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COMMITTEE ON HERBAL MEDICINAL PRODUCTS (HMPC)

DRAFT

ASSESSMENT REPORT ON *PIMPINELLA ANISUM* L.

DISCUSSION IN THE HMPC	
ADOPTION BY HMPC	

KEYWORDS	Herbal medicinal products; HMPC; Assessment report;

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Draft Assessment Report
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PIMPINELLA ANISUM L. (Aniseed and Anise oil)

Botanical species and variety	***Pimpinella anisum* L.**
Botanical family	***Apiaceae (*Umbelliferae)**
Botanical synonyms	***Anisum vulgare* Gaertn** ***Pimpinella aromatica* Bleb**
Common names	**Anise, Sweet cumin — English** **Anis vert, Petit anis, — French** **Anis d'Europe — French** **Anis, Aneis, Brotsame, — German** **Süsser Kümmel, — German** **Tauben-anis — German** **Anice verde (Anice vero) — Italian** **Anis verde, — Spanish** **Hierba dulce, — Spanish** **Matafaluga — Spanish**
Part of the plant	**Fruit (whole cremocarp)**
Pharmaceutical preparations	**Herbal drug or herbal drug preparations in solid or liquid dosage forms**
Rapporteur country	**Italy**
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I. Introduction

This assessment report reviews the available scientific data for aniseed (*Pimpinella anisum* L.). Aniseed belongs to the *Apiaceae* (Umbelliferae) botanical family. The material of interest for medicinal use is the fruit (i.e. whole cremocarp). This herbal drug is administered after crushing in solid or liquid dosages.

In preparing this report, a number of data sources have been taken into account. The main ones are as follows:

- The ESCOP monographs published in 2003.
- The results of a literature search carried out in mid 2005 by the Italian National Institute of Health in Pubmed.
- 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 AESGP at the end of 2005.
- The monographs on anisi fructus present in the current (5th edition) of the European Pharmacopoeia and that on anise oil ("A*nisi aetheroleum*") present in the 2th edition of the European Pharmacopoeia.
- The Council of Europe Monograph on *Pimpinella anisum* as a cosmetic ingredient (2002);
- The Monograph on *Pimpinella anisum* published in Teuscher et al (2005).
- 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 at the end of 2005 on phytovigilance data banks available on internet (i.e. www/farmacovigilanza.org; www.epicentro.iss.it/focus/erbe/sorv_piante_officinali.htm).

Chrushed aniseeds as infusions have found traditionally use for the treatment of a variety of symptoms including:

- Dyspeptic complaints, a broad range of adverse symptoms including, among others, 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 secrection, cleared by pneumocyte cilia, consists mainly of flaked away epithelial cells, microrganisms and mononuclear cells.

These indications are substantiated mainly by empirical data deriving from investigations carried out into the constituents and their pharmacology, while no clinical data are available.

II.Clinical Pharmacology

II.1.1. Phyto-chemical characterization

Aniseed is characterized by a content of essential oil not lower than 20 ml per kg anhydrous fruit.

The essential oil, obtained by steam distillation of crushed fruits, varies between 1.5% and 6% and contains mainly trans-anethole (80-95%) (Hänsel et al,. 1994; Schultze et al., 1987); differently by the fennel essential oil, the aniseed 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). Differently by the fennel essential oil, the aniseed oil does not contain appreciable amounts of fenchone and also contains esquiterpene and monoterpene hydrocarbons are also present (Kubeczka et al., 1976; Schultze et al., 1987; Burkhardt G et al., 1986) with a variety of other compounds including linalool and beta-farnesene (Harborne et al., 1969; Becker, 1970; Kleimann et al., 1988) (for some examples see Table 1). The quality of aniseed oil depends upon the absence anethole oxidized forms such as anisaldehyde, aniseketone and anisic acid (Zackso-Szasz and Szasz, 1966). Aniseed stored in different conditions was evaluated for deterioration in terms of trans-anethole, anisaldehyde and other compositional characteristics by Guneyli and 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.

Yield and quality of the oil obtained by supercritical fluid extraction and steam distillation were compared by Ondarza and Sanchez, 1990; 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%), gamma-himachalene (2-4%), p-anisaldehyde (<1%), estragole (0.9-1.9%, *cis*-pseudoisoeugenyl 2-methylbutyrate and *trans*- pseudoisoeugenyl 2-methylbutyrate (Rodrigues et al., 2003).

Other constituents include flavonol glycosides (El-Moghazi et al., 1979; Kunzemann and Herrmann, 1977), phenolic acid (Schulz and Herrmann, 1980; El-Wakeil et al., 1986), a phenolic glucoside (Dirks and Herrmann, 1984a; Dirks and Herrmann, 1984b), furocoumarins, mainly bergaptene (Ceska et al., 1987; Kartnig and Scholz, 1969), hydroxycoumarins, mainly umbelliferone (Hänsel et al,. 1994) and fixed oil (Kartnig and Scholz, 1969) and lipids, manly constituted of petroselinic acid (Van Loon, 1973).
Twelve new and 5 known glucosides of phenyl-propanoids,including 4 stereoisomers of anethole glycol 2'-O-beta-D-glucopyranoside and 4 stereoisomers of l'-(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, 2002a and 2002b).

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

Table 1- Compounds identified in essential oils obtained by steam distillation from anisi fructus (+) (++) (+++)

Compound	Aniseed
Trans-anethole	76.7- 93.0%
Estragole	0.5- 6.1%
Anisaldehyde	0.1- 3.5%
Linalol	0.1- 1.5%
Alpha-terpineol	0.1-1.5%
Cis-anethole	<0.5%

(+) Monograph on anise fruit oil (European Pharmacopeia-2th 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* oil at various harvest times have been studied by 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. A separate monograph is prepared for anise oil.

II.2 Absorption, metabolism and excretion

No data available for aniseed in human beings or animals.

After oral administration of radioactively-labelled trans-anethole (as the *methoxy-"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 "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 "C) was 4-methoxyhippuric acid (Sangster et al., 1984b; Caldwell and Sutton, 1988). Trans-anethole, is metabolized 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 metabolized by O-demethylation and by oxidative transformation of the C3-side chain. After low doses (0.05 and 5 mg/kg body weight) O-demethylation occurs predominantly, whereas higher doses (up to 1500 mg/kg body weight) give rise to higher yields of oxygenated metabolites (Sangster et al., 1984a; Sangster et al., 1984b) .

II.2. Pharmacodynamics

II.2.1. Mode of action

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 antagonizing several contraction-inducing agents.

In the isolated tracheal smooth muscle from guinea pig, the aniseed essential 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 and Brandt, 1985).

The relaxant effect of aniseed essential oil (0.02 ml), aqueous extract (0.6 ml equivalent to 1.5 g of aniseed) and ethanol extract (0.1ml equivalent to 0.25 g of aniseed) on methacholine pre-contracted isolated tracheal chains of guinea pig has been studied by Boskabady and Ramazani-Assari (2001). A statistically- significant bronchodilatory effect of the essential oil ($p<0.05$), acqueous extract ($p<0.005$) and ethanol extract ($p<0.001$) was detected.

Anise oil, at a dose of 0.3 ml/kg b.w., prevented decrement 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 and Aviado, 1970).

Anethole (10 to 25 ml/l of physiological solution in which an isolated mouse intestinal jejunum is

plunged) (Imaseki and Katabatake, 1962) induced intestinal motility at low concentrations, but an intestinal relaxation was observed at concentrations higher than 50 ml/.l

- *Secretolytic and expectorant effects*

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

A similar experiment in anaesthetized rats, orally dosed with the 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 and Leusink, 1953).

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

An increase of about 12% in mucociliary transport velocity was observed in isolated ciliated epithelium from the frog oesophagus 90 seconds after application of 200 /ul of an infusion from aniseed (4.6 g per 100 ml of water) (Muller-Limmroth and Frohlich, 1980).

Anethole and fenchone vapours were given by inhalation to urethanized rabbits as doses from 1 to 243 mg/kg body weight added to the steam vaporizer (the amount actually absorbed by the animals being considerably less, estimated as not more than 1% of that added to the vaporizer). 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. (Boye and Sheppard, 1971).

A water extract of a mixture of herbs including anise, was tested for its inhibitory effect on histamine released from rat peritoneal mast cells stimulated either by compound 48/80 or be IgE/anti-IgE. The effect of the herb mixture 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. The clinical results 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).

Changes in the symptoms of cough after treatment with a combined herbal preparation containing dry ivy leaf extract as main active ingredient, decoction of thyme and aniseed, and mucilage of marshmallow root and its tolerability were investigated in an open clinical trial 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.

- *Oestrogenic and anti-oestrogenic effects*

Aqueous extracts of *Pimpinella anisum* seed and from flowers of *Sideritis euboea and clandestina* and *Matricaria camomilla*, at a concentration range between 10-100 µg/ml, have been found to be active *in vitro* in stimulating the differentiation and mineralization of osteoblastic cell culture and inducing, like antiestrogens, the insulin growth factor binding

protein 3 (IGFBP3) in MCF-7 breast cancer cells, whereas no effect was observed on the proliferation of cervical adenocarcinoma (HeLa) cells by use of MTT assay (a laboratory test for measuring cellular proliferation) ((Kassi et al., 2004).The presence of estradiol inhibited the antiestrogenic effect, thus suggesting an estrogen receptor-related mechanism.

Trans-anethole administered orally to immature female rats at 80 mg/kg body weight 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 oestradiol valerate subcutaneously at 0.1 pg/ rat/day ($p<0.001$). The results confirmed that *trans* anethole has oestrogenic activity; other experiments showed that it has no anti-oestrogenic, progestational, anti-progestational, androgenic or anti-androgenic activity (Dhar, 1995).

Oestrogenic 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.

- *Antimicrobial effects*

Aniseed extracts and oil as well as some oil components, 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.

An acetone extract of aniseed 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, 1959).

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 (Mueller-Riebau et al., 1995).

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

Aniseed essential oil inhibited the growth of *Escherichia coli* (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 has also been demonstrated in other studies (Ramadan et al., 1972; Ibrahim and Ogunmodeli, 1991; Shukla and Tripathi, 1991; Okuyama et al., 1995; Sokmen, 1999).

A methanolic extract of aniseed exhibited *in vitro* an antibacterial activity against *Helicobacter pylori* at concentrations of 50 and 100 /u/ml (Mahady et al., 2000).

A methanol dry extract of aniseed reduced the resistance of *Pseudomonas aeruginosa* to a series of antibiotics. When both the extract and the antibiotics were tested at concentrations individually unable to inhibit microbial growth, the aniseed extract, in combination with chloramphenicol, gentamicin, cephalexin, tetracycline or nalixidic acid caused almost complete inhibition of growth of the standard strain of *P.aeruginosa* (Aburjai et al., 2001).

The essential oils of anise (500 ppm), fennel (2000 ppm) and other herbals showed a dose-dependent inhibitory effect on the growth of tested fungi including *Aspergillus flavus, A. parasiticus, A. ochraceus* and *Fusarium moniliformis* (Farag et al.,1989; Hasan,1994; Soher, 1999; Soliman and Badeaa, 2002). Moreover, anise oil also inhibited production of aflatoxins, Ochratoxin A and Fumosin in inoculated wheat samples (Soliman and Badeaa, 2002).

Bactericidal activities of a number of plant essential oils, including the aniseed fruit one, and of their isolated constituents were tested against *Campylobacter jejuni*, *Escherichia coli*, *Listeria*

monocytogenes and *Salmonella enterica* (Friedman et al, 2002). Aniseed oil was shown to reduce bacterial activity of all tested bacteria (*C. jejuni* > *L. monocytogene*s > *S. enterica* = *E. coli*). As far as the antibacterial activity of 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.*

The aniseed and fennel oils was found to have a high antibacterial activity against *Staphilococcus aureus* (responsible for bases, sepses and skin infections), *Streptococus haemoliticus* (causing infection of the throat and nose), *Bacillus subtilis* (infection in immunecompromised patients), *Pseudomonas aeruginosa* (causing hospital acquired infection), *Escherichia coli* (responsible for urogenital tract infections and diarrhea), *Klebsella species* and *Proteus vulgaris* (Singh et al., 2002).

Antimicrobial activity of both water and ethanol extracts of *Pimpinella anisum* fructus was tested against *Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Citrobacter koseri, Staphylococcus aureus, Streprococcus Pneumoniae, Enterobacter aerogenes, Micrococcus luteus, Staphylococcus Epidermidis and Candida albicans* (Gulcin et al.,2003). Most micro-organisms were inhibited, but no not activity of the water anise fructus extract was detected against*Pseudomonas aeruginosa* and *Escherichia coli.*

- *Anti-tumour effects*

See Section IV.1.1.

- *Local anaesthetic activity*

Trans-anethole concentration-dependently reduced electrically-evoked contractions of rat phrenic nerve-hemidiaphragm, by 10.3% at 1 pg/ml, by 43.9% at 10 pg/mI, by 79.7% at 100 pg/ml and by 100% at 1000 pg/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 and Lichtenstein, 1982).

- *Other effects*

The fruit essential oil of *Pimpinella anisum* given intraperitoneally significantly ($p < 0.001$) and dose-dependently counteracted convulsivant effects induced in male mice by injection of phenylenetetrazole or by electroshock. The ED50 of anise essential oil was 0.52 (0.35 to 0.76) milliliters/kilogram and its efficacy was lesser than i.p. ethosuximide and phenytoin (Pourgholami et al., 1999).

An aqueous extract of aniseed exhibited a weak *in vitro* cytotoxic activity against melanoma cells (Sathiyamoorthy et al., 1990).

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

A weak antiaggregant effect of human platelets was detected by testing *in vitro* an aniseed methanolic extract at a concentration of 500 μg /ml (Okazaki et al., 1998).

An aniseed water extract did not show any activity when tested *in vitro* on the activity of Na^+-K^+-ATPase from rat jejunum (Kreydiyyeh et al., 2000).

Tunc et al. (2000) studied the fumigant activity of the essential oils of *Pimpinella anisum* and other herbals against eggs of two stored products insects and found 100% mortality of the exposed eggs.

The effects of fruit essential oil of the *Pimpinella anisum* on the acquisition and expression of morphine-induced conditioned place preference in mice were studied by Saharei et al (2002) who concluded that the aniseed oil may reduce the morphine effect via a GABAergic mechanism.

Aniseed oil enhanced significantly in dose dependent manner glucose absorption from the rat perfused jejunum and increased the Na^+-K^+-ATPase activity in jejunal homogenate, but did not affect water absorption from the perfused colon and the activity of the Na^+-K^+- ATPase in the colon. When added for 24 h to drinking water, aniseed 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%) (Kreydiyyehhh et al., 2003).

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

III. Clinical efficacy

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

III.1 Dosage

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., 2000; Czygan and Hiller, 2002; Dorsch et al., 2002).

For aniseed fruit

Adult and children over 12 years:
For asymptomatic treatment of mild, spasmodic gastro-intestinal complaints including bloating and flatulence and as expectorant in cough and cold, 3,5 g of crushed anise fruits as a herbal tea.
A single dose 2-3 times daily. A single dose consists of 1-5 g of crushed fruits in 150 ml of water as a herbal tea. (ESCOP, 1996-99).
Posology for the tincture is not available. In a herbal textbook 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 RF 1985). For asymptomatic treatment of mild, spasmodic gastro-intestinal complaints including bloating and flatulence and as expectorant in cough and cold, 0.05-0.2 ml of anise oil, three times daily (British Herbal Pharmacopoiea 1983).

The use in the paediatric age is not recommended for the presence of estragol, whose exposure should be minimised in young children.

***Duration of administration*:** no restriction for herbal tea. On the basis of lack of clinical trials, preparations with high aniseed content (> 5 g) should not be taken for more than two weeks without medical advice.

***Method of administration*:** For oral administration.

<u><If the symptoms persist or worsen, a doctor or a qualified health care practitioner should be consulted></u>

Preparations marketed in Europe

No authorised/registered products are on the market in the following European countries: Austria, Belgium, Czech Republik, Ireland, Italy, Netherland, Portugal, Finland and Norway.
Herbal teas are authorised in Germany and France; an aqueous extract (oral liquid) is authorised in UK. The oldest MA is dated 1986 for the herbal tea (GE) and 27.04.1992 for the aqueous extract.
Anise oil is authorised in Germany (soft capsules) and UK (lozenges and syrup). The oldest MA is dated 01.10.1987
Various fixed combinations are authorised/registered in different European Countries.
Food supplements are on the market.

III.2 Clinical studies

Clinical trials

No data

III.3 Clinical studies in special populations

Use in children

No data.

A 12-day-old infant, who had unintentionally received multiple doses of undiluted aniseed oil as a treatment for colic, was reported at the Pediatric
Emergency Department with generalized tonic-clonic seizures. A complete Lood 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 subsequenrtly recovered with no further sequelae reported (Tuckler et al., 2002).
No metabolic data for anethole in children are reported. Therefore, as a precautional measure, aniseed is not recommended in children

Use during pregnancy and lactation

There are no clinical studies available.

Oestrogenic activity (see Section II.2.1) and antifertility and fetal cell toxicity effects (see Section IV.2) have been shown for trans-anethole (the major constituent of the aniseed essential oil) demonstrated in rats.

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

No data are available that would raise concern in relation to the use of aqueous anise infusions during pregnancy and lactation at the recommended dosages.

III.4 Traditional use

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; Czgan, 1992; Hansel et al., 1994; European Pharmacopoeia, 1997; Weiss, 1997, Wichtl, 1999. British Pharmacopoeia, 1999; Hansel et al 1999; Czygan and Hiller, 2002; Sweetmann, 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 (Bisset, 1994) as well as to neurologic disorders (Aboabrahim, 1970).

In the traditional system of Indian medicine, aniseed is used as antiseptic, stomachic, carminative, stimulant and to prevent flatulence and colic (Chopra et al., 1956).

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 and Bye, 1987; Scholz, 1998; Teuscher et al., 2005).

Plinius the old (23-79 after Christ) already described some effects of aniseed, which could be presently described as oestrogenic effects.

The Treaty "Farmacologia Teorica e Pratica", also named "Farmacopea Italiana" di Giuseppe Orosi (1851- Vincenzo Mansi Ed.-Livorno) lists anise fruit in the Materia Medica Botanica Charter (p.35).

Use of aniseed products (tincture) as an expectorant in cough and cold is not supported by scientific data, however, it is described in traditional medicine (Weiss RF 1985).

Safety

IV.1 Genotoxic and carcinogenic risk

IV.1.1 Preclinical data

Mutagenicity and carcinogenicity

- Aniseed extracts

An 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 TA98 (a frameshift mutation test), TA 100 (a base-pair substitution mutation test) and TA102 (an oxidative mutation test) (Al-Bataina et al., 2003).

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

A dry ethanolic aniseed extract was mutagenic at high concentrations (5 mg/plate) to streptomycin-dependent of *Salmonella typhimurium* TA 98 (Shashikanth and Hosono, 1986).

An ethanolic 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 (Ishidate et al., 1984).

- Anethole

From a series of studies in mice, Miller et al (1983) concluded that anethole added to female CD-1 mice diet or given orally or by i.p. injection to male pre-weanling B6C3F1 mice was not 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 rats, administered 0.25; 0.5; and 1.0% anethole in the diet for 121 weeks 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 histological changes to the liver as in those observed after enzymes inducers (Newberne et al.1989) and were considered as not caused by a direct genotoxic effect of trans-anethole (Lin, 1991). Reed et al. (1992) also showed that i.p. dosing of SD rats with anethole increased liver weight, microsomal protein and cytochrone P-450 content.

In nine Salmonella studies to detect base-pair substitutions or frameshift mutations without metabolic activation, anethole was uniformly negative and this was also the case in four studies with activation after careful consideration of all experimental conditions (Heck et al., 1989; Hsia et al., 1979; Marcus and Liechtenstain, 1982; Mortelmans et al., 1986; Nestmann et al., 1980; Sekizawa and Shibamoto, 1982; Swanson et al, 1979; To et al., 1982). The four findings suggesting a weak mutagenic potential of anethole (Marcus and Liechtenstain,1982; Swanson et al, 1979; Mortelmans et al., 1986; Sekizawa and Shibamoto, 1982; Lin, 1991) 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 and Marshall and Caldwell, 1996).

Anethole was found to be mutagenic in the mouse lymphoma assay which is known for its extreme sensitivity and poor selectivity for genotoxicity (Gorelik, 1995; Heck et al., 1989; Caldwell, 1993; Casciano et al., 1992).

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

A mouse micronucleus assay was negative, with no micronuclei found at 6 and 30 hours after anethole administration (Marzin, 1979). Similarly, in the mouse bone marrow micronucleus test, oral pre-treatment of mice with trans-anethole at 40-400 mg/kg body weight 2 and 20 hours before genotoxins, no significant increase in genotoxicity was observed (Abraham, 2001).

Very low levels of DNA adducts were evidenced 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; Muller 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 and 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 and 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 and Caldwell, 1996).

In 1999 the USA Expert Panel of FEMA (Flavour and Extract Manufacterers' 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 can be "generally recogninized as safe" (GRAS) at low level of intake (54 μg/kg body weight/day) (Newberne et al., 1999).

In the 51st meeting of the Joint FAO/WHO Expert Commitee 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-cinical studies in rats. The report of JECFA allocated the acceptable daily intake (ADI) at the dose of 0-2 mg/kg body weight on the basis of scientific pre-clinical data published on trans-anethole (JECFA, 1999).

- Estragole

Estragole, a minor constituent of aniseed 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 (EMEA/HCMP, 2005). Moreover, estragole or its metabolite, 1'-hydroxyestragole, administered to mice binds readily to DNA and several DNA adducts have been characterized. Several studies have shown the carcinogenic effects of estragole in mice (mainly malignant liver tumors); moreover, 1'-hydroxyestragole and other metabolites and synthetic derivatives were shown to be potent carcinogens in mice (EMEA/HMPC, 2005).

The EMEA/HMPC (2005) assessment is 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 studies indicate that these events are probably minimal in the dose range 1-10 mg estragole/kg b.w., which is approximately 100-1000 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.

IV.1.2. Clinical data

No data available.

IV.1.3 Conclusion

The human exposure resulting from consumption of aniseed-based herbal medicinal products (short time at recommended posology) does not pose significant cancer risk (see also the data below on anti-tumor and anti-oxidant activity of anethole).

- *Anti-tumor 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 1000 mg/kg) and body weight ($p<0.05$ to 0.01) compared to EAT-bearing controls. Mean survival time increased from 54.6 days to 62.2 days (500 mg/ kg) and 71.2 days (1000 mg/kg). Histopathological changes were comparable to those after treatment with cyclophosphamide (a standard cytotoxic drug). 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).

In the mouse bone marrow micronucleus test, oral pre-treatment of mice with trans-anethole at 40-400 mg/kg body weight 2 and 20 hours before intraperitoneal injection of genotoxins led to moderate, dose-dependent protective effects against known genotoxins such as cyclophosphamide, pro-carbazine, N-methyl-N'-nitrosoguanidine, urethane and ethyl methane sulfonate ($p<0.05$ to $p<0.01$ at various dose levels). No significant increase in genotoxicity was observed when trans-

anethole (40-400 mg/kg body weight) was administered alone (Abraham, 2001).

- *Antioxidant activity*

An activity of aniseed oil and many other essential oils was observed *in vitro* in inhibiting copper-catalyzed oxidation of human Low-Density Lipoproteins (LDL); such an activity correlated well with total phenol content of the oil (Teissedre and Waterhouse, 2000).

The antioxidant properties of water and ethanol extracts of anisi fructus were shown by using different antioxidant tests, including reducing power, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, and metal chelating activities.In general the water extract exhibited greate antioxidant activity that that of the ethanol extract (Gulcin et al., 2003).

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

IV.2. Toxicity

Acute toxicity

Oral lethal dose of aniseed oil has been reported for human beings to be in the range from 50 to 500 mg/kg b.w. (Gosselin et al., 1984).

Oral LD50 values per kg b.w. have been 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).

Intraperitoneal LD_{50} values for trans-anethole have been determined as 0.65-1.41 g/kg in mice and 0.9-2.67 g/kg in rats (Lin, 1991). Anethole activates the central nervous system and its excessive use may lead to convulsions (Zagari, 1991).

Subchronic toxicity

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 between 0.3 and 3.0% (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).

Reproductive toxicity

Trans-anethole exerted dose-dependent anti-implantation activity after oral administration to aduIt female rats on days 1-10 of pregnancy. Compared to control animals (all of which delivered normal offspring on completion of term), trans-anethole administered at 50, 70 and 80 mg/kg body weight inhibited implantation by 33%, 66% and 100% respectively. Further experiments at the 80 mg/kg dose level showed that in rats given trans-anethole only on days 1-2 of pregnancy normal implantation and delivery occurred; in those given anethole only on days 3-5 implantation was completely inhibited; and in those given *trans*-anethole only on days 6-10 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).

IV.3 Contraindications

Persons with known sensitivity to Umbelliferae or Compositae or to anethole should avoid the use of aniseed. 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 diarrhea. (Jensen-Jarolim et al, 1997; Garcia-Gonzalez et al., 2002).

IV.4 Special warnings / precautions

Aniseed's oil and alcoholic extracts should not be used during pregnancy and lactation because scientific evidence for the safe use in these conditions is not available.

If excessive doses are ingested, the oestrogenic activity of anethole may affect hormone therapy, including the oral contraceptive pill and hormone replacement therapy.

Anethole is structurally similar to myristicin, consumption of large amounts of aniseed may cause neurological effects similar to those documented for nutmeg.

Persons with known sensitivity to Umbelliferae or Compositae or to anethole should avoid the use of aniseed.

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

Patients should seek medical advice if symptoms persist for more two weeks or worsen upon administration of the drug.

IV.5 Undesirable effects

The allergenic potential of aniseed is relatively weak and it shows up occasionally with allergic reactions at dermal, respiratory and gastro-intestinal level (Stricker et al., 1986; Wuthrich and Dietsch, 1985; G39; Kommission E, BAnz 122 von 06.07.1988, 1988; Fraj et al., 1996; Garcia-Bravo et al., 1997; Garcia Gonzalez et al., 2002; Keller K. 1992). The molecular weights of the main immunoglobulin (Ig)E 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).

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. The content of furocumarins in aniseed extracts is under study (Vincieri, personal communication).

Toxic syndromes may result in infants from ingestion of aniseed oil.

IV.6 Interactions

Theoretically, anise might increase the risk of bleeding or potentiate the effects of anticoagulants. because its coumarin constituents. However, a single scientific article has been published describing "An in vitro assay of an aniseed methanolic extract 500 µg/ml showed an antiaggregant effect on human platelets (Okazaki et al., 1998)". Moreover, both the qualitative and quantitative

profile of coumarins in aniseed is not well known. Therefore, caution is necessary in case of use of drugs anticoagulant (warfarin) or antiplatelet agents or others substances or plants influencing blood coagulation (Heck et al, 2000).

Herbs with anticoagulant or antiplatelet properties may increase the risk of bleeding when combined with anise.

Excessive doses may affect hormone therapy or oral contraception.

If the patient is on other medication, he/she should seek medical advice.

IV.7 Overdose

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

VI. Overall Conclusion

The traditional indications for aniseed on *"Dyspeptic complaints such as mild, spasmodic gastro-intestinal ailments, bloating and flatulence. Catarrh of the upper respiratory tract"* are supported only 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 anise alcoholic extracts and essential oil on tracheal and ileal smooth muscles contracted by several contraction-inducing agents.

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 components 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, there should no underestimation of the likely role of a number of compounds detected in anise fruit being very active in inhibiting growth of pathogenic bacteria and fungi.

An improved version of the above indications could be as follows: *"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 and cold"

However, these indications are mainly based upon long-standing use and the almost complete lack of clinical trials must be highlighted.

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

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