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

London, 12 June 2008
Doc. Ref. EMEA/HMPC/286756/2007

OVERVIEW OF COMMENTS RECEIVED ON 'COMMUNITY HERBAL MONOGRAPH ON *PIMPINELLA ANISUM* L., AETHEROLEUM ' (EMEA/HMPC/263273/2006)

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.	INFARMED, Portugal
4.	Kooperation Phytopharmaka, Germany

7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Tel. (44-20) 74 18 84 00 Fax (44-20) 75 23 70 51
E-mail: mail@emea.europa.eu http://www.emea.europa.eu

Table 2: Discussion of comments

Line no or section and paragraph no	Comment and rationale	Outcome
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.
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 gastro-intestinal 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.	In the chapter "III.1. Dosage" it is said, that the single dosage is 0,05-0,2 ml three times daily (daily dosage 0,15-0,6 ml). This dosage is referring to the BHP while the recommended dosage by the Commission E is "daily dosage 0,3 g" (equals - 0,4 ml). We recommend changing the daily dosage in "3 times 0,1 -0,15 ml" (equals 0,3-0,45 ml/day). Proposal: Adults, elderly Single dose: indication i and ii): 0,1-0,15 ml of anise oil. Up to 3 times daily.	There are two different dosages recommended by two distinguished Scientific Bodies however due to the presence of compounds that do not have a clear toxicological profile (such as estragole and trans-anethole), it is decided to take a precautionary approach and to keep the lower dosage of BHP. This decision is considered appropriate as the monograph refers to a self-medication THMP to be used with no medical supervision.

Line no or section and paragraph no	Comment and rationale	Outcome
4.2. Posology and method of administration	In the same chapter it is mentioned, that the use in paediatric age is not recommended for the presence of estragole, whose exposure should be minimised in children. This advice should be given as follows: "No application in children and adolescents.". Duration of administration Not to be taken for more than two weeks. Method of administration No special advice.	Endorsed. The use in children and adolescents is contraindicated with cross-reference to section 4.3.
4.2. Posology and method of administration	For the **well-established medicinal use**, we propose the posology which is currently listed under "traditional use". These recommendations are justified by the references mentioned above (under Section 4.1 "Therapeutic indications"). Furthermore, we propose (for the well-established medicinal use and/or for the traditional use) the following wording**: *"The use of Anise oil in children is not recommended"*** and to delete the rest of the paragraph. Reasons: We refer to our comments under 5.3. For safety reasons, Anise oil should not be used in small children. The HMPC draft monograph itself states under 5.3 that estragole is a minor constituent of anise oil. Reference is made to rodent studies which indicate that "these events are probably minimal in the dose range of 1-10 mg estragole/kg b.w." **Duration of administration:** We propose to delete the sentence *"Not to be taken for more than two weeks"* as a restriction to two weeks cannot be deduced from preclinical data. This sentence may be replaced by **"no restriction"**.	Not agreed. The well established use is not supported by sufficient scientific data. The recommended posology has been set according to the posology of the products on the European market. No documentation to substantiate the traditional use in children has been found for anise oil. Because of the lack of available safety data on long term use of anise oil 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. If symptoms persist or worsen after two weeks it is necessary to consult a doctor.

Line no or section and paragraph no	Comment and rationale	Outcome
4.3. Contra-indications	In the chapter 4.3 "Contraindication" it should stated: “Patients with known sensitivity to Apiaceae (Umbelliferae) (fennel, caraway, coriander and dill) or to anethole should not use aniseed and its preparations.” "Because of the lack of data and because of the presence of estragole anise oil is not be used in children and adolescent."	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) Agreed.
4.4 Special Warnings and Precautions for use	We recommend to delete the information on Asteraceae because not data is given which supports this. The special warning and precaution for use concerning hormone therapy, or contraceptive pill and hormone replacement therapy can not be supported. For such extensive recommendations no information exists. Therefore this sentence should be deleted as well as the information given in chapter 4.5 "Interactions with other medicinal products and other forms of interactions". In the same chapter it is to point out, that the self-medication without progress should not exceed 1 week. So therefore we suggest to give this advice in the monograph under special warnings too. 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.	Agreed. Agreed. Because of the lack of available safety data on long term use of anise oil 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 oil monograph.
4.4 Special Warnings and Precautions for use	We strongly recommend deleting the statement on the estrogenic activity of anethol as explained under 5.3.	Agreed.
4.5 Interactions	Here we also recommend deleting the statement on the estrogenic activity of anethol as explained under 5.3.	Agreed.

Line no or section and paragraph no	Comment and rationale	Outcome
4.6. Pregnancy and lactation	Proposal of wording: There are not data from the use of anise oil in pregnant or breastfeeding patients. Studies in rats have shown anti-implantation and early abortifacient activity. It is unknown if anise oil constituents are excreted in human breast milk. Animal studies are missing. In absence of sufficient data and because of the presence of estragole, which exposure should be minimised in pregnant and breastfeeding woman, anise oil should not be used during pregnancy and lactation.	Agreed. The wording has been further simplified.
4.6. Pregnancy and lactation	I have some concerns on accepting PIMPINELLA ANISUM L., AETHEROLEUM, as a traditional herbal medicine, with the recommendation of not to be used by women of childbearing potential not using effective contraception. On my point of view, this recommendation is not in line with the conditions of the Directive, article 16(a), 1 -" intended and designed for use without the supervision of a medical practitioner", and (e) - in particular the product proves not to be harmful in the specified conditions of use".	The recommendation has been deleted.

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 anise oil in pregnant patients". We recommend replacing this sentence by the following: "Clinical data on the safety of using anise oil preparations in pregnancy are lacking. Therefore, pregnant women are recommended to seek advice from their healthcare professional before taking anise oil preparations." Furthermore we recommend deleting the statement not to use anise oil in childbearing potential without effective contraception. 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 anise oil 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-anethol, the major constituent of anise oil". For the reasons given under section 5.3, we propose to delete this statement.	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) The statement recommending not using anise oil in women with childbearing potential not using effective contraception has been deleted. 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)
4.8. Undesirable effects	We suggest to delete "and gastro-intestinal system" because there are no reports available.	Endorsed

Line no or section and paragraph no	Comment and rationale	Outcome
5.1. Pharmaco-dynamic properties	In the chapter 5.1 additionally information should be given as follows: "**The traditional medicinal use of anise oil is plausible on its antispasmodic, secretolytic and expectorant effects.**" 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 first sentence in the chapter 5.1 ("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.
5.2. Pharmaco-kinetic properties	For chapter 5.2 additionally information should be given as follows: "Anise oil contains up to 94% trans-anethol. After oral administration the compound trans-anethole is rapidly absorbed. 54-69% of the dose is eliminated in the urine and 13-17% in exhaled carbon dioxide. Transanethole is reported to be metabolized by O-demethylation and by oxidative transformation of the C3side chain. The bulk of elimination occurred within 8 hours. The principal metabolite is 4-methoxyhippuric acid"	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.

Line no or section and paragraph no	Comment and rationale	Outcome
5.3. Preclinical safety data	The first sentence in the chapter 5.3 ("Not required ") should be deleted In the monograph a lot of information is given in chapter 5, additionally with the phrase concerning the article 16c (1)(a)iii) of the Directive 2001/83/EC. It should be clear, if special information is required or not. In chapter 5.3 we propose the following wording "For transanethole anti-implantation and early abortificient activity has been reported. The genotoxic risk related to estragole (EMENHMPC/137212/2005) is not considered to be relevant for adults in the recommended dosage due to the small amount present in anise oil." Regarding the daily dosage of 0,3 g anise oil/day the equivalent dose on herbal substance is 10 g aniseed (assuming an average essential oil content of 3% in the herbal substance). According to the "Final position paper on the use of herbal medicinal products containing estragole" (EMEAHMPWP/338/03) 1 g herbal substance contains 1050 µg estragole, so 10 g contain 10,5 mg estragole. For an adult (60 kg body weight) this would mean an intake of 0,175 mg/kg/day. In the final position paper it is said, that generally in a dosage range of 110 mg estragole/kg the daily intake is considered to be safe, so that in the recommended dosage the intake is supposed to be safe. Proposal For trans-anethole anti-implantation and early abortificient activity has been reported. The genotoxic risk related to estragole (EMEA/HMPC/137212/2005) is not considered to be relevant for adults in the recommended dosage due to the small amount present in anise oil.	Partially agreed. Estragole is a constituent of anise oil and reference is made to the HMPC 'Public statement on the use of herbal medicinal products containing estragole' (EMEA/HMPC/137212/2005). 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.

Line no or section and paragraph no	Comment and rationale	Outcome
5.3. Preclinical safety data	In this section, the HMPC draft refers to studies performed with the isolated aniseed compound trans-anethol, 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-anethol, are not discussed. **Studies on reproduction/developmental studies** In the study of DHAR (1995), 50, 70 or 80 mg/kg trans-anethol (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 anethol was administered on day 1-2 or on day 3-5 of pregnancy. An anti-fertility effect was observed only on day 3-5, 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-anethol does not show any hints on adverse effects of the substance on fertility or reproduction although trans-anethol 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 anethol treatment. In a four-generation 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 pups. 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 sentence related to the estrogenic and antifertility activity of trans-anethole demonstrated *in vitro* and in laboratory animals at high concentrations has been modified, specifying that it is not considered relevant to human exposure given the recommended posology and conditions of use. Experimental data 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 anise oil 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, a reduction in body weight of the pups. Although some of these effects were noted at highest doses, they do not support anise oil safety in pregnancy. Some works cited (Argus, Le Bourhis) are not relevant to support a clear safety of trans-anethole because original data are not accessible and the studies are not mentioned in the most important data banks. Works mentioned in the assessment report, even if carried out with a limited number of animals, are the only factual source of anise and anethole toxicity. The criticisms stated are not based on scientific data, but on personal opinion.

Line no or section and paragraph no	Comment and rationale	Outcome
5.3. Preclinical safety data	The findings of the publication of DHAR seem to be of questionable relevance. They are in clear contrast 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 way and therefore are regarded to be reliable. The very weak effects seen in these well-conducted and documented experiments even in excessive doses of anethol 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 anethol 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 anethol (e.g., by 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 anethol metabolism may be responsible for the extreme differences. Thus two extensive, well-documented studies (ARGUS 1992 and LE BOURHIS 1973, both cited in JECFA 1999) suggest that anethol, 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 anethol but is very poorly documented. Teratogenic effects were not observed in any of the studies.	

Line no or section and paragraph no	Comment and rationale	Outcome
5.3. Preclinical safety data	**Estrogenicity of anethol** For trans-anethol 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-anethol and polymerisation products of anethol to be responsible for the observed activity. In an attempt to verify the hypothesis that stilbene-like dimerisation products of anethol exhibit estrogen-effects, KRAUS and HAMMERSCHMIDT (1980) subjected fennel oil (>80% anethol) to extreme storage conditions in terms of light, oxygen and temperature. These authors did not detect any anethol 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 anethol 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 which result from inappropriate storage (i.e. not in line with the storage conditions described by 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 works 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 estrogenic activity of anethole is a not still cleared possible risk for people using products containing anethole.

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 anethol or native anise oil. **<u>Conclusion</u>** • The HMPC should consider inclusion of pharmacovigilance data from EMEA, Member States or WHO data bases 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 anethol. Pharmacokinetic data from animals and humans demonstrate extensive metabolisation and fast elimination of anethol (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. **On the basis of the available data, we believe that a general restriction of use for anise oil preparations in adolescents as well as in pregnant and breastfeeding women and women with childbearing potential is inadequate. While for reasons of general precaution anise oil should be used during pregnancy only after consultation of a healthcare professional, general restrictions for the other groups are not appropriate in the light of available data.**	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. 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 anise oil 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, 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-anethol 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 x 10^{-8}. HOWES et al (2002) observed an estrogenic activity of trans-anethol 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-anethol 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-anethol were investigated in isolated rat hepatocytes by NAGAKAWA and SUZUKI (2003). At a weakly toxic concentration (0.5 mM) trans-anethol 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 anethol by *O*-demethylation is responsible for the estrogenic effects of anethol, 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 anise oil in human sensitive populations 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 x 10^{-5} M for 4OHPB, whereas neither anethol 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 anethol nor its metabolite 4MCA showed any effect. The authors concluded that 4OHPB is responsible for the estrogenicity of anethol.	
	The metabolism of trans-anethol 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 anethol 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 Anise oil in adolescents as well as in pregnant and breastfeeding women and during childbearing potential appears to be inappropriate. While for reasons of general precaution anise oil should be used during pregnancy only after consultation of a physician, general restrictions for the other groups do not seem appropriate in the light of available data.**	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 anise oil 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, 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'.