European Medicines Agency
Post-authorisation Evaluation of Medicines for Human Use

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OVERVIEW OF COMMENTS RECEIVED ON 'COMMUNITY HERBAL MONOGRAPH ON *PIMPINELLA ANISUM* L., FRUCTUS ' (EMEA/HMPC/137423/2006)

Table 1: Organisations that commented on the document as released for consultation on 7 September 2006 until 2 January 2007

	Organisation
1.	Association of the European Self-Medication Industry (AESGP)
2.	Bundesinstitut fur Arzneimittel und Medizinprodukte (BfArM), Germany
3.	Kooperation Phytopharmaka, Germany

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Table 2: Discussion of comments

General comments	Comment and rationale	Outcome
	In principle the preparation of the Community herbal monograph is welcomed. However, we are of the opinion that these drafts need some improvement because particularly the assessment of potential risks (e.g. of trans-anethole) cannot be deduced from scientific literature.	Agreement that the assessment of potential risks (e.g. trans-anethole, but also estragole) cannot be fully deduced from the existing scientific literature. The lack of complete safety suggests caution in using aniseed preparations in sensitive population groups such as children and women during pregnancy and lactation.

Line no or section and paragraph no	Comment and rationale	Outcome
2. Qualitative and quantitative composition	We suggest to add "Aniseed, crushed" under ii) b), because this is also a commonly used preparation.	Endorsed.
3. Pharmaceutical form	Herbal substance or herbal preparation as herbal tea for oral use	Endorsed.
4.1 Therapeutic indications	The term "Cough and cold" is not acceptable because it does not describes distinct the disease pattern of a simple, uncomplicated cold. By translating the phrase into other languages it turns out to describe coughs (including a lot of severe differential diagnosis) which are not desired. The following alternative wording is suggested for the indication (ii): Traditional herbal medicinal product for liquefaction of mucus in common colds.	Agreement with the need of better wording to describe the type of cough. Indication is modified as follows: Traditional herbal medicinal product used as an expectorant in cough associated with cold.

Line no or section and paragraph no	Comment and rationale	Outcome
4.1 Therapeutic indications	From our point of view, the following indications are suitable for a well-established medicinal use instead of a traditional use: • Dyspeptic complaints such as mild spasmodic gastrointestinal complaints, bloating, flatulence. • Catarrh of the upper respiratory tract. These indications are justified by the following references: BHP 1983, CZYGAN 1992 and 2002, HÄNSEL 1994, WEISS 2002. Well-documented clinical experience is available as well as supportive conclusive (human) pharmacological data which thus meet the requirements for the well-established medicinal use.	Published clinical data are insufficient to support the well established use. References mentioned by interested parties support the plausibility of the traditional use.
4.2 Posology and method of administration	For the **well-established medicinal use** we propose the same posology which is currently listed under "traditional use". These recommendations are justified by the references mentioned under "indications". In addition, we propose the following posology: i) **"3.5 g comminuted or crushed aniseed in 150 ml water as herbal tea, 2-3x daily".** This should be included under well-established use, and/or (in case the HMPC does not agree upon our proposal to describe a well-established use) under "traditional use". **"Freshly"** as well as footnote 2 should be deleted, because "freshly comminuted" is not a commercial preparation but prepared in pharmacies upon individual request. Furthermore, we propose (for the well-established medicinal use and/or for the traditional use) to **delete the statement: *"The use in children is not recommended due to the lack of adequate data for safety assessment".***	Not agreed. The well established use is not supported by sufficient scientific data. Not agreed. The essential oil is considered to be responsible of the activity of aniseed. Therefore the herbal preparation should be "freshly" comminuted, in order to limit the loss of the essential oil. Moreover its content in the commercial preparations must be controlled.

Line no or section and paragraph no	Comment and rationale	Outcome
4.2 Posology and method of administration	Reasons: Aniseed has been used for centuries both as spice and herbal medicinal product. Its therapeutic use is dedicated to diseases of the intestinal and respiratory tract. For both fields of indication the use of aniseed preparations is common in adults as well as in children. Aniseed is applied mainly in the form of a herbal infusion, alone or in combination with other herbs like, e.g., fennel, caraway, liquorice, peppermint and others. Aniseed is as well used in food, mainly as spice or infusion. It is a popular and very common infusion for babies and infants. Aniseed is the subject of various acknowledged national and international monographs (Kommission E 1988, ESCOP 2003). In these monographs there is no restriction of age (Kommission E), or a dose regimen for children is even explicitly recommended (ANDERSON et al., 1996, ESCOP 2003, CZYGAN 2002, DAB10 comment). Although there is hardly any clinical data on the tolerability of aniseed preparations in children, however, it cannot be neglected that the combination of aniseed with fennel and/or caraway is **the most popular remedy** which mothers in many parts of Europe have used for decades or even centuries both as a lactagogue for themselves and as a mild treatment for intestinal spasms of babies and small infants. It is highly unlikely that mothers would stick to using aniseed or fennel preparations for their children if these preparations had any observable adverse effects. Therefore, although data from prospective studies are lacking, it is highly reasonable to consider aniseed infusions as being safe and well-tolerated by even small babies.	Not agreed. Despite the fact that aniseed has been used for centuries, both as a spice and as a herbal medicinal product, there is not adequate data for safety assessment. On the contrary to comments provided estragole is a constituent of aniseed known to be mutagenic/carcinogenic according to non-clinical data and no vertical study exists investigating its use in relation to the occurrence of severe diseases such as genetic diseases or tumours.
	The question if aniseed infusion may have undesirable effects under repeat dose conditions is discussed in section 5.3. We propose **"no restriction"** because a restriction to two weeks cannot be deduced from preclinical data (see 5.3.).	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 a traditional herbal medicinal product. Therefore the statement 'If symptoms persist or worsen after two weeks it is necessary to consult a doctor' should remain in the monograph.

4.2. Posology and method of administration	The single dosage for indication i) is 3.5 g and a second single dosage with 1-5 g is given for indication ii). The dosage recommended by the Commission E is "daily dosage 3 g", Standardzulassung "2 times daily 1.5 g" and BHP "thrice daily 0.5-1 g dried fruits". Furthermore the recommended dosage in the HAGER-ROM2004 is 3 g as daily dosage with reference to the monograph of the Commission E. It is not clear, why the ESCOP reflects to the HAGERs handbook with 1-5 g. As a general comment, the daily dosage and the frequency of intake, respectively, needs to be clarified. We recommend changing the daily dosage in "3 times 1 g". Proposal: Adolescents over 12 years of age, adults, elderlv Single dose: indication i) and ii): 1 g of (freshly) comminuted or crushed aniseed in 150 ml of water as a herbal tea Up to 3 times daily.	A single dose of 1 g 3 times daily is recommended by the German Commission E. 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 FC 1992). The revised monograph confirms the adult average daily dose of 3 g. (ESCOP 2003: Czygan FC and Hiller K 2002, British Herbal Pharmacopoiea 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 and Leclerc. Czygan (1992) refers to the German Kommission E (1 g 3 times daily), but also to the Standardzulassung: 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 HMPC considers the following posology as usual in the practice: 1 to 3.5 g of whole or (freshly[2]) comminuted or crushed aniseed in 150 ml of water as a herbal tea.

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

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4.2. Posology and method of administration	Proposal: Because of the lack of data the use of aniseed is not recommended in children below the age of 12 years.	Data on safety in children are lacking and this aspect should be clear in the monograph. Cross-reference is made to section 4.4 Special warning and precaution for use
	The duration of administration should given with "No restriction", because the monograph only reflects on herbal teas. Method of administration “No special advice”	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 of a traditional herbal medicinal product.
4.3 Contraindications	Proposal of wording: Patients with known sensitivity to Apiaceae (Umbelliferae) (fennel, caraway, coriander and dill) or to anethole should not use aniseed and its preparations.	The statement has been modified according to the current revision of the procedure for the preparation of Community monographs for THMPs (EMEA/HMPC/182320/2005 Rev.2)
4.4 - Special warnings and precautions for use	Without progress the use of aniseed should not exceed 1 week. So therefore we suggest to give the advice concerning the use of aniseed with persisting symptoms in the monograph under special warnings. Proposal: If symptoms persist for more than 1 week or worsen during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.	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 a traditional herbal medicinal product, and is in agreement with the decision taken for the fennel monographs.

Line no or section and paragraph no	Comment and rationale	Outcome
4.6. Pregnancy and lactation	The 1st paragraph states: "There are no data from the use of aniseed in pregnant patients". We recommend replacing this sentence by the following: "Although clinical data on the safety of using aniseed preparations (infusions) in pregnancy are lacking, Aniseed may be used during pregnancy and lactation at the recommended dosage." Reasons: In this context we would like to refer to our comments on section 5.3. Furthermore, it seems inappropriate to include a contraindication for groups of patients in the absence of prospective data covering the use of a medicine in this group. It should also be considered which alternatives pregnant women do have to treat bloating and related intestinal symptoms (which they do frequently experience during pregnancy). It is reasonable to expect that if there were notable side effects of aniseed infusions in particular in pregnant women, this should have become apparent by respective reports in the literature or in pharmacovigilance systems especially when considering the close supervision of pregnant women by their doctors. Therefore, it would be useful that the HMPC considers an evaluation of pharmacovigilance data from Member States and the EMEA EudraVigilance data as well as the WHO database. In the 2nd paragraph the following wording is proposed by the HMPC: "Studies in animals have shown reproductive toxicity of trans-anethole (the major constituent of anise oil)". For the reasons given under section 5.3, we propose to delete this statement. The 1st paragraph states: "*There are no data from the use of aniseed in pregnant patients*". We recommend to replace this sentence by the following one: "*Although clinical data on the safety of using aniseed preparations (infusions) in pregnancy is missing, Aniseed may be used during pregnancy and lactation at the recommended dosage*".	Not endorsed. The sentences reported in the monograph are in agreement with the statements in annexes I and III of the 'Guideline on SPCs' and the template for a Community herbal monograph (EMEA/HMPC/107436/05 Rev. 3) See comments in section 5.3. The statement has been deleted. Not endorsed. The sentences reported in the monograph are in agreement with the statements in annexes I and III of the 'Guideline on SPCs' and the template for a Community herbal monograph (EMEA/HMPC/107436/05 Rev. 3)

Line no or section and paragraph no	Comment and rationale	Outcome
4.6. Pregnancy and lactation	Reasons: In this context we would like to refer to our comments on section 5.3. Furthermore, it has to be considered which alternatives pregnant women do have to treat bloating and related intestinal symptoms which they do frequently experience during pregnancy. In case remarkable side effects of aniseed preparations had occurred in pregnant women this would have become apparent by respective reports in the literature or in pharmacovigilance systems especially when considering the close supervision of pregnant women by their doctors. In the **2nd paragraph** the following wording is proposed by the HMPC: *"Studies in animals have shown reproductive toxicity of trans-anethole, the major constituent of anise oil"*. For the reasons given under section 5.3, we propose to **delete this statement**.	See comments in section 5.3. The statement has been deleted.
4.7. Effects on ability to drive and use machines	We propose to replace the current statement by "***No data available***."	Not endorsed The sentence is in compliance with the template for a Community herbal monograph (EMEA/HMPC/107436/05 Rev. 3)

Line no or section and paragraph no	Comment and rationale	Outcome
4.8. Undesirable effects	We suggest to delete "*and gastro-intestinal system*" because there are no reports available.	Endorsed.
5. Pharmacological properties	In the monograph a lot of information is given in chapter 5, additionally with the phrase concerning the article 15c (1)(a)iii) of the Directive 2001/83/EC. It should be clear, if special information is required or not. In our opinion additionally information is not needed to every point. From the information in chapter 5.1 only the first sentence could be given as follows: "The traditional medicinal use of aniseed is plausible on its antispasmodic, secretolytic and expectorant effects of its essential oil.". The information about the antibiotic effects can not be supported, because the examinations on antibiotic activity were not done with bacteria which are relevant for infections of the respiratory tract. Therefore this information is without relation to the claimed indication. This can be discussed in the assessment report. The information in chapter 5.2 is not needed, while in the chapter 5.3 only the last sentence is needed as information and should therefore given. In the chapters 5.1 and 5.3 the first sentence ("Not required …..") should be deleted.	HMPC agreed to delete all the information unless necessary for the safe use of the product. Information is given in the assessment report.
5.1 Pharmacodynamic properties	The traditional medicinal use of aniseed is plausible on its antispasmodic, secretolytic and expectorant effects of its essential oil.	HMPC agreed to delete all the information considering them not necessary for the safe use of the product. Information is given in the assessment report.
5.3 Preclinical safety data	The genotoxic risk related to estragole (EME/HMPC/137212/2005) is not considered to be relevant due to the small amount present in herbal infusions prepared from aniseed. In this section, the HMPC draft refers to studies performed with the isolated aniseed compound trans-anethole, in particular the study of DHAR (1995). However, further important references such as NEWBERNE et al. (1998), JECFA (1999), particularly the GRAS assessment of trans-anethole, are not discussed.	Endorsed for the specified conditions of use. The sentence referring to the dose dependent anti-implantation, early abortifacient and antifertility activity reported at high doses of trans-anethole in rats has been deleted from the monograph.

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5.3. Preclinical safety data	**Studies on reproduction/developmental studies** In the study of DHAR (1995), 50, 70 or 80 mg/kg trans-anethole (not defined) were given on day 1-10 of pregnancy (n=6/treatment), a reduction of the number of the implantations sites by 33, 66 or 100 %, respectively, was described. In further experiments anethole was administered on day 1-2 or on day 3-5 of pregnancy. An anti-fertility effect was observed only on day 3-5; but application on day 1 and 2 was ineffective. Malformations were not observed. These findings are in clear contrast to those cited in NEWBERNE et al, 1999. The FEMA GRAS Assessment of trans-anethole does not show any hints on adverse effects of the substance on fertility or reproduction although trans-anethole was studied in three experimental sets. Doses from 0, 25, 175 or 350 mg/kg b.w. were administered by force-feeding/gavage to rats (n=10/treatment) starting on day 7 prior to mating up to day 4 of lactation. Only in the highest dose group a slight increase of gestation time, increases in pup mortality and stillbirths and reductions of body weight of the pups were noted. No gross physical abnormalities were associated with anethole treatment. In a four generations study in rats (n=40), anethol was added at a concentration of 1% to the diet (corresponding to 700 mg/kg b.w.). The only effect observed was a reduced body weight and a reduced body weight increase in the pubs. In a further experiment, this delay in the growth of the pubs could be explained by the reduced palatability of trans-anethol. The authors concluded that trans-anethol did not produce any reproductive toxicity at doses which are not associated with palatability problems (LE BOURHIS 1973, cited in JECFA 1999). The findings of the publication of DHAR seem to be of questionable relevance. They are in clear opposition to those cited by Newberne who described three independent investigations (ARGUS (1992, cited in JECFA 1999, JECFA 1999, LE BOURHIS 1973, cited in NEWBERNE et al. 1999). These investigations have been performed in a sufficient number of animals and in a very elaborated and correct methodology and therefore are regarded to be reliable	Experimental data on trans-anethole cited by the interested parties are included in the assessment report. Despite the lack of human data, they do not exclude potential toxicity of trans-anethole and aniseed at higher doses and for prolonged use, especially for sensitive population groups such as children, pregnant and breastfeeding women. Experimental conditions showed a) a reduction in the occurrence of implantation b) an increase in gestation time, pup mortality and stillbirths and a reduction in body weight of the pups. Although some of these effects were only noted at high doses, they do not support aniseed safety in pregnancy.

Line no or section and paragraph no	Comment and rationale	Outcome
5.3. Preclinical safety data	The very weak effects seen in these well-conducted and documented experiments even in excessive doses of anethole up to 1400 mg/kg b.w./day clearly put a question mark behind the results of DHAR (1995) who reports a 100% inhibition of implantation at a dose of 80 mg/kg b.w./day administered p.o., i.e., 50% of the NOEL which had been determined with 175mg/kg b.w./day (ARGUS RESEARCH LABORATORIES 1992, cited in NEWBERNE et al. 1999 and JECFA 1999). The author does not adequately describe the quality and source neither of the anethole used in the study nor of any other material. Figures in the paper do not indicate standard deviations. The reported increase of implantation inhibition from 33% at 50 mg/kg b.w. to 66% at 70 mg/kg and to 100% at 80 mg/kg appears rather drastic for a biological effect. Furthermore, the number of animals per group (n=5) was rather small. However, supposing that the information given in this publication be valid, the results are only explicable by an impurity of anethole (e.g., due to inappropriate storage). The results may also be due to the use of Charles-Foster rats instead of Wistar or Sprague Dawley rats used in other studies suggesting that differences in anethole metabolism may be responsible for the large differences. Thus, two extensive, well-documented studies (ARGUS 1992 and LE BOURHIS 1973, both cited in JECFA 1999) suggest that anethole, the major constituent of aniseed oil, is safe during pregnancy and lactation for both mothers and offspring. The study of DHAR (1995) suggests a strong anti-implantation effect of anethole; however its poor documentation should be borne in mind. Teratogenic effects were not observed in any of the studies.	

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5.3. Preclinical safety data	**Estrogenicity of anethole** For trans-anethole an estrogenic activity has been discussed on the basis of in vitro findings and animal experiments. The assumption of an estrogenic activity of anise oil has a long history starting with a study of ZONDEK and BERGMANN (1938) who describe anise oil to be estrogenic in the Allen-Doisy-test (200µl/day for seven days, s.c.). In 1980 ALBERT-PULEO conducted studies with anise oil and compounds isolated after exposing the oil to excessive oxygen and UV light which made him consider desmethyl-anethole and polymerisation products of anethole to be responsible for the observed activity. In an attempt to verify the hypothesis that stilbene-like dimerisation products of anethole exhibit estrogen-effects, KRAUS and HAMMERSCHMIDT (1980) subjected fennel oil (>80% anethole) to extreme storage conditions in terms of light, oxygen and temperature. These authors did not detect any anethole dimers in the so-treated oil. MIETHING et al (1990) however found 0.39ppm of 4.4´-dimethylstilbene in aniseed oil exposed to daylight for 6 months. The authors concluded that the dimer was a reaction product of anethole and anisaldehyde. The fact that isolated anethole is practically free from anisaldehyde is a likely explanation for the contradictory results of different authors. **From these findings, it can be concluded that an estrogenic activity observed in older experiments may be due to compounds arising from inappropriate storage. (i.e. not in line with the storage conditions described in the European Pharmacopoeia).**	Trans-anethole estrogenic activity has been demonstrated both in animals (Dhar, SK., 1995) and in humans (Howes MJ et al., 2002). Both the studies are discussed in the assessment report. Miething et al (1990) found the dimer 4,4´-dimethylstilbene in aniseed oil. The contradictory work of Kraus and Hammerschmidt is a company report not published in journals subjected to peer review. In conclusion, currently the risk associated with the estrogenic activity of anethole to people using products containing anethole is a not clear.

Line no or section and paragraph no	Comment and rationale	Outcome
5.3. Preclinical safety data	In the study of DHAR a significant increase in uterus weight of juvenile rats was seen following application of 80 mg/kg b.w. for three days (DHAR, 1995). The relevance of this finding is questionable since the findings on a possible anti-fertility activity of the author were not confirmed by other, more reliable studies (NEWBERNE et al, 1999). There is no convincing evidence of an intrinsic estrogenic effect of anethole or native anise oil. **<u>Conclusion</u>** • The long-term and wide use in humans has not shown any side effects susceptible to affect infants or children. • The HMPC should consider the inclusion of pharmacovigilance data from EMEA, Member States or WHO databases in order to include all the available evidence. • Animal toxicity data show a low acute and chronic toxicity of anise oil and its major constituent anethole. Pharmacokinetic data from animals and humans demonstrate extensive metabolisation and fast elimination of anethole (JECFA 1999). • There is no conclusive evidence today of a clinically relevant estrogenic effect of anise oil. Positive results from one poorly documented study (DHAR 1995) are strongly contradictory to earlier, far more extensive and well-documented studies and may be due to a species effect (Charles-Foster vs. Wistar rats). • Results from mechanistic studies suggest that chemical artefacts, occurring only under extreme conditions in anise oil may account for the estrogenic activity observed in some earlier studies. **For these reasons, we believe that a restriction of use of Aniseed preparations in babies and children as well as in pregnant and breastfeeding women is inappropriate.**	The work of Dhar is a scientific article reporting original experiments. The Newberne article, discussed in the assessment report, is an assessment of studies on anethole not reporting new original experiments. Recent pharmacovigilance publications report that the use of herbals in pregnancy is underestimated and in this case a correct communication with the physicians does not exist. It is well known that adverse events on herbals are underreported. We agree that only a few toxicological studies were carried out on *Pimpinella anisum* and the available studies are incomplete, inconsistent and contradictory. None of them were performed according to current requirements. The studies do not lead to a clear and definitive positive evaluation of aqueous aniseed infusions during pregnancy and lactation. There are however signals from non clinical studies of potential toxicity linked to a weak mutagenic potential of anethole and a potential genotoxic risk related to estragole. While the genotoxic risk can be considered not relevant for adults in the specified conditions of use, due to the small amount present in herbal infusions prepared from aniseed, a positive statement cannot be supported for sensitive population groups such as children, pregnant and breastfeeding women, whose exposure to estragole should be minimised (Please refer to the HMPC 'Public statement on the use of herbal medicinal products containing estragole' (EMEA/HMPC/137212/2005)). This is also in agreement with the annexes I and III of the 'Guideline on SPCs'.

Line no or section and paragraph no	Comment and rationale	Outcome
5.3. Preclinical safety data	**Receptor-Binding-Studies** In two papers, results on the estrogenic activity of trans-anethole in yeast cells were published: TABANACA et al (2004) observed an estrogenic activity with an IC_{50} value of 625 µg/ml, as compared to 17 β-estradiol the effectivity was 8.6×10^{-8}. HOWES et al (2002) observed an estrogenic activity of trans-anethole only at a concentration of 10 mM, i.e. at a concentration of 1.48 mg/ml (corresponding to 1.48 g/l). All lower concentrations studied were ineffective. From these findings it can be concluded that an interference of trans-anethole with hormone therapy or oral contraceptives can be expected only at unrealistic high concentrations of the substance: in order to obtain an IC_{50} value according to TABANACA et al (625 mg/l), corresponding to an intake of at least 2.5 g would be necessary, according to HOWES et al even a higher intake of 6 g/volunteer. **In vitro-findings** The metabolism and the metabolites which were formed at different concentrations of trans-anethole were investigated in isolated rat hepatocytes by NAGAKAWA and SUZUKI (2003). At a weakly toxic concentration (0.5 mM) trans-anethole was mainly metabolized to 4-methoxycinnamic acid (4MCA), 4-hydroxy-1-propenylbenzene (4OHPB) and to the monosulfate conjugate of 4OHPB. Free unconjugated 4OHPB reached less than 0.5 µM, whereas at the toxic concentration of 1 mM unconjugated, free 4OHPB reached 10 µM. It seems to be of special interest that the rate of formation of free unconjugated 4OHPB, a minor metabolite, is only relevant at high toxic concentrations. The authors showed that only the free unconjugated metabolite 4OHPB formed from anethole by *O*-demethylation is responsible for the estrogenic effects of anethole, i.e, for the receptor binding as well as for the stimulation of the growth of MCF-7 cells (estrogen receptor positive mammary carcinoma cells).	Experiments of **Tabanca** et al. (2004), report an IC_{50} value of 625 µg/ml. They refer to *Pimpinella anisum* fruit oils (Tabanca et al 2004 Estrogenic activity of isolated compounds and essential oils of *Pimpinella* species from Turkey, evaluated using a recombinant yeast screen Planta Med. 2004; 70:728-35). The study of Howes (2002) confirming that high concentrations of trans-anethole have the potential to interact with estrogen receptors in rodents, leads to suggest caution with the use of aniseed in human sensitive population groups. Conclusions of the **Nakagawa** and Suzuki's (2003) experiments, based on studies on rodents, are the following: "These results suggest that the biotransformation of anethole induces a cytotoxic effect at higher concentrations in rat hepatocytes and an estrogenic effect at lower concentrations in MCF-7 cells based on the concentrations of the hydroxylated intermediate, 4OHPB".

Line no or section and paragraph no	Comment and rationale	Outcome
5.3. Preclinical safety data	Receptor binding was observed with IC_{50} values of 5×10^{-5} M for 4OHPB, whereas neither anethole nor its metabolite 4MCA showed interference with 17β-estradiol receptor binding up to a concentration of 10^{-3} or 10^{-4} M, respectively. 4OHPB stimulated cell proliferation of MCF-7 cells in a range of 10^{-6} to 10^{-8} M, whereas neither anethole nor its metabolite 4MCA showed any effect. The authors concluded that 4OHPB is responsible for the estrogenicity of anethole. The metabolism of trans-anethole in human volunteers has been studied (NEWBERNE et al 1999, CALDWELL 1987). In contrast to rodents there was no clear dependency of the dose on the rate and the route of elimination (doses of 1, 50 or 250 mg anethole were applied). Elimination was much faster in humans than in rodents. 8 hours after application the bulk of the dose was eliminated in expired air and urine of men, whereas in rats or mice it took 48-73 hours in high doses. 13-17 % of the metabolites in urine of the volunteers were *O*-demethylation products. Thus it obvious that neither in mice nor in rats a satisfying testing of anethole toxicity is possible; especially at higher doses the pronounced differences in metabolism may result in an overestimation of the possible risk (CALDWELL 1987). **In vivo-studies** In one study a significant increase in uterus weight of juvenile rats was seen following application of 80 mg/kg b.w. for three days (DHAR, 1995). The relevance of this finding is questionable since the findings on a possible anti-fertility activity of the author were not confirmed by other, more reliable studies (NEWBERNE et al, 1999).	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, Caldwell et al., 1987; Caldwell and 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 risk influence is lacking. These Caldwell's experiments show essentially the difference in anethole metabolism between rodents and humans. The work of Dhar is a scientific article reporting original experiments. The Newberne's article, discussed in the assessment report, is an assessment of studies on anethole not reporting new original experiments.

Line no or section and paragraph no	Comment and rationale	Outcome
5.3. Preclinical safety data	**For these reasons a restriction of use of Aniseed preparations in babies and children as well as in pregnant and breastfeeding women appears to be inappropriate.**	We agree that only a few toxicological studies were carried out on *Pimpinella anisum* and the available studies are incomplete, inconsistent and contradictory. None of them were performed according to current requirements. The studies do not lead to a clear and definitive positive evaluation of aqueous aniseed infusions during pregnancy and lactation. There are however signals of potential toxicity from non clinical studies linked to a weak mutagenic potential of anethole and a potential genotoxic risk related to estragole. While the genotoxic risk can be considered not relevant for adults in the specified conditions of use, due to the small amount present in herbal infusions prepared from aniseed, a positive statement cannot be supported for sensitive population groups such as children, pregnant and breastfeeding women, whose exposure to estragole should be minimised (Please refer to the HMPC 'Public statement on the use of herbal medicinal products containing estragole' (EMEA/HMPC/137212/2005)). This is also in agreement with the annexes I and III of the 'Guideline on SPCs'.