20 November 2019
EMA/HMPC/278706/2015
Committee on Herbal Medicinal Products

Overview of comments received on the draft revised Public statement on the use of herbal medicinal products containing estragole (EMA/HMPC/137212/2005 Rev 1)

Table 1: Organisations and/or individuals that commented on the draft public statement on the use of herbal medicinal products containing estragole as released for public consultation on December 2014 until March 2015.

	Organisations and/or individuals
1	AESGP (The Association of the European Self-Medication Industry)
2	EFSA (European Food Safety Agency)
3	EUCOPE (European Confederation of Pharmaceutical Entrepreneurs)
4	Frey + Lau GmbH, Henstedt-Ulzburg, Germany
5	Institute for Neuroimmunology and Multiple Sclerosis (INIMS), Center for Molecular Neuro-biology Hamburg, University Medical Center; Hamburg-Eppendorf, Hamburg, Germany

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Table 2: Comments

General comments to draft document

Interested party	Comment and Rationale	Outcome
AESGP	**Summary** The present exposure to estragole resulting from the consumption of herbal medicinal products used for short periods of time at the recommended posology in adults does not pose a significant cancer risk. Carcinogenicity studies use high doses of the isolated compound estragole only and do not take into account the matrix effect. Thus data gained from a pure compound may overestimate effects of the compound in the botanical matrix. For these reasons, setting a limit for the maximum daily intake of 0.5 mg per person is not justified. A limit of 1.8 mg per day (corresponding to an acceptable daily dose of estragole of 30 µg/kg per day) taking into consideration an average body weight of 60 kg, is proposed. We are of the opinion that this Public Statement is clearly related to estragole as a substance and not to herbal medicinal products which–among a large number of other substances–contain estragole.	Partially endorsed. Due to missing adequate data on the carcinogenicity of estragole (see PS) at the moment a daily limit for estragole containing active ingredients in herbal medicinal products is not suggested except for sensitive groups.
AESGP	**Problem Statement** From our point of view, the chapter “problem statement” seems misleading since the text of this chapter leads to the impression that there is not substantially more evidence available today in terms of a potential human carcinogenicity of estragole than in 2005 when the first HMPC Public Statement on the issue was published. To our opinion there is no conclusive explanation given with regard to an assessment different from that of 2005. Furthermore, we do not understand which are the reasons for setting impractically restrictive limits for an intrinsic compound of a traditional herbal drug, a compound which is abundantly present as well in many	Partially endorsed. Especially an extensive series of studies from Rietjens’ group has elucidated toxicokinetics and mechanisms of action of estragole (and other alkenylbenzenes).

Interested party	Comment and Rationale	Outcome
	different food sources. Table 2 (line 81) Regarding background food exposure Table 2 gives examples of common food sources containing estragole of natural origin demonstrating that typical portions of these will provide significantly higher amounts of estragole than the maximum doses of fennel tea as specified in the HMPC monograph. Consumption of food is not restricted by the amount or duration of intake while the maximum dosage and duration of use of a medicinal product is defined in the patient leaflet. Keeping in mind that significant fractions of the general population can be considered high and regular consumers of e.g. fennel tea or pesto, this clearly demonstrates that exposure to estragole from medicinal fennel tea use is not a matter of concern. Moreover, there are no signals of risks from the market, neither from food (pesto) nor from herbal medicinal products.	The PS is focussed on estragole in herbal medicinal products, which is more reliably assessed and controlled. Estragole exposure via food has been considered, but its quantitation may not be adequately characterised and needs a lot of more research.

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AESGP	**Transition rates of estragole** Lines 86 ff. describe different rates of transition of estragole found in publications. In general, limitations in exposure estimates are a major source of uncertainty in safety assessment. In order to get an accurate safety assessment of fennel or anise based tea preparations the extraction efficiency has to be considered. In the literature, different extraction efficiencies are discussed. The ESCO working group describes an extraction efficiency of essential oil into the infusion of 25-35%. (EFSA 2009a). Zeller and Rychlik (2006) determined the extraction efficiency for estragole in an herbal infusion of 12%, which is ½ to ⅓ of the above mentioned value. Van den Berg *et al.* (2014) examined a total of 34 fennel tea preparations in form of tea bags, unpackaged fennel consisting of whole fruits or fine material. Extraction efficiency of estragole into the infusion was 0.1%-2.3%. The German "Chemisches und Veterinäruntersuchungsamt (CVUA, 2007)" tested anise and fennel teas. The extraction efficiency was less than 2%. The transition rate of anethol in an infusion has been determined with about 10% (Iten and Saller, 2004). Estragole is an isomer of anethol, thus a chemical comparability can be assumed. As a result of all these studies, the extraction efficiency of estragole depending on different factors, e.g. the material (whole fruits or cut fruits) or the method of extraction, and if the tea bag is squeezed after preparation or not. In all studies an extraction efficiency far below 100% has been found. Thus, herbal medicinal teas can be classified as an exception, because the estragole content in an infusion is much lower as in the fennel or anise fruit. Thus a limit of 0.5 mg as maximum daily intake per person should be questioned.	Partially endorsed. Extraction efficiency determines the amount basically available for absorption and systemic amount and concentration is determined by availability in the gut and the fraction absorbed to the systemic circulation. More research is needed to characterize both extraction efficiency and systemic (bio) availability. In any case, determination of extraction efficiency and bioavailability could provide data to allow calculation whether the limit value has been exceeded or not. Information (to be) added based on Zeller and Rychlik (2006) and CVUA (2007). For limit on daily intake see above.
AESGP	**Regulatory status and official guidelines** Since the authors use background food exposure as an essential reference point, it would be desirable to find a more detailed assessment and description of food	Partially endorsed. Actually, the possibility to use ICH M7 and the LTL

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	intake sources and patterns of consumption in the document. From our point of view, state-of-the-art rules for the assessment of Less-than-Lifetime (LTL) patterns of exposure in accordance with commonly acknowledged scientific concepts as described in the ICH M7 Guideline (EMA/CHMP/ICH/83812/2013) should be followed. This would allow for a more differentiated and balanced assessment with an option to permit higher, more adequate limits in accordance with the duration and frequency of use of herbal medicinal products (ICH M7). Note: Herbal (medicinal) products like many other types of products/drug substances are excluded from the scope of the ICH M7 Guideline for reasons not specified in the document. It is obvious however that these reasons are probably more of a formal nature than based on scientific rationales. The basic scientific principles reflected in the approach of staged limits for less-than-lifetime exposure scenarios are not reserved per se for DNA-reactive substances which are impurities of chemically defined active pharmaceutical ingredients (API). These principles are a result of extensive scientific work and a long lasting discourse in the scientific community. While a guidance document may be mandatory for certain medicinal products only and even exclude other products formally, applicants (and institutions, likewise) are obliged to consider state-of-the-art scientific knowledge in their assessment of products. Obviously estragole is not a candidate for the application of a TTC in the first instance. However, since estragole-containing medicinal products with mostly short term use (even if multiple application episodes are considered) are the subject of consideration, the respective scientific publications including those considered in ICH M7 (e.g. Brigo and Müller 2011, Felter *et al*. 2011, Müller *et al.* 2006) have to be taken into account. These publications reflect the basic toxicological principles and document the fundamental rationales which the ICH committee has taken into account. In this context, ICH M7 states: "*Standard risk assessments of known carcinogens assume that cancer risk increases as a function of cumulative dose. Thus, cancer*	scheme has been mentioned in the PS. If there is a possibility to set the limit to a higher level according to the LTL scheme for treatments of shorter durations, it is certainly possible to suggest such a higher limit based on posology of a specific preparation. In the revised PS there is a paragraph: 'Taking into consideration the argumentation above, the duration of treatment by a herbal medicinal product and an increase in an acceptable daily dose may be determined by the calculating the less-than-lifetime exposure according to the ICH M7 scheme. However, the calculation has to be based on the accepted posology of the specific herbal medicinal product taking also into consideration the non-avoidable intake by food.' For limit on daily intake see above.

Interested party	Comment and Rationale	Outcome
	risk of a continuous low dose over a lifetime would be equivalent to the cancer risk associated with an identical cumulative exposure averaged over a shorter duration."	

Interested party	Comment and Rationale	Outcome
AESGP	**Medicinal versus food use of fennel** According to recent estimations performed by companies, in Germany ca. 4,500 tons of fennel tea (mono) have been sold as food in 2013 (WKF 2014). In comparison, only about 33 tons have been marketed as medicinal product which is less than 1%. This relation might serve as an example for the exposition from food compared to medicinal products. Consequently, the sporadic use of medicinal fennel tea for a specific indication is rather negligible in relation to the overall exposure via food use. The above figures also illustrate that in an exposure scenario for medicinal fennel tea the resulting estragole intake cannot be added to the total food exposure estimates since a person who uses medicinal fennel tea for a therapeutic purpose is very unlikely to consume a fennel tea marketed as food during that same period. Moreover, the estragole content of fennel for use in medicinal products is limited to 5% of the essential oil in the Ph.Eur. Monograph. This is not the case for fennel in food use where considerably higher estragole contents may occur. This data and reflections should be taken into account with regard to the HMPC document in line with the following content of the ICH M7 guideline which states under *Point* 7.5: *Exceptions and Flexibility in Approaches:* *"Higher acceptable intakes may be justified when human exposure to the impurity will be much greater from other sources e.g., food …"* This exactly applies to estragole. However, while total foodborne estragole consumption may account for a daily, i.e., long term and possibly life-long estragole intake of 0.5–5 mg or more, the amount considered acceptable by the HMPC for medicinal product use is at the very lower end of this estimate reflecting a quite contrary reasoning ("because there is already a considerable exposure of individuals through food consumption, any additional exposure must be as low as	For limit on daily intake see above.

Interested party	Comment and Rationale	Outcome
	possible"). This is contradictory to the rationale established in the ICH process. Therefore, we urgently suggest to reconsider the proportions and interrelations (fennel tea) of food/HMP exposure within the HMPC document.	

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AESGP	**Toxicological assessment** While there has been a wealth of new publications related to estragole toxicity since 2005 as stated in this chapter, not a single one of those publications can add substantially to solving the question of human carcinogenicity. Likewise, no information has been published that would change the basis for assessing the carcinogenic potency of estragole. From our point of view, there are no convincing evidence of the potential genotoxicity and cancirogenicity of estragole. A potential genotoxicity is concluded from mechanisms only. Furthermore, e.g. in the experiment described in line 319 ff. (Suzuki 2012b) only female mice have been taken into consideration in which levels of SULT1a1, a key enzyme in estragole toxification in rodents, were increased several times above normal. Experiments in such an artificial model may serve to verify mechanistic hypotheses but are not suitable to establish dose response relations. Estragole, investigated in numerous studies, has been demonstrated to be a relatively weak rodent carcinogen compared to the activity of 2-acetylaminofluorene. It is not the compound itself that can cause cancer by damaging the cellular DNA, but reactive metabolites that only develop at high concentrations between 10^{-3} and 10^{-2} M (500 and 1000 mg/kg bw in animal experiments). Only at these high doses, the metabolic toxification of estragole to a DNA-reactive compound takes place, involving a two-step mechanism: hydroxylation of the C-1' atom of the side chain generating 1'-hydroxyestragole, followed by sulphation (Howes *et al*., 1990) and other metabolic changes (Iyer *et al*., 2003). The sulfate conjugate of 1'-hydroxyestragole, 1'-sulfo-oxyestragole, forms the DNA adducts and is considered to be responsible for the hepatocarcinogenic effects of estragole and 1'-hydroxyestragole. In addition, 1'-hydroxyestragole is also known	Not endorsed. Genotoxicity is difficult, or practically impossible, to demonstrate in humans with a 100% certainty. However, studies like Suzuki's demonstrate that the mechanism thought to be behind carcinogenicity is working in rats and mice and because humans harbour in principle the same mechanism of action, it is feasible to argue that estragole may be carcinogenic in humans. Dose-response relationship is again difficult to demonstrate in humans and need preferably human data, which is difficult to obtain. Just because the concentration of a putative procarcinogen depends on formation, binding to targets (and other molecules) and further metabolism and ultimate excretion, it is premature to conclude that a tiny amount of the metabolite in the urine reflects the formation and toxicologically significant macromolecular binding. PBPK modelling by Rietjens's group suggests that probably the formation and binding of 1'-hydroxyestragole in humans can be compared to that in animals and there is no non-linearity in dose-response relationship.

Interested party	Comment and Rationale	Outcome
	to form DNA adducts via formation of 1'-hydroxyestragole-2',3'-oxide (epoxide). This metabolite, however, is probably not significant for a carcinogenicity of estragole, because it is rapidly degraded by epoxide hydrolases *in vivo*. Furthermore, 1'-hydroxyestragole also undergoes glucuronide conjugation by uridine diphosphate glucuronosyltransferases (UGTs), and the corresponding metabolites (hydrophilic glucuronides) are easily excreted into the urine, a major detoxification pathway for estragole. Some isoforms of these enzymes are expressed in the gastrointestinal and liver tissues and, hence, the conjugation of estragole can occur at both these sites (Iyer *et al*., 2003). Other detoxification pathways such as O-demethylation forming hydroxyl-allylbenzene are also known (HMPC, 2014; Sangster *et al*., 1987). Thus, the real carcinogenic metabolites of estragole are 1'-sulfo-oxyestragole and, to a very low extent, 1'-hydroxyestragole-2',3'-oxide. The carcinogenicity of 1'-hydroxyestragole is clearly dependent on the balance between formation of the active metabolites, 1'-sulfo-oxyestragole and epoxides, and the detoxification processes (Iyer *et al*., 2003). In fact, only a small amount of 1'-hydroxyestragole is produced after intake of low estragole dosages in humans. In a study, volunteers ingesting a single dose of only 100 µg estragole for 6 months produced tiny amounts of 1′-hydroxyestragole between 0.2% and 0.4% of the ingested estragole, and these very minor amounts of 1'-hydroxyestragole were excreted in urine (Sangster *et al*., 1987). At the very high doses administered to animals, 1'-hydroxyestragole is a major metabolite, accounting for approximately 10% or more of the estragole administered (Tisserand and Balacs, 1995). All these findings are very important for the safety assessment of food flavouring substances, since the dose levels used in carcinogenicity studies have been substantially larger than the estimated level of human daily intake. For these reasons, it is concluded that the present exposure to estragole resulting from the	

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	consumption of herbal medicinal products used for short periods of time at the recommended posology in adults does not pose a significant cancer risk (EFSA, 2009; Sangster *et al*., 1987). This conclusion is in line with that of Smith *et al*., who published a safety assessment of allylalkoxybenzene derivatives, including, used as flavouring substances (Smith *et al*., 2002). It should also be noted that these carcinogenicity studies use high doses of the isolated compound estragole only. This does not take into account the matrix effect, which would potentially support the safety of particular levels of compounds, since data from a pure compound may overestimate effects of the compound in the botanical matrix. The question can be raised whether studies with pure compounds dosed by gavage without the normal food matrix represent a good starting point for the risk assessment of botanical ingredients. Jeurissen *et al*. (2008) demonstrated with regard to basil, another estragole-containing plant, that the level of DNA binding of the proximate carcinogenic metabolite 1'-hydroxyestragole to DNA *in vitro* (rat and human liver S9 homogenates) but also to DNA in intact HepG2 human hepatoma cells could be inhibited by a methanolic basil extract. A similar effect could be obtained by adding the SULT (sulfotransferases) inhibitor pentachlorophenol to the incubation medium. Because the inhibition by basil extract resembles the inhibition by the SULT inhibitor pentachlorophenol and because the inhibition was not observed in incubations with the direct electrophile 1'-hydroxyestragole, it was concluded that the inhibition by the basil extract occurs at the level of the SULT mediated bio-activation of 1'-hydroxyestragole to 1'-sulfo-oxyestragole (EFSA, 2009; Jeurissen *et al*., 2008). Although it has not yet been established whether a similar inhibition will occur in the *in vivo* situation, the inhibition of SULT mediated bio-activation of 1'-hydroxyestragole by basil ingredients suggests that the possibilities for bio-activation and subsequent adverse effects may be lower when estragole is dosed in a matrix of other plant ingredients than what would be expected on the basis of	Not endorsed. Potential matrix effects regarding exposure to estragole have been extensively discussed in the PS. In the comment there are no real novel viewpoints or scientific sources presented. The SULT inhibition by various flavones (mainly *in vitro*) is a well-known finding, but its significance for *in vivo* extrapolation and prediction is not known. For limit on daily intake see above.

Interested party	Comment and Rationale	Outcome
	experiments dosing estragole as a single compound (EFSA, 2009). A more recent publication by Alhusainy and coauthors shows that the constituent responsible for the SULT inhibition by basil extract is the flavone, nevadensin (Alhusainy *et al*. 2010). While in the model used by the authors nevadensin showed a considerab inhibitory activity the SULT inhibition itself of this 5,7-hydroxylated flavone is not at all surprising. 5,7-hydroxylated flavones like apigenin, kaempferol or quercetin which are abundantly present in fruit and vegetables have long been recognised for their strong SULT inhibitory effects which have been demonstrated in numerous mammalian (including human) tissues and with a large range of model substrates including, e.g. paracetamol, dehydroepisandrosterone, minoxidil, resveratrol, salbutamol, 4-nitrophenol and dopamine. Not surprisingly, IC_{50} values varied in relation to test substrates and model tissue but reached as low as 12 pM (!) (quercetin inhibition of resveratrol sulfation, human liver) (Gilissen *et al* 1994; Harris and Waring 2008; Miller 1994; Morimitsu *et al*. 2004; Marchetti *et al.* 2001; De Santi *et al*. 2002; Pacifici 2004; Rossi *et al.* 2004). The mentioned data shows very clearly that extrapolation from animal trials (obtained with very high doses of pure estragole) to the human exposure via herbal preparations (providing low doses of estragole in a complex matrix including numerous flavonoids known as strong SULT inhibitors) is not scientifically sound. The HMPC, like other scientific bodies, has noted in the draft statement on estragole via reference to the publication by Jeurissen and co-workers that matrix effects may be of strong importance but that data were lacking to consider such effect for estragole in the fennel matrix. Obviously the literature cited above (and that cited by Jeurissen) which actually provides a different basis for the assessment of this question, has not been taken into consideration within the HMPC draft. Quercetin and apigenin are the major flavonoids of fennel fruit (Badgujar *et al*. 2014). Particular attention must therefore be drawn to the matrix issue taking into account the cited literature on SULT inhibition by dietary flavonoids.	

Interested party	Comment and Rationale	Outcome
	The metabolic activation of estragole is dose-dependent. Only at very high doses, estragole and its metabolites, respectively, cause rodent hepatocarcinogenicity. Moreover, the formation of 1'-hydroxyestragole in rats and mice is known to be not linearly related to the dose size, but shows a disproportionate increase in 1'-hydroxylation as the dose is increased. Thus, what is a minor metabolite at human dietary and low animal doses becomes a major metabolite at high, i.e. hepatocarcinogenic doses. It is likely that the use of these high doses used in animal studies results in a marked overestimation of the potential hazard to humans of low doses of allylbenzenes, which generate only very small to tiny quantities of genotoxic metabolites (Howes *et al*., 1990). The human exposure to estragole generally occurs at much lower levels than the ones used in animal studies (up to 1000 mg estragole per kg bw). At lower dose levels, estragole is detoxified more efficiently by various enzyme systems than at higher doses, where these pathways become saturated and alternative routes become increasingly employed that lead to the formation of toxic metabolites (Tisserand and Balacs, 1995).	

<table>
<tr><th>Interested party</th><th>Comment and Rationale</th><th>Outcome</th></tr>
<tr><td>AESGP</td><td>Body weight of 50 kg no appropriate basis for calculation

The HMPC is requested to reconsider its body weight (bw) assumptions and take into account to more recent data on body weight distribution in the European population based on 21 dietary surveys as reflected in the Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data (EFSA 2012). These data clearly demonstrate that the calculation based on an adult body weight of 50 kg is overtly conservative for the assessment of mostly short exposure scenarios of herbal medicinal products and the fact that most herbal medicinal products are indicated for mild to moderate conditions, i.e., these indications do not correspond to a population subgroup at the lower end of the bw distribution frequent in those affected by severe conditions.

Table 1: Body weight (kg) statistics for adult subjects in all surveys of the EFSA Comprehensive database

<table>
<tr><th>Age (years)</th><th>Gender</th><th>N</th><th>Mean</th><th>SD</th><th>Median</th><th>P5</th><th>P95</th><th>% ≤ 70kg</th><th>% > 70kg</th></tr>
<tr><td>18-64</td><td>♀</td><td>22556</td><td>67.2</td><td>12.8</td><td>66.0</td><td>50.0</td><td>90.7</td><td>70.9</td><td>29.1</td></tr>
<tr><td>18-64</td><td>♂</td><td>18736</td><td>82.0</td><td>13.1</td><td>82.0</td><td>63.0</td><td>105.0</td><td>18.4</td><td>81.6</td></tr>
<tr><td>18-64</td><td>♀+♂</td><td>41294</td><td>73.9</td><td>14.9</td><td>72.0</td><td>52.0</td><td>100.0</td><td>47.1</td><td>52.9</td></tr>
<tr><td>65-75</td><td>♀</td><td>2420</td><td>70.6</td><td>12.0</td><td>71.0</td><td>53.0</td><td>92.0</td><td>49.2</td><td>50.8</td></tr>
<tr><td>65-75</td><td>♂</td><td>2132</td><td>82.2</td><td>11.5</td><td>82.5</td><td>65.0</td><td>102.0</td><td>14.8</td><td>85.2</td></tr>
<tr><td>65-75</td><td>♀+♂</td><td>4552</td><td>76.0</td><td>13.1</td><td>75.0</td><td>55.0</td><td>98.0</td><td>33.1</td><td>66.9</td></tr>
<tr><td>≥75</td><td>♀</td><td>1080</td><td>66.4</td><td>11.6</td><td>66.2</td><td>49.0</td><td>85.0</td><td>63.0</td><td>37.0</td></tr>
<tr><td>≥75</td><td>♂</td><td>890</td><td>77.1</td><td>12.4</td><td>77.0</td><td>58.0</td><td>96.0</td><td>32.7</td><td>67.3</td></tr>
<tr><td>≥75</td><td>♀+♂</td><td>1970</td><td>71.2</td><td>13.1</td><td>70.1</td><td>50.0</td><td>92.0</td><td>49.3</td><td>50.7</td></tr>
</table>
N: number of individuals in the database
SD: standard deviation
P5: 5th percentile
P95: 95th percentile

Source: EFSA 2012</td><td>Not endorsed.
e.g. ICH Q3C and ICH M7 use 50 kg.

Furthermore, in the PS, we give the following justification for the use of 50 kg as a body weight.

'For ~18% (average) of the European population the body weight is given with less than 60 kg (EUROPEAN COMMISSION 2006). These numbers would increase to up to 30%, if only taking into account woman. Therefore the calculation is linked to a body weight of 50 kg.'</td></tr>
<tr><td>AESGP</td><td>Acceptable limit for daily dose

The HMPC draft recommends an acceptable daily dose of 0.5 mg daily for herbal medicinal products as short-term (maximum 14 days) intake. We consider such a limit as too low, especially as compared to the daily exposure of estragole from the</td><td>For limit on daily intake see above.</td></tr>
</table>

Interested party	Comment and Rationale	Outcome
	food intake (0.5 – 5 mg per day). A NOAEL of 37.5 mg/kg per day has been given in the draft Public Statement, coming from a sub-chronic toxicity study, where mice were given estragole by gavage for 3 months. The NOAEL value has been based on increased liver weights in males and incidences of oval cell hyperplasia in females at 75 mg/kg. Calculation of PDE is the following: 37.5x50/12x10x5x2x1=1.5 mg per day with: F1=12 (extrapolation from mice to humans) F2=10 (variability between individuals) F3=5 (3-month study in rodents) F4=2 (toxicity) F5=1 (no-effect level established) Thus, we propose to increase the limit to 1.5 mg per day (corresponding to an acceptable daily dose of estragole of 30 µg/kg per day) or better to 1.8 mg per day, taking into consideration an average body weight of 60 kg.	

Interested party	Comment and Rationale	Outcome
AESGP	**Consequences for marketed products** The following calculation shows potential consequences of the HMPC draft for products containing Fennel oil. Fennel oil is used for the symptomatic treatment of cough associated with cold. This is clearly a self-limiting indication. For clarification, the use is restricted to two weeks in the HMPC monograph. Based on the dosage recommended in the HMPC monograph (200 µl per day≈18 0mg) the daily estragole intake would be 9 mg assuming 5% estragole in the oil or 180 µg/kg bw, respectively, for an adult person of 60 kg bw. While this level of exposure is considerably higher than that recommended in the HMPC draft it is still comparable to exposures resulting from normal food consumption, e.g. a portion of 25 g of pesto genovese (Table 2), i.e., 250µg/kg bw per day based on the information provided in the 2009 JECFA assessment of alkoxybenzene derivatives cited by the HMPC. Still, in this case JECFA has considered only the mean estragole content in basil oil, 40%, while this content may be up to 90% as stated in the same report. In that case a single portion would provide more than 500 µg/kg bw of estragole. Consequently, a single consumption as described may result in intakes 1–2 orders of magnitude greater than the mean estragole intake of 80 µg per day reported in the same JECFA publication. JECFA comments: "*However, because pesto is not consumed daily even for these specialized consumer groups, their average lifetime intake when calculated on a daily basis would approach mean or maximum daily intake levels for non-specialised consumer groups"*. This is applicable to the medicinal use of fennel oil as well. Consequences for an existing product are shown in the following: a traditional pharmaceutical preparation against inflammations of the oral mucosa is containing Star Anise oil (Anisi stellati aetheroleum with an average content of 3.5% estragole) and Fennel oil (Foeniculi vulgare aetheroleum with an average content of	For limit on daily intake see above.

Interested party	Comment and Rationale	Outcome
	3% estragole) in a defined mixture of different other essential oils. The daily dose (20 drops 3 times per day) results in an amount of estragole of 1.5 mg per day, which exceeds the proposed limit for estragole tree times. It appears highly questionable whether the estragole contents in the natural essential oils of Fennel and Anise etc. can be further reduced. Therefore the future of this well-known and extensively used preparation (in Germany) would be at risk. Similarly, herbal teas with fennel fruit as the sole ingredient or with considerable proportions of fennel fruit in a combination which have been used both as a beverage and as medicinal products for centuries, even on the basis of a transition rate of 10% would exceed the limit proposed in the draft document and thus would no longer be marketable as medicinal products. Such consequences would appear not only disproportionate but definitely irrational in view of the overall low proportion of potential estragole exposure through medicinal products as compared to overall and–even more so-peak exposures from food sources. (Bringing to mind that medicinal fennel tea accounts for only ca. 1% of total fennel tea consumption (Germany)–in other EU member states the consumption of fennel tea both as food and medicinal tea is much lower anyway).	

Interested party	Comment and Rationale	Outcome
AESGP	**Conclusion** The draft HMPC Public Statement proposes a maximum daily intake of 0.5 mg per person. For the reasons explained above, a limit for the maximum daily intake of 0.5 mg per person is not justified from our point of view. We propose a limit of 1.8 mg per day (corresponding to an acceptable daily dose of estragole of 30 µg/kg per day and taking into consideration an average body weight of 60 kg.	For limit on daily intake see above.
EUCOPE	**Metabolism and Toxicokinetics** *Metabolism* Estragole, investigated in numerous studies, has been demonstrated to be a relatively weak carcinogen compared to the activity of 2-acetylaminofluorene. It is not the compound itself that can cause cancer by damaging the cellular DNA, but reactive metabolites that only develop at high concentrations between 10^{-3} and 10^{-2} M (500 and 1000 mg/kg bw in animal experiments). Only at these high doses, the metabolic toxification of estragole to a DNA-reactive compound takes place, involving a two-step mechanism: hydroxylation of the C-1' atom of the side chain generating 1'-hydroxyestragole, followed by sulphation (Howes *et al*., 1990) and other metabolic changes (Iyer *et al*., 2003). The sulfate conjugate of 1'-hydroxyestragole, 1'-sulfo-oxyestragole, forms the DNA adducts and is considered to be responsible for the hepatocarcinogenic effects of estragole and 1'-hydroxyestragole. In addition, 1'-hydroxyestragole is also known to form DNA adducts via formation of 1'-hydroxyestragole-2',3'-oxide (epoxide). But this metabolite is probably not significant in the carcinogenicity of estragole, because it is rapidly degraded by epoxide hydrolases *in vivo*. Furthermore, 1'-hydroxyestragole also undergoes glucuronide conjugation by uridine diphosphate glucuronosyltransferases (UGTs), and the corresponding metabolites (hydrophilic glucuronides) are easily excreted into the urine, a major detoxification pathway for	Most of the comments from EUCOPE are practically the same as from AESGP (see above), sometimes even *ad verbatim*. Only those comments that require a specific reply have been responded to.

Interested party	Comment and Rationale	Outcome
	estragole. Some isoforms of these enzymes are both expressed in the gastrointestinal and liver tissues and, hence, the conjugation of estragole can occur at both these sites (Iyer *et al*., 2003). Other detoxification pathways such as O-demethylation forming hydroxyl-allylbenzene are also known (HMPC, 2014; Sangster *et al*., 1987). Thus, the real carcinogenic metabolites of estragole are 1'-sulfo-oxyestragole and, to a very low extent, 1'-hydroxyestragole-2',3'-oxide. The carcinogenicity of 1'-hydroxyestragole is clearly dependent on the balance between formation of the active metabolites, 1'-sulfo-oxyestragole and epoxides, and the detoxification processes (Iyer *et al*., 2003). In fact, only a small amount of 1'-hydroxyestragole is produced after intake of low estragole dosages in humans. In a study, volunteers ingesting a single dose of only 100 µg estragole for 6 months produced tiny amounts of 1′-hydroxyestragole between 0.2 % and 0.4 % of the ingested estragole, and these very minor amounts of 1'-hydroxyestragole were excreted in urine (Sangster *et al*., 1987). At the very high doses administered to animals, 1'-hydroxyestragole is a major metabolite, accounting for approximately 10% or more of the estragole administered (Tisserand and Balacs, 1995). All these findings are very important for the safety assessment of food flavouring, since the dose levels used in carcinogenicity studies have been substantially larger than the estimated level of human daily intake. For these reasons, it is concluded that the present exposure to estragole resulting from the consumption of herbal medicinal products used for short periods of time at the recommended posology in adults does not pose a significant cancer risk (EFSA, 2009; Sangster *et al*., 1987). This conclusion is in line with that of Smith *et al*., who published a safety assessment of allylalkoxybenzene derivatives, including, used as flavouring substances (Smith *et al*., 2002).	

Interested party	Comment and Rationale	Outcome
	Matrix effects It should be noted that these carcinogenicity studies use high doses of the isolated compound estragole only. This does not take into account the matrix effect, which would potentially support the safety of particular levels of compounds, since data from a pure compound may overestimate effects of the compound in the botanical matrix. The question arises whether studies with pure compounds dosed by gavage without the normal food matrix represent a good starting point for the risk assessment of herbal preparations. Jeurissen *et al*. demonstrated in 2008 with regard to basil, another estragole-containing plant, that the level of DNA binding of the proximate carcinogenic metabolite 1'-hydroxyestragole to DNA *in vitro* (rat and human liver S9 homogenates) but also to DNA in intact HepG2 human hepatoma cells was effectively inhibited by a methanolic basil extract. A similar effect was obtained by adding the known SULT (sulfotransferases) inhibitor pentachlorophenol to the incubation medium. Because the inhibition by basil extract resembles the inhibition by the SULT inhibitor pentachlorophenol and because the inhibition was not observed in incubations with the direct electrophile 1'-hydroxyestragole, it was concluded that the inhibition by the basil extract occurs at the level of the SULT mediated bioactivation of 1'-hydroxyestragole to 1'-sulfo-oxyestragole (EFSA, 2009; Jeurissen *et al*., 2008). Although it remains to be established whether a similar inhibition will occur in the *in vivo* situation, the inhibition of SULT mediated bio-activation of 1'-hydroxyestragole by basil suggests that the possibilities for bio-activation and subsequent adverse effects may be lower when estragole is dosed in a matrix of other plant ingredients than what would be expected on the basis of experiments dosing estragole as a single compound (EFSA, 2009). This is supported by a more recent publication of Alhusainy and co-authors who have found that the constituent responsible for the SULT inhibition by basil extract	

Interested party	Comment and Rationale	Outcome
	is the flavone, nevadensin (Alhusainy *et al*., 2010). While in the model used by the authors nevadensin showed a considerable inhibitory activity the SULT inhibition itself of this 5,7-hydroxylated flavone is not at all surprising. 5,7-hydroxylated flavones like apigenin, kaempferol or quercetin which are abundantly present in fruit, herbs and vegetables have long been recognized for their strong SULT inhibitory effects which have been demonstrated in numerous mammalian (including human) tissues and with a large range of model substrates including, e.g. paracetamol, dehydroepiandrosterone, minoxidil, resveratrol, salbutamol, 4-nitrophenol and dopamine. IC_{50} values varied in relation to test substrates and model tissue but reached as low as 12 pM (!) (quercetin inhibition of resveratrol sulfation, human liver tissue) (Gilissen *et al.,* 1994; Harris and Waring, 2008; Miller, 1994; Morimitsu *et al.,* 2004; Marchetti *et al*., 2001; De Santi *et al.*, 2002; Pacifici, 2004; Rossi *et al*., 2004). The mentioned data shows very clearly that extrapolation from animal trials of toxicological observations obtained with very high doses of pure estragole to the exposure of humans with herbal preparations providing low doses of estragole in a complex matrix including numerous flavonoids known as strong SULT inhibitors is highly speculative. The HMPC like other scientific bodies has recognized in the draft on estragole with reference to the publication by Jeurissen and co-workers that matrix effects may be of strong importance but that data were lacking to consider such effect for estragole in the herbal matrix. Obviously the literature cited above (and that cited by Jeurissen) which actually provides a different basis for the assessment of this question, has not been available to the HMPC when the draft was established. Of note, quercetin and apigenin are the major flavonoids of fennel fruit (Badgujar *et al.,* 2014) and occur also in many other plants. More in-depth attention should therefore be drawn within further assessment to the matrix issue taking into account the cited literature on SULT inhibition by dietary flavonoids.	

Interested party	Comment and Rationale	Outcome
	Toxicokinetics The metabolic activation of estragole is dose-dependent. Only at very high doses, estragole and its metabolites, respectively, cause rodent hepato-carcinogenicity. Moreover, the formation of 1'-hydroxyestragole in rats and mice is known to be not linearly related to the dose size, but shows a disproportionate increase in 1'-hydroxylation as the dose is increased. Thus, what is a minor metabolite at human dietary and low animal doses becomes a major metabolite at high, i.e. hepatocarcinogenic doses. It is likely that the use of these high doses used in animal studies results in a marked overestimation of the potential hazard to humans of low doses of allylbenzenes, which generate only very small to tiny quantities of genotoxic metabolites (Howes *et al*., 1990).The human exposure to estragole generally occurs at much lower levels than the ones used in animal studies (up to 1000 mg estragole per kg bw). At lower dose levels, estragole is detoxified more efficiently by various enzyme systems than at higher doses, where these pathways become saturated and alternative routes become increasingly employed that lead to the formation of toxic metabolites (Tisserand and Balacs, 1995).	

Interested party	Comment and Rationale	Outcome
EUCOPE	**Exposure** The HMPC Draft Public Statement states: "*… The intake of 0.5 mg/person per day (even if the limit presents the overall intake from all sources) can be accepted for herbal medicinal products as short-term (maximum 14 days) intake*." Comment: 0.5 mg per day actually does not generally "*present the overall intake from all sources…*"! As expressed several times in the HMPC document published, institutional intake estimates range from 0.5 to 5 mg per day! The approach of choosing any possible worst case assumption (here: 0.5 mg per day) leads to an overly conservative limit! and "*Although rigorous and comprehensive estimates of estragole intake via food are not available, values of 0.5–5 mg daily* (sic!) *have been presented by various authorities in the EU and the USA (see Table 2). The dietary intake estimates are thus up to 10-fold higher that the above limit value of 0.5 mg per person per day. However, the extraction efficiency of estragole from food items may be considerable less than 25-35%, assumed by EFSA (2009). Assuming the maximum extraction value of 2.5% taken from Van den Berg et al. (2014) and the maximum intake of 5 mg via food items, the calculated "real" intake is 0.125 mg per person per day and probably much less. This theoretical calculation demonstrates that it is very important to investigate extraction efficiencies of estragole from various commodities and products.*" Comment: The relations established in the underlined sentence are incorrect because they are based on the assumption that the whole food intake of estragole is based on fennel tea consumption. Obviously this is not the case because a large variety of food, i.e. spices, natural aromas, confectionery, bakery, pesto and-of	Partially endorsed. We made an assumption, which in the lack of adequate research may be applied to food items. As commented, there is probably a large variability between different foods and even if extraction efficiency is not of importance for those foods consumed "as such", bioavailability is probably also variable and of importance for the systemic presence of estragole.

Interested party	Comment and Rationale	Outcome
	course, fennel and other herbal teas-contribute to the total intake. In fact, transition rates are completely irrelevant for most estragole containing food items because these are simply consumed in total. Transition rates are important exactly for herbal teas, fennel tea being the most important one in quantitative terms. Therefore the construed relation between the estimated maximum intake of 5 mg per day and the putative “real” intake should be thoroughly revisited. Most remarkably, for individuals whose major food estragole source is fennel tea the consumption of a medicinal fennel tea will hardly make a difference because it is not reasonable to assume that a regular (food) fennel tea consumer will actually add a large portion of fennel tea with (T)HMP status to his daily portion in case he is ill but would either not change his product at all or substitute one with the other. In this connection figures from the German market depict the relations quite clearly: While 4,600 tons of food grade fennel tea was sold in 2013 (WKF, 2014), fennel tea with medicinal product status accounted for less than 50 tons (including HMP sales outside pharmacies). The HMPC is kindly requested to reconsider its assessment of the relation between food and HMP exposure to estragole with particular attention to fennel tea which represents a major intersection between food and HMP exposure sources.	

Interested party	Comment and Rationale	Outcome
EUCOPE	**Daily limit and duration of use** We consider the acceptable daily dose of 0.5 mg per day for estragole in herbal medicinal products as far too low, especially when compared to the daily exposure of estragole from food sources and with regard to the known toxicokinetic particularities. This limit dose is based on the lowest of a range of $BMDL_{10}$ values which were calculated by analysis of data from a carcinogenicity study in mice. In this study (Miller *et al*., 1983), animals were fed with grain supplemented by very high dosages of estragole (2,300 or 4,600 mg/kg administered *ad libitum*, corresponding to, according to EFSA (2009) and SCF (2001), an estimated intake of 150-300 or 300-600 mg/kg bw per day). It remains unclear how the estimated daily intake of estragole in this study was assessed by SCF (2001), as the publication of Miller *et al*. (1983) does not provide this information. As a consequence, it is highly questionable whether the calculation of the $BMDL_{10}$ as performed by EFSA (2009) based on estimated dosage ranges and assuming several worst case estimates reflects any reliable value. Therefore, and due to the reasons specified in the following, it seems neither sound nor realistic to use this study design as a basis for an actual limit dose for estragole in herbal medicinal products. It is well-known that the metabolic activation of estragole in rats and mice shows a disproportionate increase as the dose is increased. As also referred to in the HMPC draft, O-demethylation predominates at low doses in the range of 0.05 to 50 mg/kg bw. At these dosage levels, which correspond to those in herbal medicinal products or food, estragole is detoxified efficiently by various enzyme systems (see above). Moreover, as rightly stated in the HMPC Draft Public Statement, there is sufficient evidence that the toxification of estragole may be largely reduced or even	For limit on daily intake see above.

Interested party	Comment and Rationale	Outcome
	completely absent when applied in its complex herbal matrix instead as single compound (see above). Therefore, if a limitation of estragole below the level already ensured by the respective Ph. Eur. monographs is considered necessary at all, it would appear reasonable to calculate a Permissible Daily Exposure (PDE) for estragole in herbal medicinal products based on a NO(A)EL. This regulatory approach is recommended amongst others by the ICH Expert Working group for mutagenic compounds with a mechanism leading to a dose response that is non-linear or for which a practical threshold can be derived (ICH Expert Working Group, 2014). When data are available, the ICH guideline refers to the ICH Q3C(R5) document for the calculation of a PDE. For estragole, an NO(A)EL of 37.5 mg/kg per day has been referred to in the Public Statement, derived from a sub-chronic NTP toxicity study, where mice were administered estragole by gavage at several dosage levels for 3 months (Bristol, 2011). The NO(A)EL value has been based on increased liver weights in males and incidences of oval cell hyperplasia in females at 75 mg/kg. The study is deemed to be of high quality and the results seem robust. Thus, based on this NO(A)EL and following the approach proposed by the ICH (ICH Expert Working Group, 2011), the calculation of the PDE for estragole is as follows: 37.5x50/12x10x5x1x1=3.0 mg per day with: F1=12; F2=10; F3=5; F4=1; F5=1 to the resulting PDE of 3.0 mg per day, corresponds to an acceptable daily dose of estragole of 60 µg/kg per day. This level is well within the range of intake estimates for total estragole from food sources.	

Interested party	Comment and Rationale	Outcome
	Limited duration of use The HMPC states: "*The intake of 0.5 mg per person per day (even if the limit presents the overall intake from all sources) can be accepted for herbal medicinal products as short-term (maximum 14 days) intake*." The limited duration of use is considered as inappropriate and not in line with the long-standing therapeutic use of many estragole-containing medicinal products. Estragole and its metabolites do not accumulate in the human body. According to Sangster *et al.* (1987) the elimination in humans is faster than in rodents, with the bulk of doses equivalent to normal human dietary levels being excreted in the urine and as CO_2 within 12 hours. For most (T)HMP containing fennel fruit, fennel oil or other fennel fruit preparations the duration of use is restricted anyway. Based on the exposure estimates referred to in the HMPC Draft Public Statement an average adult person has a daily intake of estragole of 0.5-5 mg. In relation to this, almost any reasonable HMP use scenario will not contribute significantly to the lifetime estragole exposure. Therefore a general limitation of the duration of use to 14 days is not considered reasonable and would appear disproportionate. It would particularly discriminate products with a low (<0.5 mg per day or <3 mg per day, respectively) but measurable estragole level.	

Interested party	Comment and Rationale	Outcome
EUCOPE	**Conclusion** For the reasons explained above, a limit value of 0.5 mg per day is overly conservative and not practicable with regard to pharmacopoeial quality requirements and available herbal drug qualities, respectively (e.g., fennel, star anise). If an acceptable dose is to be established, a limit of 3 mg per day would appear appropriate based on the PDE model. This would still be sufficiently conservative in view of the low level of bio-activation expected on the basis of experimental data in the dose range relevant for herbal medicinal products, the matrix effects that are reasonably expected from compounds of herbal preparations as such or from the vegetable portion of an average diet and with regard to the relation between estragole exposure from food and medicinal products. If the HMPC adheres to the position that a restriction to the duration of use is necessary it is kindly requested to take into consideration scientifically established and regulatory accepted toxicological models for less-than-lifetime exposure scenarios as reflected, e.g. in the ICH Harmonised Tripartite Guideline (ICH Expert Working Group, 2014). Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk.	See above.
INIMS	**Background Information:** Introduction Estragole is a by-product in BOSWELAN® BSR 001 (For the manufacturing of BOSWELAN® BSR 001 the resin of *Boswellia serrata* (the herbal drug) is extracted in ethanol 80% water 20% (m/m) to obtain a fluid extract. The average yield of extractable matter is 25-50% (m/m) of the herbal drug, resulting in drug extract ratio (DER) of 2-4:1 (with reference to the native extract). Subsequently, the fluid extract is carefully mixed with propylene glycol monolaurate (50%, m/m), calculated to the extractives, and then evaporated under reduced pressure until a	The information has been taken into account.

Interested party	Comment and Rationale	Outcome
	solvent free oleo resin is obtained) which is used for the treatment of patients suffering from multiple sclerosis in the clinical SABA trial (study details see below). Potential Estragol Intake Under Boswelan BSR001 Medication In The Clinical SABA Trial Boswelan BSR001 is used for the treatment of patients suffering from multiple sclerosis in the clinical SABA trial. The total daily dose are 4,800 mg Boswelan BSR001 consisting of twelve capsules with each containing 400 mg Boswelan BSR001. The batch currently used (BSR001 "neu") contains 698 µg estragole per g Boswelan BSR001, resulting in a potential total daily estragole intake of 3.35 mg. Preliminary human metabolism data after Boswelan BSR001 intake showed that neither estragole (with an LOQ of 540 ng/ml plasma) nor 1-hydroxyestragole (with an LOQ of 9 ng/ml plasma) were detectable through all the plasma and serum samples (Mail Pharmacelsus, 17th March 2015). Any potential adverse effects of the daily Boswelan BSR001 intake on the liver of patients in the SABA trial up to a treatment duration of twelve months were checked by the evaluation of the gamma-GT-, AST-, ALT-, total bilirubin-and LDH levels (own data) in the cohort of n=28 MS patients. There was a transient significant increase of gamma-GT values within the reference range during month 2 and 3 with mean values still below the upper normal range and a significant decrease of bilirubin values from month 5–12. Data are presented in Appendix 1. In conclusion, there were no signs of liver injury in the SABA trial. In summary, the potential estragole intake resulting from Boswelan BSR001 treatment in the SABA trial is 0.15 fold that of the acceptable daily intake concluded by the HPMC for short-term intake, but in the same range (1.5 fold) as estimated for the highest estragole intake via food. The dose factors based on animal data yield a margin of 55fold for the 3-month NTP toxicity study in mice where no adverse effects occurred and 867 fold for the 3-month NTP toxicity study	

Interested party	Comment and Rationale	Outcome
	in rats where liver toxicity, but no liver tumours were seen. No signs of liver injury were seen in patients of the SABA trial. Neither estragole nor its metabolite 1-hydroxyestragole was detectable in the plasma and serum samples of patients. In conclusion, based on the data presented, there is low likelihood that the patients under Boswelan BSR001 treatment in the SABA trial were exposed to a carcinogenic risk. All of the above mentioned data and discussion has been implemented in an amendment to the study protocol that has been approved by the local ethics committee as well as the federal authority, the Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM) in March 2015.Therefore extension of the study is ongoing. We would ask the regulatory authorities to carefully discuss and define new safety references. Boswelan seems to offer a safe and achievable treatment in multiple sclerosis which deserves further studying. EMA recommendations will strongly impact its development.	

<table>
<tr><th>Interested party</th><th>Comment and Rationale</th><th>Outcome</th></tr>
<tr><td>Frey + Lau GmbH</td><td>Estragole in fennel oils and medicinal herbal teas

The reduction of naturally occurring genotoxic carcinogens in active ingredients and herbal medicinal products should be generally supported as far as possible.

Bitter fennel fruit oil is an active pharmaceutical substance containing estragole which is described in the European Pharmacopeia in an own detailed monograph (Ph. Eur. 1826). A monograph on Sweet Fennel Fruit oil is currently drafted.

<table><tr><td>Ph. Eur.</td><td>Estragole</td></tr><tr><td>Bitter Fennel fruit oil</td><td>max. 6.0%</td></tr></table>
The GC profile given in the individual monograph defines limits for the typical compounds and quality markers of an essential oil as part of the tests on purity and authenticity. Those limits consider a natural variability of batches across the years.

Modern analytical testing relies on quality markers to qualify genuine essential oils against falsifications, e.g. nature identical reconstitutes (flavours). Fennel oils matching the chromatographic profile given in the monograph can be considered as genuine.

Removing or lowering the natural estragole content in natural bitter fennel oils e.g. by established physical separating techniques such as rectification is technically not possible, if the complete essential oil according to the monograph should be maintained at the same time.

With regard to the increasing number of falsified medicines in Europe, it is therefore recommendable to keep defined values of estragole in in the monograph for genuine bitter fennel fruit oil. A lower limit should be discussed.

Existing and traditionally established herbal medicinal products containing fennel fruit oils or fennel fruit as part of medicinal teas should be able to refer to the</td><td>Partially endorsed.
For limit on daily intake see above.

For the usage in children and pregnant woman, we refer to the EU monographs of herbal substances / herbal preparations containing estragole and the newly drafted PS on estragole.</td></tr>
</table>

Interested party	Comment and Rationale	Outcome
	European monograph. Bitter fennel fruit is traditionally often used for children against abdominal pain, and for breast-feeding women as part of breast-feeding teas. Especially new born babies traditionally profit by medicinal bitter fennel teas as one of the only remedies against colics in the early days of their lives, without any serious alternative at hand. Fennel fruit is considered in the essay to contain a maximum of 6% of essential oil with a minimum of 3.5% estragole in the oil. The proposed maximum intake is 0.5 mg estragole per day in general and 0.2 mg estragole per day for sensitive groups such as children. Based on this calculation, the consumption of a cup of a long established, customary medicinal herbal tea for children, containing bitter fennel fruit tea bags of 1.5 gr of powdered fruit, would be no longer recommendable without assuming the proposed suitable extraction value. It is therefore proposed to establish official drug extraction factors along with maximum daily intake recommendations.	

Specific comments on text

Section number and heading	Interested party	Comment and Rationale	Outcome
Page 4, line 72	AESGP	On page 4, in the table starting on line 72: *Artemisia dranunculus* L. (3rd entry) should be corrected to *Artemisia dra**c**unculus* L.	Endorsed and corrected.

Section number and heading	Interested party	Comment and Rationale	Outcome
	EUCOPE	Artemisia dra**c**unculus L. instead of dra**n**unculus	
	AESGP	We do not understand the content (maximum 0.04%) of estragole in *Pimpinella anisum* fruit. This value is not logical with the content of essential oil in plant and the content of estragole in essential oil which are respectively of 4% and 5%.	Endorsed. Corrected maximum 0.2%
Page 4, line 72	EUCOPE	We don't understand the content (%) of estragole in part of plant used in *Pimpinella anisum* fruit. The value "max. 0.04%" is not logical with the content of essential oil in plant and the content of estragole in essential oil, respectively 4% and 5%. The correct numerical value should be **maximum 0.2%**.	See above.
1.2. and 1.3.	EFSA	Sections 1.2, lines 83-88 and 102-103; section 1.3 lines 116-126; and recommendations section: you use for your assessment the EFSA ESCO Report (2009), in which Appendix D illustrates how the tiered approach described in the EFSA SC guidance for the risk assessment of botanicals and botanical preparations (2009) could be used for the assessment of Foeniculum vulgare MILL. At the beginning of this appendix, there is a disclaimer (in bold) explaining that "The document is not intended to provide a formal safety assessment of the botanical or botanical preparation, and therefore the outcome of the assessment cannot be used to legally support the safety of the botanicals and botanical preparations evaluated (…). Data evaluated were collected for the purpose of this testing exercise and are not intended to be complete". We therefore disagree with the fact that you use/discuss in your statement some of the data/assumptions used in this	Partially endorsed. We have removed all references (see above-mentioned sections of the draft statement) to Appendix D of the EFSA ESCO Report, but we have preserved the data of Miller *et al*. and used it as a material for BMDL or NOAEL "eye-balling".

Section number and heading	Interested party	Comment and Rationale	Outcome
		illustrative case study, which once again is in no way a safety assessment of Foeniculum vulgare or estragole. We therefore kindly request that you remove all references (see above-mentioned sections of your draft statement) to Appendix D of the EFSA ESCO Report and the information it contains.	
1.3.	EFSA	Section 1.3: it would be appropriate for EMA to refer to the Regulation (EC) 1334/2008 in which estragole in food is regulated. It is stated in Annex III, part A, that 1-allyl-4-methoxybenzene (estragole) is one of the substances that "*shall not be added as such to food*". Annex III, part B, provides maximum levels for occurrence in certain foodstuffs when flavourings etc. have been used that naturally contain estragole. In the same section (1.3), there are errors in what is referenced to the Council of Europe (CoE): - To begin with, the year is wrong; it should be 2005, as it is in the reference list. - EMA has erroneously construed that: the CoE "*…evaluated estragole and recommended a limit of 0.05 mg/kg (detection limit). Whether this limit is of intake or of content in a herbal substance is not clear*." In its report, the CoE recommends the following limits: "*General limits in foods and beverages (not traditionally flavoured with estragole containing herbs and spices): non-detectable based on modern analytical test methods.*"	Corrected.

Section number and heading	Interested party	Comment and Rationale	Outcome
		Footnote: "*The limit of determination should be taken into consideration as general limit*." Limits (mg/kg of foodstuff) are presented for the following exceptions: "*Anise flavoured confectionery 100, Anise flavoured alcoholic beverages 35, Alcoholic beverages traditionally flavoured with tarragon, fennel or chervil 70, Flavoured baked goods 10, Cheese with herbs 10, Flavoured preserves (with vegetable, fish) 35, Flavoured ready meals (with fish, meat) 35, Tarragon/basil flavoured oil 175, Condiments 200, Sauces 100*." - The (at the time) proposed limit of determination of 0.05 mg/kg food/beverage was only used for calculation of theoretical maximum intake levels from foodstuffs in general, in the evaluation from CoE. See http://www.coe.int/t/e/social_cohesion/soc-sp/public_health/flavouring_substances/Active%20principles.pdf for further details; the evaluation of estragole is on pp. 75-86 of this report.	
Page 7, line 168 ff	EUCOPE	"*Concerning humans it has been reported that after oral administration of estragole to two volunteers (100 µg per day for 6 months) the excretion of 1'hydroxyestragole in the urine amounted to 0.2 and 0.4% of the administered dose*." At this point HMPC cited the results published by Sangster *et al.* (1987) in the wrong way. In this study, volunteers ingesting **a single dose of only 100 µg estragole** for 6 months produced tiny amounts of 1'hydroxyestragole between 0.2% and 0.4% of the ingested estragole. These very minor amounts	Corrected.

Section number and heading	Interested party	Comment and Rationale	Outcome
		of 1'hydroxyestragole were excreted in the urine. That means, that only a small amount of 1'hydroxyestragole is produced after intake of low estragole dosages in humans.	
References	AESGP	References not included in the HMPC reference list Alhusainy W, Paini A, Punt A, Louisse J, Spenkelink A, Vervoort J *et al*. Identification of nevadensin as an important herb-based constituent inhibiting estragole bioactivation and physiology-based biokinetic modeling of its possible *in vivo* effect. *Toxicology and Applied Pharmacology* 2010; 245:179–190 Badgujar SB, Patel VV, Bandivdekar AH. Foeniculum vulgare Mill : A Review of Its Botany Phytochemistry, Pharmacology, Contemporary Application, and Toxicology. *BioMed Research International* 2014, ID 842674 Brigo, A, Müller L. Development of the Threshold of Toxicological Concern Concept and its Relationship to Duration of Exposure, in Genotoxic Impurities (Ed. A. Teasdale). 2011, John Wiley & Sons, Inc., Hoboken, NJ, USA. doi: 10.1002/9780470929377.ch2 De Santi C, Petrabissa A, Mosca F, Rane A, Pacifici GM. Inhibition of phenol sulfotransferase (SULT1A1) by quercetin in human adult and foetal livers. *Xenobiotica; the fate of foreign compounds in biological systems* 2002, 32(5):363-368 EFSA Scientific Opinion: Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal	We thank for the submission of additional literature. It has been taken into account and added to the PS if considered relevant.

Section number and heading	Interested party	Comment and Rationale	Outcome
		2012;10(3): 2579 Felter SP, Conolly RB, Bercu JP, Bolger PM, Boobis AR, Bos PM *et al*. A proposed framework for assessing risk from less-than-lifetime exposures to carcinogens. *Critical reviews in toxicology* 2011, 41 (6):507-544 Gilissen RAHJ, Hume R, Meerman JHN, Coughtrie MWH. Sulphation of N-hydroxy-4-aminobiphenyl and N-hydroxy-4-acetylaminobiphenyl by human foetal and neonatal sulphotransferase. *Biochem. Pharmacol.* 1994, 48:837-840 Harris RM, Waring RH. Sulfotransferase inhibition: potential impact of diet and environmental chemicals on steroid metabolism and drug detoxification. *Current drug metabolism* 2008, 9(4):269-275 Howes AJ, Chan VS, Caldwell J. Structure-specificity of the genotoxicity of some naturally occurring alkenylbenzenes determined by the unscheduled DNA synthesis assay in rat hepatocytes. *Food Chem Toxicol* 1990; 28:537-542 ICH Harmonised Tripartite Guideline. Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk. M7. Current *Step 4* version dated 23 June 2014) http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Multidisciplinary/M7/M7_Step_4.pdf Iten F, Saller R. Fencheltee: Risikoabschätzung der phytogenen Monosubstanz Estragole im Vergleich zum natürlichen	

Section number and heading	Interested party	Comment and Rationale	Outcome
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