*, 2002).

4.2.2. Clinical studies (case studies and clinical trials)

- *Secretolytic and expectorant effects*

A combined herbal preparation containing dry ivy leaf extract as the main active ingredient, a decoction of thyme and aniseed, and mucilage of marshmallow root was investigated in an open clinical trial for its effects on the symptoms of cough and its tolerability. The trial was carried out on 62 patients with a mean age of 50 years (range 16-89) with irritating cough in consequence of common cold (n = 29), bronchitis (n = 20) or respiratory tract diseases with formation of viscous mucus (n = 15). The mean daily intake was 10 ml (range 7.5-15) of syrup, and the mean duration of treatment was 12 days (range 3-23 days). All symptom scores showed an improvement as compared to baseline (Buechi *et al.*, 2005).

- *Combinations*

Randomised clinical trial of a phytotherapic compound containing *Pimpinella anisum*, *Foeniculum vulgare*, *Sambucus nigra*, and *Cassia augustifolia* for chronic constipation

The laxative efficacy of a phytotherapeutic combination containing *Pimpinella anisum* L., *Foeniculum vulgare*, *Sambucus nigra* L., and Cassia *angustifolia* largely used in Brazil for the treatment of constipation has been tested in a randomised, crossover, placebo-controlled, single-blinded clinical trial including 20 patients with chronic constipation according to the criteria of the American Association of Gastroenterology. Half of the subjects received the combination for a 5-day period, whereas the other half received placebo for the same period. Both treatment periods were separated by a 9-day washout period followed by the reverse treatment for another 5-day period. The primary endpoint was colonic transit time (CTT), measured radiologically. Secondary endpoints included number of evacuations per day, perception of bowel function, adverse effects, and quality of life. Mean CTT assessed by X ray was 15.7 hours (95%CI 11.1-20.2) in the active treatment period and 42.3 hours (95%CI 33.5-51.1) during the placebo treatment ($p < 0.001$). Number of evacuations per day increased during the use of active tea; significant differences were observed as of the second day of treatment ($p < 0.001$). Patient perception of bowel function was improved ($p < 0.01$), but quality of life did not show significant differences among the study periods. Except for a small reduction in serum potassium levels

during the active treatment, no significant differences were observed in terms of adverse effects throughout the study period. The authors concluded that the combination assessed has laxative efficacy and is a safe alternative option for the treatment of constipation (Picon *et al.* 2010).

Open pilot study of a herbal tea mixture on allergic asthma

An open pilot study was carried out with a herbal tea mixture of eight herbs including anise (2.5 g each of powdered anise, fennel and caraway fruits, 7.5 g powdered chamomile flowers, 0.3 g each of powdered licorice roots and saffron flowers, 2.5 g and 1.3 g of freshly ground cardamom and *Nigella sativa* seeds).The mixture had the same composition as the water extract used to test the *in vitro* inhibitory effect on histamine released from rat peritoneal mast cells stimulated either by compound 48/80 or by IgE/anti-IgE (see section 3.1.). Forty female patients, aged 30 to 50 years, suffering from allergic asthma, taking the antihistaminic drug ketotifen, 1 mg twice a day, plus the selective β_2 stimulant salbutamol, 2 mg once a day, were divided into two groups of twenty each: Group I taking one cup of the herbal tea mixture twice daily and Group II taking regular tea of *Camellia sinensis* as an active placebo. The antihistaminic medication was reduced to one daily in all patients. The clinical results reported by the authors showed significant improvements of sleep discomfort, cough frequency and cough intensity in addition to increased percentages of FEV1/FVC in patients suffering from allergic asthma, who used the herbal tea compared to those who used the placebo tea (Haggag *et al.*, 2003).

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

No data available.

4.3. Overall conclusions on clinical pharmacology and efficacy

Medicinal use of aniseed and anise oil is not supported by clinical evidence. On the basis of the long standing use reported in scientific literature (see section 2.1) traditional medicinal use can only be proposed.

5. Clinical Safety/Pharmacovigilance

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

No data available

5.2. Patient exposure

No data available

5.3. Adverse events and serious adverse events and deaths

The allergenic potential of aniseed is relatively weak and it shows up occasionally with allergic reactions at dermal, respiratory and gastro-intestinal level (Hänsel *et al.*, 1994; Wüthrich & Dietsch, 1985; Blumenthal *et al.*, 1998; Fraj *et al.*, 1996; Garcia-Bravo *et al.*, 1997; Garcia Gonzalez *et al.*, 2002). The molecular weights of the main immunoglobulin IgE binding proteins in aniseed extracts were approximately 48, 42, 39, 37, 34, 33 and 20kD. Enzyme immunoassay inhibition studies with one patient's serum revealed cross-reactivity among the IgE components deriving form aniseed, fennel, caraway, coriander and dill extracts (Garcia Gonzalez *et al.*, 2002).

A case of aniseed-induced tongue angioedema has been reported. Skin prick tests to foods was positive only to aniseed. Serum-specific immunoglobulin IgE was determined by enzyme allergosorbent

test to aniseed extract. This was the first report of a case of type I hypersensitivity due to aniseed liqueur ingestion (Gázquez García 2007).

Rare cases of contact dermatitis to anethole containing preparations (Andersen, 1978; Franks, 1998) have been reported.

Anise contains furocoumarins which can cause photosensitivity reactions (Newall *et al.*, 1996). No furocumarins were found in aniseed herbal teas.

Toxic syndromes may result in infants from ingestion of anise oil (see Section 5.5 Safety in special populations and situations – Overdose).

5.4. Laboratory findings

No data available

5.5. Safety in special populations and situations

Contraindications

People with known sensitivity to aniseed or to Apiaceae (Umbelliferae) (caraway, celery, coriander, dill and fennel) or to anethole should avoid the use of aniseed preparations and anise oil. A common allergen called Bet v 1, also bound to fennel, possibly accounting for the observed cross-sensitivity was found in subjects showing allergic symptoms as rhinitis, angioedema, asthma, wheezing, urticaria, eczema, abdominal pain, vomiting, and diarrhoea (Jensen-Jarolim *et al.*, 1997; Garcia-Gonzalez *et al.*, 2002).

The use of anise oil in children and adolescents is contraindicated because of the lack of data and because of the presence of estragole, whose use should be minimised in young children. Moreover, no metabolic data for anethole in children is reported, posing toxicological concern.

Special warnings and precautions for use

As a precautionary measure, the use of aniseed is not recommended in children under 12 years of age due to the lack of adequate data for safety assessment.

Preparations with high aniseed content (> 5 g) should not be taken for more than two weeks without medical advice.

Patients should seek medical advice if symptoms persist for more than two weeks or worsen during the use of the medicinal product.

Drug interactions

It has been suggested that anise might increase the risk of bleeding or potentiate the effects of anticoagulants. However, a single scientific article has been published reporting that “An *in vitro* assay of an aniseed methanolic extract 500 µg/ml showed an antiaggregant effect on human platelets (Okazaki *et al.*, 1998)”. Heck *et al.*, 2000 stated in his article entitled “Potential interactions between alternative therapies and warfarin” that anise "is thought to contain coumarin". However “there have been no documented case reports of an interaction of warfarin with aniseed". Thus only a potential interaction may be supposed although “caution could be useful when using anticoagulants (warfarin), antiplatelets or other substances or plants influencing blood coagulation”.

The quali-quantitative profile of coumarins in aniseed is not well known. The coumarins described in literature for aniseed are: bergaptene, scopoletine, umbelliferone and umbelliprenine (Newall *et al.*, 1996). None of these are known for "coumarin-like" actions (influence on the platelet aggregation) because they are furo- and hydroxycoumarins, while anticoagulant activity is bound to dicoumarole. For this reason no particular caution may be required.

In case of prolonged use or if excessive doses are ingested, the estrogenic activity of anethole may affect hormone therapy, including the oral contraceptive pill and hormone replacement therapy (see section 3.1. Overview of available pharmacological data- Estrogenic and antiestrogenic effects), but this potential interaction has not been confirmed by factual data.

Experiments carried out with laboratory animals indicate that concomitant intake of anise oil and medicinal products with action on the CNS may cause herb–drug interactions. However, this finding needs further clinical confirmation.

Use during pregnancy and lactation

There are no clinical studies available.

It is unknown if aniseed and anise oil constituents are excreted in human breast milk.

Estrogenic activity (see section 3.1. Overview of available pharmacological data - Estrogenic and antiestrogenic effects) and antifertility and foetal cell toxicity effects (see section 3.3 Overview of available toxicological data – Reproductive toxicity) have been shown for trans-anethole (the major constituent of the anise oil) in rats.

In view of the above-mentioned data, as a precautionary measure, anise oil and aniseed extracts should not be used during pregnancy and lactation.

In the absence of sufficient data, the use of aniseed and aniseed preparations during pregnancy and lactation is not recommended.

No fertility data are available.

Overdose

Ingestion of 1 to 5 ml of anise oil was associated with nausea, vomiting, seizures and pulmonary oedema (Newall *et al.*, 1996).

A 12-day-old infant, who had unintentionally received multiple doses of undiluted anise oil as a treatment for colic, was reported at the Paediatric Emergency Department with generalised tonic-clonic seizures. A complete blood cell count, electrolytes, spinal fluid analysis with culture, blood cultures, CT Scan of the brain, and EEG were all normal. No further seizure activity was noted after admission to the hospital. The infant subsequently recovered with no further sequelae reported (Tuckler *et al.*, 2002).

5.6. Overall conclusions on clinical safety

No data available

6. Overall conclusions

The traditional uses of aniseed for *"dyspeptic complaints such as mild, spasmodic gastro-intestinal ailments, bloating and flatulence" and "catarrh of the upper respiratory tract"* are supported mainly by experimental data and by experts opinion, while no clinical data are available.

The medicinal use of aniseed is largely due to antispasmodic, secretolytic, secretomotor and

antibacterial effects of its essential oil.

Pharmacological data show a significant relaxing effect of aniseed alcoholic extracts and essential oil on tracheal and ileal smooth muscles contracted by several contraction-inducing agents (e.g. metacholine and carbachol).

The above-mentioned effects are also likely to play a beneficial role in the treatment of inflammation of mucous membranes of the upper respiratory tract. Moreover, this indication is also made plausible by the secretolytic and expectorant effects exhibited by anethole, a main component of anise oil.

Lastly, when considering the plausibility of the above indications, particularly with reference to the inflammation of mucous membranes of the upper respiratory tract, bloating and flatulence, the likely role of a number of compounds detected in aniseed and very active in inhibiting growth of pathogenic bacteria and fungi should not be underestimated.

On the basis of long-standing use and experience, the HMPC recommends the following traditional-use indications for aniseed and anise oil: *"Traditional herbal medicinal product*

i) for symptomatic treatment of mild, spasmodic gastro-intestinal complaints including bloating and flatulence,

ii) used as an expectorant in cough associated with cold"

The above recommended indications are exclusively based upon long-standing traditional use of aniseed and not on clinical trial data.

No other traditional medicinal uses of aniseed are supported by adequate data.

Estrogenic activity described for trans-anethole is not confirmed for aniseed alcoholic extracts on the basis of epidemiological data related to the common use of aniseed alcoholic beverages. Therefore it is not expected that trans-anethole could exert estrogenic effects when taken as a herbal infusion at the recommended posology.

Also in case of use of anise oil the data on estrogenic activity and antifertility activity of trans-anethole demonstrated *in vitro* and in laboratory animals at high concentrations are not considered relevant to human exposure given the recommended posology and conditions of use (short term use only in adults and elderly).

Ethanolic aniseed extracts are mutagenic at high concentrations and results from studies carried out in the laboratory animals showed a weak mutagenic potential of anethole. However an aniseed extract prepared with water was tested in an Ames test on *Salmonella typhimurium* strains TA98, TA100, TA102 and turned out as negative; trans-anethole is reported as "generally recognised as safe" (GRAS) at the intake of 54 μg/kg b.w./day) and the acceptable daily intake is about 0.2 mg/kg b.w.

Several studies have shown the carcinogenic effects of estragole and some of its metabolites in mice (mainly malignant liver tumours). The EMEA/HMPC 'Public statement on the use of herbal medicinal products containing estragole' (EMEA/HMPC/137212/2005) states that the profiles of metabolism, metabolic activation and covalent binding of estragole are dose-dependent and tend to markedly decrease at low levels of exposure. The genotoxic risk related to estragole is not considered to be relevant for adults and elderly in the recommended dosage due to the small amount present in anise oil. However the risk cannot be calculated with high doses or prolonged use or in children.

An anti-tumour activity of anethole has also been reported (see section 3.1 Overview of available pharmacological data).

Considering the above-mentioned data and all the uses of aniseed, it is concluded that human exposure resulting from short term use of aniseed-based medicinal products, complying with the proposed specifications, is unlikely to pose any significant cancer risk. However, the use of aniseed in children under 12 years of age is not recommended due to the lack of adequate data for safety assessment.

The content of estragole in anise oil does not pose concern in adult and elderly, because the intake with traditional herbal medicinal products, given the specified condition of use, can be considered negligible compared to the background exposition to foods and beverages containing anise. The use in children and adolescents under 18 years of age is contraindicated because of concerns due to the presence of estragole and because of the lack of adequate data to evaluate the safe use in this population taking into consideration also to the presence of anethole.

A list entry for aniseed is proposed only for adolescents over 12 years, adults and elderly, considering the small amount of estragole and constituents of essential oil present in herbal infusions prepared from aniseed.

Annex

List of references