22 September 2021
EMA/HMPC/482974/2020
Committee on Herbal Medicinal Products (HMPC)

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 – 2nd Draft)

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 15 February 2020 until 15 May 2020

	Organisations and/or individuals
1	The Association of the European Self-Medication Industry (AESGP)

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000

General comments to draft document

Table 2: Discussion of comments

Interested party	Comment and Rationale	Outcome
AESGP	**Summary** Background dietary exposure to estragole is an essential factor to be considered when reasonable limits for estragole in HMPs are discussed. However, since robust exposure data from the food sector do hardly exist, the issue of consumption patterns of food vs HMPs over the lifetime is of high relevance. In this context, the recommendations of ICH M7 play an important role for an appropriate assessment of a compound with estimated cumulative (lifetime) exposure levels by food consumption in comparison to amounts of estragole that can reasonably be expected by the intake of medicinal products. The expert report of Professor Schrenk confirms the HMPCs conclusion that the available toxicity data do not enable establishing an ADI by departing from a $BMDL_{10}$, but equally puts into question the derivation of an ADI from a TD_{50} by applying a safety factor of 50.000 because it is based on a high degree of uncertainty since data from animal experiments using two dose levels only are used. Furthermore, the dose range applied in the respective study was several-fold higher than the relevant exposure in humans. Instead, since DNA adduct formation in the liver is accepted as the key event in estragole carcinogenicity, DNA adduct formation is recommended as a surrogate marker. A benchmark dose approach reveals a $BMDL_{2x}$ value of 5 mg estragole/kg b.w. per day as the lowest. Application of an uncertainty factor of 100 results in an interim TDI of 50 µg/kg b.w. and day. Accordingly, calculation for an adult person of 50 kg b.w. leads to a maximum daily intake of 2.5 mg estragole.	Specific issues (e.g. maximum daily intake) are discussed below.

Interested party	Comment and Rationale	Outcome
	As can be shown by calculations for various marketed products, implementation of the HMPC recommendations as drafted would have dramatic consequences for preparations of estragole-containing essential oils and herbal drugs, in particular for those used in sensitive patient groups like pregnant and breastfeeding women as well as children. As robust, up-to-date exposure data for food is not available, an immediate implementation of the HMPC recommendations into regulatory practice for herbal medicinal products is considered inappropriate. **General Comments** We appreciate the opportunity to comment on the 2nd Draft Revision and we wish to comment on the toxicological considerations and conclusions drawn in this draft. Moreover, we refer to our comments that we had submitted to the 1st Draft Revision of this document that had been released for public consultation in December 2014. As a lot of arguments and new references that we had submitted were not taken into consideration in the 2nd Draft Revision, we find it useful to open the discussion again on some of these issues.	The outcome of the public consultation on the second draft varies considerably from the first one (e.g. proposed limits). Therefore, it is not clearly specified, which argumentation is meant. Single points are discussed below, when specific arguments are brought forward.

Specific comments on text

Section number and heading	Interested party	Comment and Rationale	Outcome
Introduction and Problem Statement (Chapter 1)	AESGP	<u>Melissae folium</u> In Table 1 (in-between lines 75-76, 7th line below header line of table), it is not clear why *Melissa officinalis* L. (Lamiaceae) is part of the list. Reference to the listed estragole content of	Not endorsed. There seems to be a misunderstanding. The table is a copy of a table from EFSA Scientific Cooperation (ESCO) Report. Advice on the EFSA 550 guidance

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Estragole in plants and plant preparations (Chapter 1.1.)		6.3% in the essential oil in *Melissa* leaves is not supported by any respective references. In relevant literature, neither the listed content of 6.3% estragole in the essential oil nor even any estragole content in *Melissa* leaves could be retrieved. Across all these references, estragole is not listed as a constituent (e.g. HMPC Assessment Report *Melissa officinalis* L., folium (EMA/HMPC/196746/2012) as well as WHO monograph Folium Melissae (2002), ESCOP monograph Melissae folium (2003), Hager's Handbook (2006), Wichtl (2016). In the literature, values of 6.3% were reported only for caffeic acid in an alcoholic (70%) extract of *M. officinalis* L. dry leaf powder (EFSA Journal 2020) and for citronellal in lemon balm essential oils (Chizzola 2018). EFSA in its Scientific Opinion notes that "lemon balm *(Melissa officinalis* L.) *may* also contain estragole" (EFSA 2010) and states in its 2020 report that "the applicant made a literature search on the composition of *M. officinalis* L. and its extracts. The presence of estragole and methyleugenol *was* reported in lemon balm (SCF 2001, EFSA 2010). Estragole and methyl eugenol *were not detected* in one batch of the extract (limit of detection 1 mg/kg). The FEEDAP Panel notes that the analysis of more batches (at least three) would be needed to exclude the presence of these compounds in the extract" (EFSA 2020). Thus, in both evaluations of EFSA the presence of Estragole in lemon balm is unclear and analytical testing showed no presence of estragole.	document for the safety assessment of botanicals and botanical preparations intended for use 551 as food supplements, based on real case studies. *EFSA Journal* 2009, 7(9):280, as to be seen from the headline. "Modified from EFSA, 2009" means it was brought into alphabetical order. The data from EFSA based Council of Europe publications: Council of Europe (2006). Active principles (constituents of toxicological concern) contained in natural sources of flavourings. Approved by the Committee of Experts on Flavouring Substances, October 2005, Health Protection of the Consumer Series. Council of Europe Press, Strasbourg. Therefore, *Melissa* cannot be removed from the table.

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		Mimica-Dukic *et al*. (2004) mention estragole as a constituent of the essential oil of Melissae folium (Table 1, page 3) with 0.1%. The yield of essential oil was 0.2% (distilled according to Ph. Eur.). In conclusion, the study does not mention any hint on a potential risk but demonstrates antimicrobial, particularly antibacterial, activity of the investigated essential oil and a strong protective activity in lipid peroxidation processes. According to the authors' conclusion the results indicate that essential oils could serve as flavouring agents and safe antioxidant and antiseptic products. In this context it may be recommendable to consult the EDQM Group of Experts 13A (Herbal Drugs and Herbal Drug Products) as this group is currently working on a Monograph for *Melissae aetheroleum.* As there is no evidence that estragole is a constituent of *Melissa officinalis* L., folium at any significant level, it would be misleading if *Melissa* leaves are listed in Table 1. "Main occurrence of estragole in plants and/or essential oils" based on assumptions only. Proposed change: *Melissa officinalis* L. (Lamiaceae) should be removed from the list.	
Exposure to estragole from herbal medicinal	AESGP	The HMPC states that "*A major factor of relevance for the risk assessment and actions to take, is to evaluate the background exposure to alkenylbenzenes (and other related and relevant substances) from foodstuffs and food commodities of the*	Not endorsed. It should be noted that HMPC did not derive a general limit. It is given (lines 474-486):

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products and food (Chapter 1.2.)		*consumer*." and "*Until further data on estragole carcinogenicity are available, an exposure limit of estragole in herbal medicinal products should be based on the background of human exposure via food*." As the HMPC also correctly states, "… *rigorous and comprehensive estimates of estragole intake via food are not available…*". Consequently, it is not possible to establish "*an exposure limit of estragole in herbal medicinal products … based on the background of human exposure via food"* Exposure through products other than medicinal products can hardly be quantified since estragole occurs in many food plants and robust figures on the intake of, e.g., estragole-containing aromas, spices, vegetables or spirits are currently not available. Of note, as the HMPC states, it is reasonable to assume that the European Regulation on flavourings and certain food ingredients with flavouring properties for use in and on foods (EC 2008) has probably reduced the overall exposure via food to some extent by imposing a ban on the addition of pure estragole and by limiting the estragole content in some product categories. While the allowable levels in the regulated food categories are not a sufficient basis for an exposure assessment, they may give an impression of putative individual exposure scenarios, depending on dietary habits. Of note, these regulated food categories do not represent an	"Until further data on estragole carcinogenicity are available, an exposure limit of estragole in herbal medicinal products should be based on the background of human exposure via food. European bodies as CoE (2005) or national agencies as BfR (2002) have recommended consumers to restrict consumption of estragole-containing herbs and spices beyond their occasional use in kitchen and have demanded industry to reduce the amount of estragole in food as far as possible. This implicates that there is a tolerable estragole exposure from food products, while additional estragole exposure via medicinal products should be kept as low as reasonably possible. To date, the increase in carcinogenic risk from the life-time intake of estragole containing products is not known. To calculate this tools and data are needed which are not readily available. Involvement of experts from the food area and epidemiologists would be necessary to proceed. Thus, given the uncertainties mentioned above, at current time an exact limit cannot be defined. Nonetheless, it is concluded, that the intake of estragole from (traditional) herbal medicinal products ((T)HMPs) in the general population should be as low as possible. It was pointed out that for sensitive patient groups

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		exhaustive list of food potentially containing significant amounts of estragole. The following table shows allowable levels (mg/kg) of estragole in certain food categories (EC 2008): • Dairy products • Processed fruits, vegetables (incl. mushrooms, fungi, roots, tubers, pulses and legumes), nuts and seeds • Fish products • Non-alcoholic beverages This EU regulation has been applied from 20 January 2011 and clearly led to a reduction of foodborne estragole exposure from added pure estragole. However, this does not mean that overall estragole exposure via food has decreased. First, fennel oil or anise oil have certainly replaced the pure compounds in a variety of food to which the former had previously been added. Second, certain food items particularly rich in estragole, e.g., among others, fresh and dried basil, pesto, but also certain aniseed-based liqueurs have gained increasing popularity and more widespread use throughout Europe. Plant-based food supplements were formally inexistent prior 2002 and have not been considered as a source of estragole exposure in any previous exposure estimation. However, they may significantly contribute to the background estragole exposure from food with levels above 0.5 mg/day as shown by Uusitalo *et al*. (2016). Besides changes in dietary habits/availability of food	and for the usage of estragole containing preparations as excipients only the calculation according to the guideline M7, should be regarded as a helpful tool (lines 459-461).

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		items and food composition, consider the refinement of exposure survey methodologies. Therefore, the level of 1 mg/day postulated by the Council of Europe in its publication from 2005, cited in the revised HMPC draft remains as speculative a figure as "0.5" or "5.0" or other options from other outdated but frequently cited sources. As regards fennel tea, market data shows that the use of HMP fennel tea is rather negligible in relation to the overall exposure via food use. According to recent market figures, approx. 4.300 tons of fennel tea (mono) have been sold as food in Germany in 2018 (WKF 2019). In comparison, only about 30 tons are normally marketed as medicinal products which is less than 1%. This relation might serve as an example for the exposure from food compared to medicinal products. 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 clearly 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. To sum up, we share the HMPC's view that background dietary exposure to estragole is an essential factor to be considered when aiming to set reasonable limits for estragole in HMPs. Without the availability of robust exposure data from the food sector, the fact of a significant background exposure from food	

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		as well as the distinct timely consumption/use patterns of food vs HMPs link up the discussion to the ICH M7 guideline, apart from its relevance for the choice of an appropriate basis for deriving a safety margin.	
Transition rates of estragole (Chapter 1.2.)	AESGP	The question as to how much of the estragole usually contained in fennel fruit migrates into an infusion during the preparation of medicinal tea is highly significant. Although the data published on the subject so far do not permit the formulation of an authoritative answer to this question, widespread experience – as also described by the HMPC (2014) - implies that the 25 to 35% level assumed by the EFSA (2009) is too high by a long way. A working group under the umbrella of BAH, the German Medicines Manufacturers' Association therefore conducted a systematic analysis of the migration rate of estragole in tea infusions and, to this end, examined tea infusions from three different quality forms (whole fennel fruits, whole + freshly crushed fennel fruits, fine-cut fennel fruits) in three different pharmaceutical laboratories in parallel. Apart from this, three different batches of fine-cut fennel fruits were analysed as the most widely used preparation of fennel fruits. The essential oil content in the drug substance was determined in % m/V and the estragole content in the fennel oil was determined in % (normalisation method) in accordance with the monograph on bitter fennel of Ph. Eur. 9.0. Each participating laboratory conducted a triplicate determination of the oil content and the estragole content in the fennel oil. The complete experimental	Not endorsed. For the time being a general transfer rate of 5% is not supported. While the data provided by industry are seen supportive for the general conclusions in the PS, some uncertainties remain, e.g. with regard to the validation of the entire approach, matrix effects in mixtures, etc. It is suggested that, in specific application for marketing authorisation, such data can be provided by individual applicants to support their claims / patient groups and might therefore be taken into account by the NCA.

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		set-up and results are compiled in more detail in the attached report (BAH 2019). As expected, uncrushed whole fennel exhibited the lowest migration rate at 1.4%. The freshly crushed, whole fennel exhibited that highest migration rate at 7.0%. The migration rates for the fine-cut fennel ranged between 3.7 and 4.9%. In spite of the wide variation ranges in the results for estragole in the infusions, and notwithstanding the estragole content in uncrushed, whole fennel, migration rates ranging between 3.7 and 7.0 were found in four different finished products containing bitter fennel. This shows that only a small amount of estragole from medicinal tea containing bitter fennel, with the typical oil content of around 5% and approx. 2.5% estragole content in the oil, migrates into the tea infusion following extraction with hot water with a maceration period of 15 minutes. The different products all exhibited similar behaviour with respect to the migration rate. The results of the analyses show that the migration rates for the most abundant fine-cut teas are all lower than 5%. The nature and scope of the analyses are such that this value may be regarded as being a sufficiently reliable approximate value for exposure calculations. This applies to the migration rates for whole fennel fruits (approx. 1.5%) and freshly crushed whole fennel fruits (approx. 7%) to a similar extent. In qualitative terms, the results are in good accordance with information presented in chapter 1.2. of the recent HMPC draft. In quantitative terms, however, this study is the first in which	

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		official methodology (Ph. Eur.) was consequently applied and exclusively medicinal fennel (bitter) of defined quality was used. Analyses were performed in parallel by three different GMP certified pharmaceutical labs on the basis of a prospective study plan. Therefore, the data presented here may be considered adequate to establish a default transfer rate of estragole from bitter fennel fruit into herbal infusions of 5%, independent from cut size.	
Toxicological assessment (Chapter 2)		In his expert report of April 2020, Professor Schrenk (2020) addresses the following issues: • Is the current scientific knowledge with respect to toxicological findings sufficient to apply benchmark dose modelling and which would be a state-of-the art approach to derive an acceptable daily intake of estragole? • Which is the rationale to apply a derivation of an ADI from TD50 data for estragole in animal experiments and which would be the preferable approach in order to sufficiently protect patients? In accordance with HMPC the expert concludes that the limited data available from carcinogenicity studies with estragole do not provide an adequate basis for a benchmark dose modelling - which would be the preferred approach today - due to the fact that only two dose levels were applied in the relevant	Not endorsed. The concept of $BMDL_{2x}$ (doubling of DNA adduct level as compared to background) for the calculation of a limit for the human usage is not covered by the regulatory guidelines in the field of authorisation of medicinal products. For the proposed procedure in PS regarding limits etc. see above.

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		studies. However, according to his report, the derivation of an ADI from a TD_{50} by applying a safety factor of 50.000 is not recommended either, because it is based on a high degree of uncertainty when data from animal experiments using two dose levels only are used. Furthermore, the dose range applied in the respective study was several-fold higher than the relevant exposure in humans. In view of the totality of the data available today – and their obvious limitations which have also been stipulated by the HMPC – the expert recommends a third approach, particularly taking into account that • estragole, based on its TD_{50} is classified as a weak carcinogen • based on its occurrence in many food plants the background exposure to estragole via food, although not exactly known, is to be considered high in relation to the mostly sporadic and timely limited exposure via medicinal products • an increasing body of evidence from animal and human data as well as from *in vitro* studies, including so far unpublished results from the experts´ working group, supports the hypothesis that estragole carcinogenicity is subject to a threshold mechanism Therefore, since DNA adduct formation in the liver is accepted	

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		as the key event in estragole carcinogenicity the expert recommends the use of DNA adduct formation as a surrogate marker. A detailed analysis of DNA adduct formation in rats (Paini, 2012) using a benchmark dose approach revealed a $BMDL_{2x}$ (doubling of DNA adduct level as compared to background) value of 5 mg estragole/kg b.w. per day as the lowest. Application of an uncertainty factor of 100 results in an interim TDI of 50 µg/kg b.w. and day. Accordingly, calculation for an adult person of 50 kg b.w. leads to a maximum daily intake of 2.5 mg estragole.	

Recommendations (Chapter 3) 3.1. Relevance of experimental toxicities for human risk assessment		The summarising assessment of the HMPC in this paragraph focuses nearly exclusively on qualitative aspects of the toxicokinetic behaviour of estragole, ending with the statement that "Further *in vitro* and *in vivo* human studies are needed, but it is anticipated that with the help of a refined PB-toxicokinetic/dynamic model scientifically satisfactory view of estragole toxicokinetics and related dynamics could be developed to help human risk assessment." The HMPC rightly states that "Further … studies are needed, …". However, we do not share the Committee's view expressed in the same sentence, that "refined PB modelling" could close this gap, given that these models are of an experimental nature with no regulatory standards for their validation or interpretation. At the same time it is surprising that important publications – even human PK data - have not been taken into consideration at all, such as the study of Zeller and co-authors from 2009, who demonstrated that only a very small proportion of an oral estragole dose administered by way of a fennel fruit infusion appears to be metabolized to the crucial 1´-hydroxyestragole and that the extent of this metabolic step is dose dependent, i.e., with a lower dose, a lesser proportion of the critical metabolite is formed. Of note, these findings do not stand for their own but must be read in conjunction with previous human PK data obtained under similar conditions by Sangster and with animal data by the groups of Zangouras and Smith. The study of Zeller *et al.* significantly supports the hypothesis of a non-	Not endorsed. The quote from the PS uses the words "anticipated" and "could" to show that one option is conceivable. This will depend on the totality of the data and the increasing knowledge of such PK models. From the point of view of the HMPC, the 4 publications to which reference is made do not contribute to answering the open questions. Indeed, linearity or non-linearity is a crucial point in safety evaluation. Here the right endpoint of measurement should be chosen. While all references that were highlighted by AESGP [Sangster *et al*., 1987 (not in submitted LoR); Smith *et al*., 2002; Zangouras *et al*., 1981; Zeller *et al*., 2009] measure plasma or urine levels of 1´-hydroxyestragole, the question by Suzuki *et al*. (2012) and Paini *et al*. (2010) is about DNA adduct formation in the liver. This is also the base for the assessment by Schrenk, which was provided by AESGP. No documentation was submitted to show the correlation between literature data on plasma and urine levels and data on DNA adducts. Therefore, the different statements should not be correlated. Further critical points of the cited references:

		linear dose response in the low dose range (Sangster 1987; Smith 2002; Zangouras 1981; Zeller 2009). The inobservance of these important human data and an overemphasis of PB modelling approaches may lead to a factual undervaluation of the existing total evidence accounting for the non-linear-dose-response hypothesis. As a matter of fact, hardly any of the eligible studies reported so far in the literature – including the PB modelling studies - has applied low dose levels in the range relevant for medicinal product intake scenarios. In addition, beyond the information so far accessible to the HMPC, very recent data obtained by the working group of Schrenk obtained for the parameter of DNA adduct formation, i.e., a parameter much more downstream in the process of carcinogenesis than metabolite formation and, thus, more predictive, clearly support the nonlinearity hypothesis. (Note: Further studies investigating this question are currently in process or planned) The question of whether or not (potential) carcinogenic mechanisms of estragole materialise with a linear or non-linear dose-response relationship is crucial for the whole safety assessment of this substance. Therefore, in the light of the information and considerations outlined above we very much expect that the HMPC will dedicate adequate endeavour to a (re)assessment of the totality of the evidence regarding the nature of the dose-response relationship of estragole carcinogenicity. We appreciate that the HMPC has made reference to ICH M7 (2015), particularly in Chapter 3.3. Recommendations, which is a substantial step towards a matter-of-factly assessment of a compound with estimated cumulative (life-time) exposure	Sangster *et al*. (1984): 2 test persons received defined amounts of estragole. This cannot answer the questions concerning linearity, regardless from the question of DNA-adducts. Zeller *et al*. (2009): In the publication it is written: "Test persons consumed estragole by drinking fennel tea made from freshly broken fennel fruits on an empty stomach. Doses were adjusted by gavage of different amounts of fennel tea. …. After the consumption of 1 L of fennel tea, which contained 3.5 mg of estragole, …." Therefore, it appears that only a fixed amount of estragole was taken which will not answer the questions concerning linearity, regardless from the question of DNA-adducts. Zangouras *et al*. (1981): animal data; dosages used: 0.05, 5, 500 and 1000 mg/kg estragole; the low doses, which are decisive for the question of linearity of small quantities, are separated by a factor of 100, thus no questions about linearity can be answered.

		levels by food consumption surpassing by far the cumulative doses that can reasonably be expected for medicinal use scenarios even encompassing multiple treatment episodes over a lifetime. Thus, taking into account the increasing evidence of a non-linear dose-response relationship in estragole carcinogenicity, the predominantly limited (less than lifetime) cumulative duration of use of medicinal products containing estragole, and the significant background exposure via food, it would be an adequate and proportionate alternative to the limit currently proposed in the revised HMPC draft, as outlined by Professor Schrenk in his expert report (Schrenk 2020), to establish a provisional approach to a limitation of estragole exposure through medicinal products. Based on an assessment using a $BMDL_{2x}$-value of 5 mg estragole/kg b.w. per day and an uncertainty factor of 100, he proposes an interim TDI of 50 µg/kg b.w. and day. I.e. calculation for a person of 50 kg b.w. would lead to a maximum intake of 2.5 mg estragole per day. Consequences for marketed products Implementation of the HMPC recommendations would have dramatic consequences for fennel oil in general and for fennel tea used in sensitive patient groups like pregnant and breastfeeding women as well as children. Availability of medicinal products in this indication will probably decrease and patients will change to food teas containing such ingredients. The following calculations show potential consequences of the HMPC draft for marketed products.	Smith *et al.* (2002): In the publication it is written: "No studies have been reported on the levels of DNA adducts in target or non-target tissues for methyl eugenol, estragole or safrole at typical levels of human exposure." "However, chemical characterization of this reaction and information on the concentration at which these biochemical events initiate hepatotoxicity are as yet unknown. Additional dose-response and metabolism data at dose levels (<10 mg/kg bw) would provide key data for a physiologically-based pharmacokinetic model." After discussion with SWP the non-linearity was not seen proven. For excipients (essential oils), it would be possible to remove estragole.

		Fennel oil and Star Anise Oil The following calculations are based on the HMPC monograph by comparison of estragole exposure with food intake. One indication for Fennel oil is the symptomatic treatment of cough associated with cold. This is clearly a self-limiting indication, and the use is restricted to two weeks in the HMPC monograph. Based on the dosage recommended in the HMPC monograph (200 µl per day ≈ 180 mg) the daily estragole intake would be 9 mg assuming 5% estragole (worst-case) in the oil or 180 µg/kg b.w., respectively, for an adult person of 60 kg b.w.. 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, i.e., 250 µg/kg b.w. per day based on the information provided in the JECFA assessment of alkoxy benzene derivatives cited by the HMPC (JECFA 2009). 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 b.w. 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-specialized consumer groups.“ By analogy, this reasoning is applicable to the medicinal use of fennel oil as well. Example of a medicinal product with Star Anise oil and Fennel	

		oil Consequences for an existing product are shown in the following: A traditional pharmaceutical preparation against inflammations of the oral mucosa contains Star Anise oil (Anisi stellati aetheroleum with an average content of 3.5% estragole) and Fennel oil (Foeniculi vulgaris aetheroleum with an average content of 3% estragole) in a defined mixture of different other essential oils. The daily dose (20 drops 3 times a day) results in an amount of estragole of 1.5 mg per day. It appears highly questionable whether the estragole contents in the natural essential oils of Fennel and Anise etc. can be further reduced. Herbal teas (Fennel and Aniseed) The following examples show calculations for individual tea products made by manufacturers of these products. <u>Company 1*)</u> Medicinal Tea Fennel Tea (100%) 2.2 g filterbag Cough-Cold Tea (27.5% Aniseed) 2.0 g filterbag Cough-Cold Tea (30% Aniseed) 1.5 g filterbag Stomach Tea (20% Fennel, 20% Aniseed) 1.8 g filterbag Food Tea Herbal Tea (58% Fennel; 13% Aniseed) 1.75 g filterbag Herbal Tea (60% Fennel, 20% Aniseed) 2.0 g filterbag COMPARATIVE LABORATORY TEST (BAH 2019)	

		Medicinal Tea Whole Fennel Whole Fennel, freshly crushed Fine-cut Fennel 2.0 g filterbag (1) Fine-cut Fennel 2.2 g filterbag (2) Fine-cut Fennel 2.2 g filterbag (3) *) Calculation is based on transition rates determined individually for each product. <u>Company 2</u> Fennel Tea 1 Traditional herbal medicinal product for use in mild spasmodic discomfort in the gastrointestinal area with a feeling of fullness and bloating and for mucus release in the case of cough in connection with a cold. Tea bags containing 2.0 g of bitter fennel fruits for preparation of a tea infusion in about 150 ml of boiling water. In practice 1 g fennel fruit contain max. 0.15% of estragole* corresponding to 1.5 mg. Transfer rates between 3.2% and 4.8% have been determined for this product regarding estragole. A calculation with 5% transfer rate is used as a worst-case scenario which corresponds to 0.15 mg / cup of tea. Adults and adolescents from the age of 12: 3 times a day one cup of tea corresponding to a max. intake of 0.45 mg estragole per day. A physician has to be consulted, if symptoms do not improve after 14 days. Children from 4 to 11 years drink 2 times a day one cup of tea	

		corresponding to a max intake of 0.3 mg estragole per day. A physician has to be consulted, if symptoms do not improve after 7 days. Children from 6 months to 3 years drink one cup of tea 1 to 2 times a day corresponding to a max intake of 0.15 mg to 0.3 mg estragole per day. A physician has to be consulted, if symptoms do not improve after 7 days. *According to the Ph. Eur. monograph of "fennel, bitter" the content of essential oil in bitter fennel fruits is min. 4.0%. Estragole content of essential oil is max. 5.0%. Assuming a very high content of 6.0% essential oil and the max content of estragole leads to a theoretical worst case of 0.3% of estragole content in fennel fruits. This clearly overestimates values in real life. Considering the real values of batches that have been marketed over the last six years the worst case is instead represented by 0.15% of estragole content in fennel fruits. Calculations in this document are therefore based on this realistic worst-case scenario for estragole content of bitter fennel fruits. Fennel Tea 2 Herbal medicinal product for the treatment of gastrointestinal complaints and respiratory diseases. It is used for indigestion like light, spasmodic gastrointestinal discomfort, bloating and flatulence and in case of catarrhs of the upper airways. Tea bags containing 2.5 g of bitter fennel fruits for preparation of a tea infusion in about 150 ml of boiling water. In practice 1 g fennel fruit contain max. 0.15% of estragole* corresponding to 1.5 mg. Transfer rates between 3.2% and 4.8% have been determined for this product regarding	

		estragole. A calculation with 5% transfer rate is used as a worst-case scenario which corresponds to 0.1875 mg estragole / cup of tea. Adults, adolescents and children: 2 to3 times a day one cup of tea corresponding to a max intake of 0.375 mg to 0.5625 mg estragole per day. Do not take this product without medical advice if the symptoms persist for more than 7 days or return periodically. Tea mixture consisting of Fennel fruit, Anise fruit and Caraway fruit Herbal medicinal product for gastrointestinal complaints. It is used for gastrointestinal complaints such as feeling of fullness, flatulence and mild spasmodic gastrointestinal disorders as well as nervous heart and stomach complaints. Tea bags containing 2.0 g of a mixture of bitter fennel fruits (0.6 g), anise fruits (0.6 g) and caraway fruits (0.8 g) for preparation of a tea infusion in about 150 ml of boiling water. In practice 1 g fennel fruit contain max. 0.15% of estragole* corresponding to 1.5 mg. 1 g anise fruits contain max 0.2% of estragole corresponding to max 2 mg. Considering a transfer rate of 5% results in a daily intake of 0.105 mg estragole / cup of tea with one tea bag and 0.21 mg estragole / cup of tea with two tea bags. Adults and adolescents from the age of 12: serval times (i.e. 3 to 4 times) a day between the meals one to two tea bags in one cup of tea corresponding to an intake of 0.315 mg to 0.84 mg of estragole per day. Do not take this product without medical advice if the symptoms persist for more than 7 days or return periodically.	

		Tea mixture consisting of anise fruits, marshmallow leaves, marshmallow root and liquorice root This herbal medicinal product is used to relieve irritation in the case of inflammation of the mucous membranes of the upper airways and associated dry cough. Tea bags containing 1.75 g of a mixture of anise fruits (0.26 g), marshmallow leaves (0.61 g), marshmallow root (0.44 g), and liquorice root (0.44 g) for preparation of a tea infusion in about 150 ml of boiling water. 1 g anise fruits contain max. 0.2% of estragole corresponding to max. 2 mg. Considering a transfer rate of 5% results in a daily intake of 0.026 mg estragole / cup of tea. Adults and adolescents from the age of 12: 3 times a day one cup of tea corresponding to an intake of 0.078 mg of estragole per day. Cough tea for children This herbal medicinal product is used for symptoms of bronchitis and to relieve irritation in catarrhs of the upper airways with dry cough Tea bags containing 1.5 g of a mixture of anise fruits (0.6 g), linden flowers (0.6 g) and thyme (0.3 g) for preparation of a tea infusion in about 150 ml of boiling water. 1 g anise fruits contain max. 0.2 % of estragole corresponding to max. 2 mg. Considering a transfer rate of 5% results in a daily intake of 0.06 mg estragole / cup of tea. Children from 1 to 4 years drink 2 to 3 times a day one cup of tea corresponding to an intake of 0.12 mg to 0.18 mg of estragole per day.	

		Children from 4 to 10 years drink 3 to 4 times a day one cup of tea corresponding to an intake of 0.18 mg to 0.24 mg of estragole per day. Children from 10 years on drink several times (3 to 5 times) a day one cup of tea corresponding to an intake of 0.18 mg to 0.30 mg of estragole per day. Do not take this product without medical advice if the symptoms persist for more than 7 days or return periodically. Excipients Other medicinal products are already on the market for a long time with Star Anise oil or Fennel oil as an excipient. Current medicinal products have already considered a threshold which normally can be accepted within the given dosage. A further restriction, however, would not be possible for a lot of products containing natural oil. As a consequence, a change in the composition with all efforts included are necessary. This may result in the loss of some of the products. The following examples show calculations for individual products containing Star anise oil (obtained by steam distillation from the dry ripe fruits of *Illicium verum* Hook. fil., percentage of estragole in Star anise oil between 0.5% and 6.0% according to Ph. Eur.) Company 3 Lozenges 1 (preparation in the German market) The product contains 4.7 mg of Star anise oil per tablet as flavour. With the maximum daily dose of 6 tablets the daily amount of Star anise oil is 28.2 mg.	

		Within the range of the share of estragole in Star anise oil the minimum amount is between 0.14 mg of estragole per day (Star anise oil with 0.5% of estragole) and 1.7 mg of estragole per day (Star anise oil with 6.0% of estragole). Lozenges 2 (preparation in the German market) The product contains 2.5 mg of Star anise oil per tablet as flavour. With the maximum daily dose of 6 tablets the daily amount of Star anise oil is 15.0 mg. Within the range of the share of estragole in Star anise oil the minimum amount is between 0.075 mg of estragole per day (Star anise oil with 0.5% of estragole) and 0.9 mg of estragole per day (Star anise oil with 6.0% of estragole). **Conclusion** In the second draft of the public statement on the use of herbal medicinal products containing estragole the recommendation for a body weight of 50 kg is 0.05 mg of estragole per day. This limit is exceeded for both products even by a Star anise oil containing the minimum content of estragole according to Ph. Eur. Since both products are widely used as OTC products with a proven acceptance and tolerance in the indication "sore throat", this limitation would reduce their use dramatically. In the view of the fact that these products are used only for a restricted time (normally 1 to two periods of 3-4 days treatment within two cold periods per year) this limitation seems - especially in comparison to the burden due to the food intake - not justified.	
		Overall Conclusion Based on the totality of toxicological data available today	Not endorsed.

		estragole can be considered a gentoxic carcinogen. However, the evidence from carcinogenicity studies points to a significant contribution to the observed tumour formation of non-genotoxic, i.e., hepatotoxic effects at the high doses tested. While adequate information on the dose-response relationship of tumour formation is still missing, there is an increasing body of data supporting the hypothesis that the initial key events of its genotoxic effects deviate from a linear dose-response at dose levels relevant for human intake from herbal medicinal products. Therefore, the derivation of an ADI from very limited carcinogenicity/dose response data obtained in 1976 comprising only two carcinogenic dose levels, by application of the utmost conservative algorithm for a safety margin, appears both unsatisfactory and disproportionate against that background. Clearly there is a need for further data providing a broader basis for quantitative risk assessment. Therefore, and taking into account the predominantly very limited duration of use of estragole-containing herbal medicinal products, an interim limit for estragole in herbal medicinal products as recommended by Professor Schrenk in his expert report would appear adequate and proportionate (Schrenk, 2020). Based on an assessment using a $BMDL_{2x}$-value of 5 mg estragole/kg b.w. per day and an uncertainty factor of 100 (accounting for inter- and intraspecies variability), a TDI of 50 µg/kg b.w. and day leading to a maximum daily intake of 2.5 mg estragole per day for adult persons is proposed. What´s more, robust information regarding dietary exposure to estragole – an important element to consider as stipulated by the ICH M7 guideline – is currently lacking.	New dose-response data could not be found. The publication (submitted later) from Schulte-Hubbert *et al*. [Schulte-Hubbert R, Küpper JH, Thomas AD, Schrenk D. Estragole: DNA adduct formation in primary rat hepatocytes and genotoxic potential in HepG2-CYP1A2 cells. Toxicology 2020; 444: 152566] provides evidence from *in vitro* studies that there may be a threshold below 1 µM estragole for DNA adducts. However, *in vitro* MNT, which recognizes DNA damage as a consequence of the adducts, could not be evaluated at low concentrations below 1 µM due to high variances as pointed out in the publication. The authors finish the paper themselves with the sentence: "*Thus the default assumption of dose-linearity of carcinogenicity of estragole strongly based on dose-response data at high concentrations/doses is probably inadequate, at least based on our in vitro findings, and needs to be replaced by a thorough dose-response analysis at relevant dose levels including additional in vivo studies*." This means that *in vivo* experiments with correspondingly low doses are needed to possibly derive a threshold. It should also be noted that DNA-adducts as such are to be seen as alert, not as a stand-alone point for quantitative derivations. It is acknowledged that there is DNA repair taking place and it is also likely that a threshold exists in humans. However, it cannot be deduced from the

		An immediate implementation of the currently drafted HMPC recommendations into regulatory practice would have dramatic consequences for marketed products from estragole-containing essential oils and herbal drugs, particularly those used in sensitive patient groups like pregnant and breastfeeding women as well as children. In the light of the aforesaid and the additional data provided with the collaborative laboratory study on estragole transfer rates and with the expert report of Prof. Schrenk, including new dose response data on adduct formation, a re-consideration of the conclusions of the (2nd) Draft Public Statement on the Use of Herbal Medicinal Products Containing Estragole by the HMPC would appear well-founded.	reported data at what concentration the threshold value can be established in humans.
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