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Committee on Herbal Medicinal Products (HMPC)

Overview of comments on draft Public statement on the use of herbal medicinal products containing pulegone and menthofuran (EMA/HMPC/138386/2005 Rev. 1)

Final

Table 1: Organisations and/or individuals that commented on the draft public statement on the use of herbal medicinal products containing pulegone and menthofuran as released for public consultation on 24 November 2015 until 31 March 2015.

	Organisations and/or individuals
1	Association of the European Self-Medication Industry (AESGP)
2	Association for Natural Medicine Europe e.V. (ANME)
3	European Directorate for the Quality of Medicines (EDQM)
4	European Food Safety Authority (EFSA)
5	European Confederation of Pharmaceutical Entrepreneurs (EUCOPE)
6	Frey + Lau GmbH, Germany
7	Johnson & Johnson, United Kingdom, Ireland
8	Tillotts Pharma AG, Switzerland
9	Dr. Willmar Schwabe GmbH & Co. KG, Germany

30 Churchill Place ● Canary Wharf ● London E14 5EU ● United Kingdom
Telephone +44 (0)20 3660 6000 **Facsimile** +44 (0)20 3660 5555
Send a question via our website www.ema.europa.eu/contact

General considerations upon comments

1. Effects of single ingredients may be modified in the complex herbal preparation ("matrix effect")

A general theme in responses is the statement that 'complex herbal preparations in their entirety are considered as active pharmaceutical ingredients (API). Hence, the toxicity of these substances needs to be analysed in its entirety and must not be reduced to single ingredients.' This concept has been used and is still being used to downplay findings of single components. With emerging concepts of systems or network pharmacology there seems to be, at least theoretically, some promise in this 'entirety' claim (see e.g. recent research on TCMs), but there is currently very little experimental or clinical evidence to support the 'entirety' claim. At present, speculation on matrix effect resulting in an absence of toxicity by pulegone in Mentha preparations remains rather hypothetical.

2. Are pulegone / menthofuran genotoxic?

The overall conclusion remains that despite some marginal positive observations, genotoxicity of pulegone/menthofuran has not been convincingly demonstrated. The draft public statement (dPS) stated that conventional genotoxicity tests may not be adequate for proving or disproving genotoxicity potential of short-living reactive metabolites formed in the liver (or bladder) and suggested that liver- and bladder-derived tests such as the Comet assay or transgene assays would provide a more convincing outcome.

However, reactive metabolites of this kind would most likely be disarmed by glutathione conjugation and thus be capable of binding to DNA only during prolonged and high exposure, when glutathione resources are depleted.

To address the problems in the available genotoxicity tests, i.e. their appropriateness vis-à-vis short-lived liver-produced reactive metabolites a GLP study (Boehringer Ingelheim International GmbH, 2015) was conducted in which oral doses of 187.5, 375 and 750 mg/kg peppermint oil (European Pharmacopeia quality containing 1.9% pulegone and 3.7% menthofuran), or 75, 150 and 300 mg/kg pulegone or menthofuran were given 3 times to 5 female Crl:CD (SD) rats, respectively. The combined bone marrow micronucleus test and the Comet assay with liver, kidney and urinary bladder urothelium as target organs were performed according to appropriate OECD guidelines. In the Comet Assay, there was no dose-dependent increase in DNA strand breaks in the liver, kidney and urinary. Menthofuran exposure did also not result in Comet induction in kidney and urinary bladder. However, statistically significant slight increases in the median tail intensity were observed in the liver cells of animals treated with 150 and 300 mg/kg/day menthofuran. The dose-dependent increase in hedgehog "ghost" cells observed in animals administered menthofuran across all tissues (predominantly liver) indicates cytotoxicity. In the micronucleus analysis, peppermint oil, pulegone and menthofuran did not cause any increase in the induction of micro nucleated polychromatic erythrocytes or bone marrow cell toxicity.

Despite some (weak) positive findings in some studies the overall conclusion is that pulegone and menthofuran do not possess genotoxic potential.

3. Selection of a proper study for setting NOAEL

In the dPS, the calculation of limit values of pulegone/menthofuran is based on a proposed LOAEL at 20 mg/kg/day (more exactly, 18.75 mg/kg/day) for the presence of hyaline glomerulopathy in the NTP rat carcinogenicity study. However, males and females were administered different doses. Only males received 18.75 mg/kg/day as the low dose, and at this dose level no hyaline glomerulopathy was observed. Thus this dose level is a NOAEL, but only in males. At the low dose in females (37.5 mg/kg/day) hyaline glomerulopathy was present; thus this was the LOAEL in females.

However, the main aim of rodent carcinogenicity studies is to determine the carcinogenic potential of a chemical substance. Although non-neoplastic findings are recorded in a lifetime rodent bioassay their incidence and character may be influenced by the age of the animals and increasing spontaneous background pathology. Therefore a subchronic repeat-dose toxicity study is considered more suitable for the evaluation of non-neoplastic toxicological effects. However, it can be argued that potentially severe toxicological findings in a chronic study should be taken into consideration. In the case of pulegone and menthofuran, even considering in the public statement that rodent carcinogenicity findings may not be very relevant to human risk assessment, a doubt of genotoxicity remains (see above), and this would argue for adopting a NOAEL value of the chronic study.

With an additionally provided in-vivo Comet assay the remaining uncertainty regarding short-lived, DNA-reactive liver metabolites was seen to be resolved therefore a threshold approach when calculating limits of pulegone/ menthofuran in medicinal products seemed to be justified. This is in line with the revised version of IHC M7, which opens up for a practical threshold for DNA-reactive compounds whose effects may be modulated by rapid detoxification or effective repair of induced damage. Therefore the NTP 3-month repeat-dose toxicity study in rats is considered the most relevant study for establishing a NOAEL.

4. Setting the limit value

The doses of pulegone in the NTP 3-month rat study were 0, 9.375, 18.75, 37.5, 75 and 150 mg/kg/day. The toxicologically most important findings were hepatotoxicity and renal hyaline glomerulopathy. Both had a NOAEL of 37.5 mg/kg/day. The only histopathological finding at 37.5 mg/kg/day was bone marrow hyperplasia in males. This finding can be linked to the presence of a small, dose-related decrease in red blood cell parameters and increased reticulocyte counts. Thus the finding of bone marrow hyperplasia is considered to be a secondary, adaptive response and non-adverse. At the lower dose levels, there were increased liver and kidney weights, which are not considered to be adverse effects. The NOAEL of 37.5 mg/kg bw/day is the most reliable and relevant dose level to use for the limit calculations.

Applying a NOAEL of 37.5 mg/kg/day, and an uncertainty factor of 50, results in a limit dose of 0. 75 mg/kg/day, or 37. 5 mg in a 50 kg person for a life-long exposure. For treatment durations of less than 1 year an intake (pulegone + menthofuran) of 75.0 mg/day can be accepted.

5. Sum limit dose value for pulegone and menthofuran

Some stakeholders commented that it is not possible to use a sum parameter for pulegone and menthofuran, because toxicological potency of menthofuran in rats was only about one third to one half of that of pulegone (Thomassen *et al*., 1988). The stakeholders suggest an adjustment factor of 0.5 for menthofuran.

The complexity of metabolism and metabolic activation of pulegone and menthofuran are amply addressed in the PS. Also the latest adduct and metabolomics studies (Rousu *et al*., 2009; Li *et al*., 2011) demonstrating these complexities and also the potential role of menthofuran is described in chapter 2.3 of the PS. In terms of potency, hepatotoxicities of pulegone and menthofuran in rats are similar enough to create a sum parameter. This is not to deny that there are other toxification pathways than metabolism of pulegone to menthofuran.

Table 2: Discussion of comments

General comments to draft document

Interested party	Comment and Rationale	Outcome
AESGP	The entire assessment in the Public Statement is clearly related to pulegone and menthofuran. We are of the opinion that this Public Statement should not generally relate to herbal medicinal products containing these substances because the properties of individual components cannot be uncritically attributed to multi-component mixtures containing the respective substances. Moreover, new data is only available for pulegone and not for menthofuran. Thus an explicit differentiation regarding toxicology and acceptable exposure limits of both individual substances is required from our point of view.	Not endorsed. Although it can be assumed that multicomponent matrix may affect the behaviour and effects of individual components, there is no reason to neglect potential adverse outcome of these individual components even if these adverse outcomes have been studied as isolated single substances. Not endorsed. Menthofuran, besides being a genuine substance in herbal medicinal products and other plant-derived commodities, is an abundant metabolite of pulegone in humans and animals in in-vitro and in-vivo conditions. Both pulegone and menthofuran are converted into reactive metabolites by the liver enzymes. Exposure to pulegone means always also the exposure to menthofuran. Consequently, it is counterproductive to separate pulegone and menthofuran from the risk assessment point of view.
AESGP	**Options to reduce the content of pulegone and menthofuran** Significant reduction of the content of pulegone and menthofuran is technically difficult, if at all possible. Moreover, the essential oil would be significantly modified. Such	Measures to reduce content of pulegone and menthofuran are outside the scope of this public statement.

Interested party	Comment and Rationale	Outcome
	modifications would not be in line with the Ph.Eur. monograph requiring a minimum content for menthofuran (span of 1-8%) and with the HMPC monograph requiring use of peppermint oil according to the Ph.Eur. monograph. It is doubtful that the contents of pulegone and menthofuran in natural Peppermint oil can be reduced or kept on this low level. Therefore the future of well-known preparations which have been extensively used for decades in some countries is considered to be uncertain.	
Johnson & Johnson McNeil	**Safety Summary of Clinical Use of Peppermint Oil** **Conclusion** Peppermint oil is an effective treatment for symptoms of IBS. The product is generally well-tolerated at the approved doses for treatment duration up to 3 months. Post-marketing safety data shows that benefit/risk ratio in patients with irritable bowel syndrome is positive.	The aspects of benefit risk assessment are taken into account in relevant individual products and related EU herbal monographs. The ability of post marketing surveillance and clinical experience to detect signals related to genotoxicity or carcinogenicity is rather low.
Tillotts Pharma AG	In the draft Public statement (dPS), a rodent study is identified as pivotal study (NTP 2011), a LOAEL value is extracted from the corresponding study results, and a safety factor of 300 is applied. Eventually a limit value of 3.5 mg per person and day (pulegone + menthofuran) is determined based on the finding that menthofuran is a major metabolite of pulegone. The limit derived based on the proceeding outlined above should, however, not directly and automatically be applied to any herbal medicinal product. We propose instead that **all** evidence that is suitable to contribute to the assessment of the safety of a specific product be considered, including the established safety profile of existing herbal medicinal products. This approach is in line with the principles applied in the recent ICH Guideline for Elemental Impurities (ICH 2014). Such principles dictate that, amongst other factors to be considered when establishing PDEs (Permitted Daily Exposures), both human exposure and safety data, in addition to the most relevant animal study, have to be considered (see	A new NOAEL value (37.50 mg/kg bw) based on the NTP 3-month study is taken as a starting point of the calculation of the limit value (*see #3 in the general considerations*).

Interested party	Comment and Rationale	Outcome
	ICH 2014, p. 2). All “qualified by use”-concepts that are in particular applicable to generics rely on this principle (EMA 2007, p.5). If the limit stated in the dPS was applied as sole criterion for defining the safe use of pulegone- and/or menthofuran-containing herbal medicinal products, many products would have to be discontinued. Peppermint oil products with a well-established use that are effective and that have a favourable side effect profile (Vanuytsel 2014) would not be available to patients any more. Instead, use of anticholinergics to treat Irritable Bowel Syndrome (IBS) symptoms would increase, including their known side effects such as constipation, dry mouth, visual disturbances and urinary retention (Occhipinti 2012). Even by restrictions in the duration of use, e.g. introduction of a time limit of 14 days for short-term intake, a meaningful medical therapy in certain indications (e.g. IBS) would become doubtful.	
Tillotts Pharma AG	Herbal preparations like peppermint oil are complex mixtures of natural constituents. Care should be taken when extrapolating the toxicological findings obtained with isolated constituents to the toxicological properties of the entire herbal preparation. The matrix contained in the herbal preparation may allow cellular protective mechanisms to prevail, especially at low exposure levels (see also p. 11-12 of dPS). This hypothesis is supported by the good safety profile of herbal medicinal products containing peppermint oil or mint oil (see also chapter 2.4 of dPS, no confirmed cases of liver damage reported), even though pulegone was recently classified by IARC as “possibly carcinogenic to humans (2B)” (Grosse, 2013). Tillotts Pharma conducted three genotoxicity studies with peppermint oil (Ph. Eur. grade), namely an in vitro bacterial reverse mutation study (Ames test), an in vitro mammalian cell gene mutation study (mouse lymphoma assay), and an in vivo mammalian study (rat bone marrow micronucleus test) (Tillotts Pharma AG, 2012-2013). The rat bone marrow micronucleus test provided unequivocal evidence of a lack of genotoxicity for peppermint oil when administered orally. Overall, the three tests demonstrated that peppermint oil	*See #1 in the general considerations.* *See #2 in the general considerations*.

Interested party	Comment and Rationale	Outcome
	shows no potential for genotoxic effects. Such results from toxicological studies performed with essential oils should be regarded as of higher relevance to herbal medicinal products containing such oils than results obtained with individual constituents. Application of a safety factor of less than 300 should be considered as more appropriate for essential oils. Cumulative data from post-market surveillance (PMS) (Jan 1996 – Feb 2015) do not reveal any safety signal for Colpermin™ (gastro-resistant capsules for oral use), the peppermint oil product of Tillotts Pharma. Moreover, the absence of a potential for genotoxic effects of peppermint oil (Tillotts Pharma AG, 2012-2013) is reflected in a lack of evidence of toxic or even carcinogenic effects in the PMS data. All in all, a balanced and pragmatic approach should be followed as described in pertinent guidelines (see p. 5 of EMA 2006). This encompasses discussion of the relevance of data on isolated constituents for the assessment of a herbal preparation and additional non-clinical testing, if applicable, but also the awareness that the documented experience gathered during the long-standing use should be the main basis of the non-clinical assessment of well-established herbal medicinal products.	The ability of post marketing surveillance to detect signals related to genotoxicity or carcinogenicity is rather low.
Tillotts Pharma AG	Based on the absence of safety signals from human use, a sufficient transition period should be foreseen for herbal medicinal products covered by the dPS. This is required to develop modifications of the manufacturing process to reduce the levels of pulegone and menthofuran in peppermint oil and to create the necessary data for a variation. In this context it should also be considered that the boiling points of menthofuran and menthol are very similar (211 °C and 212 °C, respectively). Therefore, a fractional distillation process alone will most likely not suffice for achieving removal of menthofuran from peppermint oil to comply with the levels set forth in the dPS.	Measures to reduce content of pulegone and menthofuran or definition of transition periods are outside the scope of this public statement.
Schwabe	The opportunity to comment on the draft public statement on the use of herbal medicinal	A new NOAEL value (37.5 mg/kg bw) based on the NTP 3-month study is taken as a

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	products containing pulegone and menthofuran is appreciated. Having evaluated all available preclinical and clinical data, we strongly disagree with the HMPC's risk assessment and the proposed limitation to a daily intake of 3.5 mg pulegone + menthofuran mg/person/day from herbal medicinal products containing peppermint oil and the limitation of treatment to a maximum of 14 days. The rationale for the proposed limitations is exclusively based on equivocal non-clinical data on pulegone and entirely disregards the comprehensive clinical and post-marketing experience with medicinal products that are authorised in the European Union and widely used. However, human data have much more relevance for the safety assessment of medicinal products. Moreover, important aspects such as dietary (background) exposure, and normal physiological metabolic pathways should be taken into consideration, in particular. A detailed argumentation is provided below. Based on our assessment of all available data and in view of the lower toxicity of menthofuran compared to pulegone, a maximum daily intake of 20 mg/person/day can be accepted as safe for herbal medicinal products for short to medium term intake (maximum 3 months). In view of the safe use of peppermint oil products under the strict European pharmacovigilance legislation over decades, it would not appear that "focused pharmacovigilance" or "increased awareness in the medical community" provides any additional benefit. **We would therefore kindly request the HMPC to re-consider the proposed limitations and regulatory actions.**	starting point of the calculation of the limit value. (*see #3 in the general considerations*).

Specific comments on text

Interested party	Comment and Rationale	Outcome
Introduction (problem statement)		
AESGP Lines 29-35	**Data from isolated substances cannot be transferred to complex mixtures such as peppermint oil** The title of the public statement relates to safety issues regarding herbal medicinal products containing pulegone and menthofuran. However, not a single study cited concerns an herbal medicinal product (HMP) in terms of its toxicological effects, but rather the statement focuses only on two of many more substances contained in concerned HMPs. In accordance with their established status, complex herbal preparations in their entirety are considered as active pharmaceutical ingredients (API). Hence, the toxicity of these substances needs to be analysed in its entirety and must not be reduced to single ingredients. Taking into account the complexity of HMPs, interactions between the varieties of constituents should not be neglected. Accordingly, it is established practice in toxicology that risk assessment for complex mixtures of chemicals should be performed with the entire mixture whenever it is readily available (Groten *et al.,* 2001). For example, in this context, it has been reported that menthol, a major constituent of peppermint oil, has potent antioxidant activity (Yang *et al.,* 2010) and upregulates glutathione (Bhadania *et al.,* 2012; Rozza *et al.,* 2014). By both of these modes of action it is to be expected that menthol will counteract potential toxic effects of pulegone and menthofuran.	Not endorsed *See #1 in the general considerations*.
ANME	The introduction refers to the definition of pulegone as a potential hepatotoxin, with no clear NOEL defined. The Public Statement therefore describes the call for more data on the oral toxicity and genotoxicity of pulegone and menthofuran. It would appear that the focus on the effects of pure substances may be misleading, especially when at the end of the debate a conclusion is drawn which does not take into account that peppermint oil and mint oil are not pure pulegone and menthofuran but complex multicomponent mixtures. Experience over many decades has never shown a	*See #1 in the general considerations*.

Interested party	Comment and Rationale	Outcome
	problem with the recommended dose schemes of peppermint oil and mint oil. Pulegone and menthofuran as pure substances may display toxicity different from that encountered in essential oils. A recent example was published by Escobar *et al*. (2015) [1].	
EUCOPE	**Data of isolated substances cannot be transferred to multicomponent mixtures such as peppermint oil** Even though the HMPC Draft Public Statement is related to safety issues of herbal medicinal products containing pulegone and menthofuran, not a single scientific publication is considered dealing with the toxicological assessment of such products or active substances, respectively, such as peppermint oil. Active substances of herbal medicinal products are complex plant extracts and should therefore be regarded in its entirety from a toxicological perspective and not as single, isolated substances. In the latter case possible interactions between the various compounds would be neglected (Groten *et al*., 2001)	*See #1 in the general considerations.*
Frey + Lau	**1. Matrix effects of Pulegone and Menthofuran in Mint and Peppermint oils** There are two important active pharmaceutical substances containing pulegone and menthofuran which are used in herbal medicinal products: Mint oil, partially dementholised (Ph. Eur. 1838) and Peppermint oil (Ph. Eur. 405). In contrast to Pennyroyal oil, which is not used in herbal medicinal products, there is no hint on potential cases causing liver damages from mint and peppermint essential oils. In contrast there is literature providing evidence, that the complex matrix of those natural complex substances might be even liver protective. (*see* Lacroix M; Caillet S; Lessard S. UMU applied for screening herb and plant extracts or pure phytochemicals for antimutagenic activity. Pharmaceutical Biology; 50 (5); p.537, May 2012). Moreover former investigations have shown that the toxicity of pulegone is suppressed in the presence of menthone. (*see* Franzios G, Mirotsou M, Hatziapostolou E, Kral J; Scouras ZG,	*See #1 in the general considerations*. Matrix effects are certainly possible, but they could be into both directions, antagonistic, inhibitory, or additive, synergistic, activating or potentiating.

Interested party	Comment and Rationale	Outcome
	Mavragani T. Insecticidal and genotoxic activities of mint essential oils. Journal of Agricultural and Food Chemistry (1997), p.2690-2694). This corresponds to tests performed on the essential oil matrices which are listed under REACH: **Mint oil** Key study: not mutagenic in Ames test, Notox, 2010, one supporting study: (Salmonella typh. and Bacillus subtilis, vehicles: DMSO + water). Result: negative in Salmonella for both vehicles, negative for Bacillus (water), positive for Bacillus (DMSO). Conclusion: 100 mg/mL. Rel 2 (based on abstract), Morimoto, 1982 http://apps.echa.europa.eu/registered/data/dossiers/DISS-dffb4072-e3b4-47ae-e044-00144f67d031/AGGR-a7f40826-b7c4-4a96-aab1-b17311d9ce36_DISS-dffb4072-e3b4-47ae-e044-00144f67d031.html#AGGR-a7f40826-b7c4-4a96-aab1-b17311d9ce36 **Peppermint oil** Key study: Ames: negative. Rel 1 (based on abstract), Supporting study: Lorillard, 1983 (supporting Andersen, 1984) http://apps.echa.europa.eu/registered/data/dossiers/DISS-dffb4072-e430-47ae-e044-00144f67d031/AGGR-961f3626-6550-4117-86f8-87a58e2fe1ce_DISS-dffb4072-e430-47ae-e044-00144f67d031.html#AGGR-961f3626-6550-4117-86f8-87a58e2fe1ce All the above suggest that synergistic or antagonistic phenomena may be involved that alter the toxicity of the whole essential oil in comparison to the single compounds pulegone and menthofuran.	
AESGP Lines 47-51	**Clinical signals related to liver-damaging effects of peppermint oil do not exist** Post-marketing authorisation surveillance did not reveal cases of liver toxicity in humans by consumption of peppermint oil or mint oil (Jaeger 2015), neither in the existing Public	Not endorsed Post-marketing surveillance is not very sensitive to detect signals of chronic,

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	Statement (EMEA/HMPC/138386/2005) nor ever since. The same applies to bladder tumours. The only reported cases of liver toxicity were observed following inappropriate use of pennyroyal oil (*Mentha pulegium*: pulegone content 62-97%). A review of 18 documented case reports of pennyroyal oil intoxication established moderate to severe toxicity only in patients who had been exposed to at least 10 ml of the oil (Anderson *et al*., 1996). This dose corresponds to about 5.6-8.7 g pulegone (ca. 112 -174 mg/kg bw for a 50 kg person) when calculated with a relative density of 0.9 for pennyroyal oil. This dose exceeds the now suggested threshold by a factor of 1600-2500. At present the safety assessment of pulegone in the Public Statement is entirely based on animal tests although it is known that their predictability is often poor and their relevance is limited. After extended and safe human use of peppermint oil, it appears inappropriate to use only experimental data as core information for risk assessment. The best opportunity to generate true evidence is to match all available information. Instead, cross matching methodology that combines the different fields of knowledge and types of data (e.g. in vitro and in vivo experiments, clinical observations, clinical and epidemiological studies, and daily life observations) should be applied and would give adequate weight to individual findings (Heinonen & Gaus, 2015).	hideous toxicity outcomes such as chronic liver injury. It has to be noted, however, that mechanisms of liver toxicity, production of reactive intermediates and their targets, are similar in experimental animals and humans and it remains to be shown that humans may be relatively more resistant to liver toxicity due to some protective factors. The cross matching approach is well known but it is not considered to be that different from WOE approach used in the dPS. It should be mentioned that some of the basic criticisms Heinonen & Gaus (2014) expressed in their analysis (Gaus, 2014; Heinonen & Gaus, 2015) concerning *Ginkgo biloba* have recently been addressed and refuted (Kissling *et al*., 2015). In the analyses of the dPS, ample space has been given to relevance and predictability of observations in animal experiments. Concerns created by animal toxicities can be neglected only after careful analysis of evidence-based pros and cons. Gaus, W., 2014. Which level of evidence does the US National Toxicology Program provide? Statistical considerations using the Technical Report 578 on Ginkgo biloba as an

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		example. Toxicol. Lett. 229 (2), 402–404. G.E. Kissling *et al*., 2014. Proper interpretation of chronic toxicity studies and their statistics: A critique of "Which level of evidence does the US National Toxicology Program provide? Statistical considerations using the Technical Report 578 on Ginkgo biloba as an example", Toxicol. Lett. http://dx.doi.org/10.1016/j.toxlet.2014.09.016
Pulegone and menthofuran in plants and plant preparations		
ANME	The quality of peppermint oil and of mint oil (partly dementholised) is defined by monographs in the European Pharmacopoeia. To recapitulate: The monograph on peppermint oil [2] defines a maximum of 3.0% pulegone and a range of 1.0 to 8.0% menthofuran. The monograph on mint oil (partly dementholised) [3] defines a maximum of 2.5%pulegone, but does not give values for menthofuran. These quality definitions must be met by oils used as active substances in medicinal products. The parameters cannot be easily changed, as then the quality of the active pharmaceutical ingredient would no longer correspond to the definition of the European Pharmacopoeia: Such APIs would be considered new active constituents for which the clinical experience obtained over many decades would no longer be applicable. The pulegone exposure is a function of dosing. The applied dose of peppermint oil and of mint oil is, however, also defined by monographs. The Community Herbal Monograph on *Mentha x piperita* aetheroleum (EMEA/HMPC/349466/2006) defines the well-established and the traditional use of peppermint oil. Traditional use allows the cutaneous and transdermal application of	The focus of the Public Statement is the toxicological assessment. Measures to reduce content of pulegone and menthofuran are outside the scope of this public statement. The recently published 3-month study in rats using peperina oil (Escobar *et al*., 2015) is considered inferior to the NTP 3-month study based on (i) dietary administration instead of oral gavage (more uncertain exposure); (ii) lack of complete histopathological evaluation (only three tissues examined). In addition, a different rat strain (Wistar) was used in the Escobar study as compared with the NTP studies,

Interested party	Comment and Rationale	Outcome
	peppermint oil, the use for inhalation, and the oromucosal use. In the latter case a daily dose of 2-3 drops (0.08-0.12 ml) 3-4 times daily, corresponding to 0.2-0.5 ml, is defined. Well-established use includes oral use in addition to cutaneous use. The oral dose is defined with 0.2-0.4 ml, up to three times daily, in adolescents and adults. The upper limit would therefore be 1.2 ml daily, as cited in the Draft Public Statement on the use of herbal medicinal products containing pulegone and menthofuran. In Germany, the Commission E monographs defined the average oral dose of mint oil [4] with 3-6 drops daily, and of peppermint oil with 6-12 drops [5]. In addition, both essential oils enjoy the advantage of a facilitated marketing authorisation through a so-called standard marketing authorisation. This monograph defines for mint oil [6]: Oral use for the treatment of functional complaints of the gastrointestinal tract and of upper respiratory tract infections. Dosage: 1-3 times daily 2 drops of mint oil. In the case of peppermint oil the German Standard Marketing Authorisation [7] states an oral use against gastrointestinal complaints, respiratory tract infections and inflammations of the oral mucosa. The dosing scheme is indicated with 2-3 times daily 3-4 drops, i.e., max. 12 drops/day. As described in section 1.2 of the Draft Public Statement, the highest recommended daily dose of peppermint oil would be 1.2 ml daily, corresponding to an upper limit of 1099 mg/day, and containing up to 32.97 mg pulegone and up to 87.92 mg menthofuran. From these figures an exposure of a 60 kg person of up to 0.540 mg/kg pulegone and 1.46 mg/kg of menthofuran was derived, calculated using the limits defined by the European Pharmacopoeia. This already exceeds the TDI of 0.1 mg/kg established by the Committee of Experts on Flavouring Substances (CEFS). So far the situation described in section 1.2 of the Public statement. Similarly, the exposure can be calculated for mint oil. In the case of the German-	which used F344 rats.

Interested party	Comment and Rationale	Outcome
	authorized medicinal product JHP Rödler, 1 g of fluid (consisting of 95% mint oil and 5% ethanol 96%) corresponds to 36 drops. The daily dose of six drops according to the German Commission E monograph would therefore correspond to 1/6th of 1 gram = 167 mg of mint oil preparation or approximately 160 mg of mint oil. With a maximum of 2.5% of pulegone, this quantity corresponds to an exposure of 4 mg of pulegone per day. As menthofuran is not specified in the European Pharmacopoeia monograph on mint oil (partly dementholised), the exposure to menthofuran cannot be calculated, but would in any case exceed the newly proposed threshold. This latter calculation was made with the traditional dose scheme, which is already by far lower than the well-established dose scheme of peppermint oil. Even then the conditions of a maximum daily exposure of 3.5 mg pulegone + menthofuran cannot be met. As a consequence, the new proposal on a restriction of pulegone exposure to less than 3.5 mg daily would not be in accordance with the definitions of the Ph.Eur.-monographs on mint oil (partly dementholised) and peppermint oil, as with the quality definition of Ph. Eur. in combination with the dosing recommendations of the HMPC monograph on peppermint oil; the German Commission E monographs and the Standard Marketing Authorisations for mint/peppermint oil, the officially defined dose scheme (dating back to at least 1986) would automatically exceed the proposed exposure limit to pulegone + menthofuran. As the quality of the active ingredients cannot be changed without losing decades of experience with respect to safety of application, the only way to achieve a limit of 3.5 mg/day of pulegone + menthofuran would be a reduction of the individual dose. This, however, would be in contrast with the clinical experience of efficacy documented through the HMPC community herbal monograph, the Commission E monograph and German Standard marketing authorisation. Even in case the doses were to be reduced, the question of feasibility still remains. The well-established use of peppermint oil with 1.2 ml = 1100 mg of peppermint oil can	

Interested party	Comment and Rationale	Outcome
	provide up to 11% of pulegone (max. 8 percent) and menthofuran (max. 3 percent) = max. 121 mg of both compounds daily, which is 34.6 times more than proposed by the Draft Public Statement. Reducing the daily dose by this factor of 34.6 would result in a daily dose of 31.8 mg of essential peppermint oil (= 1.25 drops per capsule and less). The well-established use is based on clinical studies performed with the recommended dose of 1100 mg/day: there is no proof of efficacy with 34.6 mg per day. Correspondingly, a reduction of the dose would at the same time lead to inefficacious products. If, however, the quality were changed, e.g., by extraction procedures taking out the fractions of pulegone and menthofuran, the dose could remain unchanged, but the active pharmaceutical ingredient would no longer correspond to the quality specified in the European Pharmacopoeia. Again, the clinical studies and the tradition of use would no longer be applicable, so the products would be considered new entities by the regulatory authorities. The situation is even worse with the traditional use of mint and peppermint oil. The daily oral dose of peppermint oil prescribed by the German Standard marketing Authorisation is up to 12 drops, which corresponds to approximately 160 mg of essential oil. With up to 11 percent pulegone + menthofuran the daily dose would contain up to 17.6 mg of the two substances, hence the limit of 3.5 mg would be exceeded by the factor of 5. Reduction of a dose measured in drops with single doses of 3-4 drops results in 0.6-0.8 drops, which cannot be applied, as a drop cannot be divided. Even the least possible dose of 1 drop is already borderline to exceeding the recommended threshold of 3.5 mg of pulegone + menthofuran. There is no observation of toxicity of mint oil or peppermint oil with the currently recommended dose schemes. The definition of 3.5 mg of pulegone daily is entirely based on hypothetical considerations concerning isolated pulegone. Isolated pulegone is not the same as the essential mint/peppermint oils as described by the European Pharmacopeia. In addition, the publication of Escobar *et al.* (2015) underlines that the toxicological profile	

<table>
<tr><th>Interested party</th><th>Comment and Rationale</th><th>Outcome</th></tr>
<tr><td></td><td>of an essential oil cannot be extrapolated from data obtained with the isolated substances pulegone and menthofuran. Escobar et al. (2015) used an essential oil with 64.7% of pulegone, and still there was no toxicity found despite the high dose. Quite obviously the overall composition of the essential oil plays an important role. Of note: In peppermint and mint oil pulegone does not exceed 3%, not 64.7% as with the Minthostachys oil tested by Escobar et al. (2015).</td><td></td></tr>
<tr><td>EDQM</td><td>The European Directorate for the Quality of Medicines would like to support the discussion on the public statement on the use of herbal medicinal products containing pulegone and menthofuran by providing quality data on the two active substances which have an own detailed monograph in the European Pharmacopoeia: Mint oil, partially dementholised (1838) and Peppermint oil (405).

The profiles of mint and peppermint oils are defined in the European Pharmacopoeia as well as in the ISO standards. The ISO standard distinguish between US and other origins.

<table>
<tr><td>Ph.Eur.</td><td>Menthofuran</td><td>Pulegone</td></tr>
<tr><td>Peppermint oil</td><td>1.0-8.0%</td><td>max. 3.0%</td></tr>
<tr><td>Mint oil</td><td>not defined</td><td>max. 2.5%</td></tr>
</table>

<table>
<tr><td>ISO</td><td>Menthofuran</td><td>Pulegone</td></tr>
<tr><td>Peppermint oil US</td><td>1.5-6.0%</td><td>0.5-2.5%</td></tr>
<tr><td>Peppermint oil other</td><td>1.0-8.0%</td><td>0.5-3.0%</td></tr>
<tr><td>Mint oil</td><td>not defined</td><td>not defined</td></tr>
</table>

The biosynthesis of pulegone and menthofuran is scientifically well investigated in peppermint plants. (see S. S. Mahmoud, R. B. Croteau PNAS 2003, Vol 100 (24), 14481-14486).

Pulegone is a major intermediate in the biochemical pathway of menthol and menthone synthesis in plants, leading either to menthofuran or to menthol. This is influenced by</td><td>We thank EDQM for these data.

Data have been considered for the revision of the Public Statement, as appropriate.</td></tr>
</table>

Interested party	Comment and Rationale	Outcome
	natural growth factors such as light, humidity and temperature, leading to an increased menthofuran level typically for the second annual harvest (second cut) of peppermint oils in India (see table 3). Typically, mint oils show practically no menthofuran, whereas genuine peppermint oils are characterised by the presence of menthofuran, as this substance is not added artificially. Thus, menthofuran in addition to isopulegol is one of two main markers, to distinguish between mint and peppermint oils and to discriminate natural, genuine peppermint oils from oils adulterated with cheaper mint oil. Currently no other analytical test is available, to detect potential falsifications of natural peppermint oil with mint oils. Batch data (table1, table 2) of hundred batches are provided, showing the natural distribution of menthofuran and pulegone in both mint and peppermint species over the last ten years in batches purchased from India, China and US (2005-2015). Batch data (table 3) from Indian suppliers show, that batches of the second cut of peppermint oil often show even higher menthofuran contents than permitted in the specification of the Ph. Eur. monograph. In conclusion menthofuran is an identity marker for Peppermint oil and its presence cannot be avoided in natural oils. Pulegone is also a typical natural constituent of Mint and Peppermint oils and according to batch data it is always present in both oils.	
Frey + Lau	**2. Pulegone and menthofuran as natural quality markers in genuine Mint and Peppermint oils** The profiles of the natural essential oils are described in the European Pharmacopeia in an own detailed monograph as well as in the ISO norms (see tables below). The individual monograph defines purity and identity tests as well as a typical chromatographic profile for each active substance. Natural mint and Peppermint oils matching these parameters as	The focus of the Public Statement is the toxicological assessment.

Interested party	Comment and Rationale	Outcome
	well as the chromatographic profile can be considered as authentic. 	Ph.Eur.	**Menthofuran**	**Pulegone**
Peppermint oil	1.0-8.0%	max. 3.0%
Mint oil	not defined	max. 2.5% | | ISO	**Menthofuran**	**Pulegone**
Peppermint oil US	1.5-6.0%	0.5-2.5%
Peppermint oil other	1.0-8.0%	0.5-3.0%
Mint oil	not defined	not defined	 Mint oils show typically no menthofuran and higher pulegone levels, whereas natural peppermint oils are characterized by their significant menthofuran level. Pulegone as a major intermediate in peppermint plants is scientifically well investigated *(see* S. S. Mahmoud, R. B. Croteau: A systems biology approach identifies the biochemical mechanisms regulating monoterpenoid essential oil composition in peppermint. (2007), 14481-14486). Pulegone enters either into a biochemical pathway resulting in levomenthol and menthone or into a biochemical pathway resulting in menthofuran in the peppermint plant. Both pathways are co-existent and competitive. The emphasis on the one or the other pathway in the plant depends on natural growth conditions, e.g. humidity, light or temperature. Those factors lead to a natural variability in pulegone and menthofuran contents due to the fact that the plants are cultivated in the open land (*see* Grulova D, De Martino L, Mancini E, Salamon I, De Feo V. Seasonal variability of the main components in essential oil of *Mentha* × *piperita* L. Journal of the science of food and agriculture; p.621-7,201502) In contrast, essential oils expected from controlled in vitro breeding are suspected to show even higher pulegone and menthofuran levels *(see* Bricout J, Paupardin: The essential oil composition of *Mentha piperita* cultured in vitro: influence of some factors on its synthesis.(1975), p.383-386*)*.	

Interested party	Comment and Rationale	Outcome
	The chromatographic profile limits given in the monographs of the European Pharmacopeia consider a typical spectrum of menthofuran and pulegone according to this natural variability of mature peppermint or mint plants. It should be mentioned that the monographs already exclude essential oils obtained from plants under extreme conditions, e.g. from very dry periods as they naturally occur in some years and which lead to an increased menthofuran content. A realistic spectrum with regard to natural variability is considered to be mandatory to guarantee a European supply with authentic essential oils. Moreover menthofuran is not added artificially. Thus, menthofuran next to isopulegol is one of two main quality markers to distinguish between mint and peppermint species. Modern analytical testing relies on such quality markers to qualify genuine peppermint oils against falsifications altered with cheaper mint oil or nature identical peppermint flavours. *With regard to the increasing number of falsified medicines in Europe, it is recommendable to maintain such quality markers, as there is currently no other sufficient analytical test installed, to detect potential falsifications of natural peppermint oil with mint oils.*	
Exposure to pulegone and menthofuran		
AESGP Lines 98-101; 159-168	**Due to the differences in metabolic pathways and in potential toxicity of pulegone and menthofuran, a sum parameter cannot be established. Menthofuran should be taken into consideration to a lower extent only.** Menthofuran is an important metabolite of pulegone and contributes to its toxicity. The toxic effects of menthofuran and pulegone are considered similar on a qualitative level but for a number of reasons it is not justified to regard them under identical quantitative aspects. For pulegone multiple pathways of metabolism exist and formation of menthofuran is only one of them. Besides menthofuran, hepatotoxic intermediates can also be formed by other	*See #5 in the general considerations*. The complexity of metabolism and metabolic activation of pulegone and menthofuran are amply addressed in the dPS. Also the latest adduct and metabolomics studies (Rousu *et al*., 2009; Li *et al*., 2011) demonstrating these complexities and also the potential role of menthofuran are described in 2.3. In terms of potency, hepatotoxicities of pulegone and menthofuran in rats are

Interested party	Comment and Rationale	Outcome
	pathways, e.g. via 5-hydroxylation of pulegone (Thomassen *et al*., 1990; Chen *et al*., 2011). Lines 98-99: Studies on the toxicological effects of pulegone and menthofuran in rats showed a toxicological potency which was only about one third to one half of that of pulegone. When the exposure of rats to menthofuran derived from pulegone or from synthetic menthofuran were matched with respect to the concentration and time course in plasma, pulegone produced more than twice the increase in plasma alanine transferase and hepatocellular necrosis than did menthofuran. The results demonstrate that events other than those associated with the disposition of menthofuran contribute to the hepatotoxicity observed after ingestion of pulegone (Thomassen *et al*., 1988). Lines 98-99: Menthofuran arises from pulegone only, if the administered dose exceeds the physiological detoxification capacity of the liver. The main metabolic pathway of pulegone in rodents and most likely in humans is the conjugation to glutathione. Its hepatotoxic effect is dependent on the level of detoxification in the liver. Following oral high level exposure of pulegone, therefore, hepatotoxicity is only to be expected at substantial glutathione depletion (Armstrong, 1987). Lines 98-99: After administration of 0.5 mg/kg bw (R)-(+)-pulegone to 6 human volunteers, menthofuran was not identified as a main metabolite. The major urinary metabolites identified were 8-hydroxymenthone, 1-hydroxymenthone, menthol, and 10-hydroxypulegone; minor metabolites included piperitone and 3-p-menthene-8-ol. Further experiments indicated that low concentrations of menthofuran observed may have arisen as artefact from 10-hydroxypulegone during extraction and hydrolysis (Engel, 2003). Lines 99-101: Menthofuran shows a quantitative toxicological potential that differs from that of pulegone. Therefore an adjustment factor should be taken into account. On the basis of the investigations of Thomassen *et al*. (1988) a factor of 0.5 or lower is considered appropriate.	similar enough to create a sum parameter. The possibility of a relative threshold of conversion of pulegone to menthofuran has been addressed in the dPS. An in-vivo study on urinary metabolites cannot provide definitive evidence that menthofuran is NOT a significant metabolite of pulegone, because metabolic routes go on until most distal metabolites. A study to cover metabolites, both proximal and distal, more comprehensively is needed for convincing evidence and this coverage has to take into consideration of in-vitro studies.

Interested party	Comment and Rationale	Outcome
EUCOPE	**Establishment of a sum parameter for pulegone and menthofuran is inappropriate** Due to the differences of the toxic potential of pulegone and menthofuran, a sum parameter cannot be established. Menthofuran is indeed quite an important metabolite of pulegone and contributes to its toxicity. On a qualitative level menthofuran is known to develop similar hepatotoxic effects as pulegone. Nevertheless, the establishment of a combined threshold considering both, pulegone and menthofuran, is not appropriate due to following reasons: Besides menthofuran also other hepatotoxic metabolites can be formed of pulegone by other pathways, e.g. metabolites from 5-hydroxypulegone. Toxicological studies showed that after administration of pulegone and menthofuran in rats at doses resulting in identical plasma concentration – time curves for menthofuran, hepatotoxic effects, measured as increase of alanin-aminotransferase (GPT) and hepatocell necrosis, after administration of menthofuran resulted in only one third to half the toxic effect of pulegone (Thomassen *et al*. 1988). Menthofuran is only developed from pulegone, if pulegone is administered at concentrations exceed the detoxification capacity of the liver. The main metabolic pathway of pulegone in rodents and most likely in humans is a conjugation with gluthatione. Menthofuran is only produced as hepatotoxic product after administration of high concentrations of pulegone. As long as the concentrations of pulegone and its numerous metabolites are not sufficient to deplete gluthatione in the liver, no hepatotoxicity of menthofuran will occur (Armstrong, 1987). After administration of 0.5 mg/kg pulegone to six human subjects menthofuran could not be identified as substantial metabolite. The author supposes, that the low concentration of menthofuran may have risen as artefact during sample preparation e.g. from 10-	*See #5 in the general considerations.*

Interested party	Comment and Rationale	Outcome
	hydroxypulegone (Engel, 2003). **Since menthofuran shows another quantitative toxicological potential compared to pulegone, an adjustment factor should be taken into account. We suggest to use the** factor 0.5 **which is based on the investigations of Thomassen *et al*. (1988).**	
Schwabe lines 99-100	Pulegone and menthofuran display qualitative similar hepatotoxicities in rodents and thus it is reasonable that these substances are evaluated together. **Comment** The study published by Thomassen *et al*. (1988) shows that the potential hepatotoxicity of menthofuran is much lower (about 50%) than that of pulegone. This is essential for the risk assessment and should therefore be stated. **Proposal for revision** Pulegone and menthofuran display similar qualitative but markedly different quantitative hepatotoxicities in rodents and thus it is not reasonable that these substances are evaluated together.	A 2-fold difference in the hepatotoxic doses between pulegone and menthofuran in one rat strain may not be enough to evaluate these two substances together. In addition, potential genotoxicity is a critical consideration. *See #5 in the general considerations.*
Regulatory status		
EFSA	Section 1.3 refers to Regulation 1334/2008 and to the limits set in this regulation, however EMA omits to mention that pulegone and menthofuran are both substances which "shall not be added as such to food". In Section 1.3, the Committee of Experts on Flavouring Substances (CEFS), which is a Committee of the Council of Europe, is mentioned but the references given link it to the European Commission. The reference should be Natural Sources of Flavourings Report No. 3, 2008, Council of Europe Publishing, ISBN 978-92-871-6422-3, p.42 (see enclosure), or alternatively Active principles (constituents of toxicological concern) contained in natural sources of flavourings, Council of Europe, 2005, p. 18.	Added to the PS. Public Statement modified accordingly. Reference changed.

Interested party	Comment and Rationale	Outcome
	In both reports of the CoE, a joint MDI (maximum daily intake) for pulegone and menthofuran of 0.1 mg/kg bodyweight is set, and the following limits in foodstuffs are proposed: **Menthofuran:** foods and beverages in general: 20 mg/kg. Exceptions (mg/kg): Mint/peppermint flavoured alcoholic beverages 100, Mint/peppermint flavoured confectionery 200, Mint/peppermint flavoured chewing gum 1000. **Pulegone:** foods and beverages in general: 20 mg/kg. Exceptions (mg/kg): Mint/peppermint flavoured alcoholic beverages 100, Mint/peppermint flavoured confectionery 100, Intensely strong mint/peppermint flavoured confectionery 200, Mint/peppermint flavoured chewing gum 30.	
2.2. Metabolism of pulegone and menthofuran		
Tillotts Pharma AG	Pulegone is metabolized to several compounds along different pathways, i.e. not only to menthofuran and its subsequent metabolite (see chapter 2.2 of dPS). Therefore, establishment of a combined limit [pulegone + menthofuran] based only on the toxicological data of pulegone is questionable and appears to be not adequate.	Not endorsed. Metabolisms of pulegone and menthofuran are so intimately intertwined that it is preferential to combine them. *See general comment 5.*
Schwabe lines 183-184	Generally, the metabolism of pulegone and menthofuran has been elucidated in a considerable detail in *in vivo* and *in vitro* studies (Fig. 2). Pathways leading to metabolic activation, covalent binding and hepatic effects have been investigated also in various *in vivo* animal studies. **Comment** In fact the data on metabolism originate predominately from nonclinical studies, which were carried out at extremely high doses (see above and below). Since toxicokinetics is dose dependent, this has to be mentioned in order to allow an appropriate risk assessment. Furthermore it should be stressed that at least 2 out of 4 described metabolic pathways lead to detoxification of pulegone or menthofuran by binding to glucuronic acid	The studies involve also experiments performed with human (and animal) liver preparations which give the most reliable first look at important metabolic pathways and constitute a framework on which to base in vivo pathways and species comparisons. Not endorsed

Interested party	Comment and Rationale	Outcome
	and/or glutathione. This is the biological detoxification process by which the liver physiologically eliminates potentially harmful substances. The information is very important for the risk assessment and should therefore be added. **Proposal for revision** Generally the metabolism of pulegone and menthofuran has been investigated in considerable detail in nonclinical *in vivo* and *in vitro* studies (Fig. 2), however with high pulegone exposures in the range of acute human toxicity. Pathways leading to detoxification, metabolic activation, possibly covalent binding and hepatic effects have been investigated also in various *in vivo* animal studies.	
Schwabe lines 198-199	It should be noted that the order of metabolic reactions in the above pathways may not be obligatory, but for example reduction of pulegone may follow hydroxylation or vice versa. More distal metabolites are nevertheless identical. **Comment** From the scientific literature, 4 metabolic pathways have been elucidated. Metabolic pathways 2 and 3 lead to detoxification of pulegone or menthofuran by binding to glucuronic acid and/or glutathione. This is the biological detoxification process by which the liver physiologically eliminates potentially harmful substances. The information is very important for the risk assessment and should therefore be added. **Proposal for revision** It should be noted that metabolic pathways 2 and 3 lead to conjugation with glucuronic acid and/or gluthatione, which are biological detoxification mechanisms. The conjugates are excreted with the bile. Furthermore, the order of metabolic reactions in the above pathways may not be obligatory, but for example reduction of pulegone may follow hydroxylation or vice versa. More distal metabolites are nevertheless identical.	Certain pathways lead to detoxification and others to reactive metabolites. Actually also activation pathways involve detoxifications, usually by conjugation reactions, and still at least partially, reactive metabolites bind to their targets. Metabolism is rather complex and data are too limited to draw firm conclusions. Not endorsed
Schwabe	There is some evidence that in the metabolism of pulegone conjugation reactions	This option was considered but experimental

Interested party	Comment and Rationale	Outcome
lines 210-215	predominate over menthofuran pathway at lower doses of pulegone (Chen *et al*., 2001), i.e. the formation of menthofuran would not be significant at lower, more "realistic" doses. Also the only available human study (Engel, 2003) seems to point to a similar scenario. **Comment** Four metabolic pathways have been elucidated of which pathways 2 and 3 lead to detoxification of pulegone or menthofuran by binding to glucuronic acid and/or glutathione. This is the biological detoxification process by which the liver physiologically eliminates potentially harmful substances. Only if the detoxification process is exhausted or overloaded due to high exposure, are the pathways leading to the formation of furane rings followed. This is supported experimentally by the human study published by Engel (2003). It is further in line with the fact that pharmacovigilance and epidemiological data do not indicate any human risk related to low exposures of pulegone. Moreover this is confirmed in the drafted HMPC public statement stating that at lower realistic exposure levels cellular protective mechanisms, trapping by glutathione and other scavengers of reactive metabolites, would constitute a practical threshold below which no genotoxicity would become manifest (see lines 373-375). **Proposal for revision** There is some evidence that in the metabolism of pulegone, conjugation reactions predominate over the menthofuran pathway at lower doses of pulegone (Chen *et al*., 2001), i.e. the formation of menthofuran would not be significant at average dietary (2 µg per day or 0.04 µg/kg bw) or therapeutic exposure of peppermint oil (pulegone: 33 mg / 0.55 mg/kg bw; menthofuran 88 mg / 1.5 mg/kg bw). The human study (Engel, 2003) points to a similar scenario since menthofuran was not detected as metabolite of pulegone at exposures with low concentrations.	evidence is far from being strong. The ability of post marketing surveillance and clinical experience to detect signals related to genotoxicity or carcinogenicity is rather low. Not endorsed. See several replies above.

Interested party	Comment and Rationale	Outcome
2.3. Bio activation of pulegone and menthofuran		
Schwabe lines 242-254	In a recent experimental study, several oxidative metabolites of menthofuran were characterized in rat and human liver microsomes and in rat liver slices exposed to cytotoxic concentrations of menthofuran (Khojasteh *et al*., 2010). **Comment** The cited study of Khojasteh *et al*. (2010) was conducted by exposing the animals to pulegone concentrations of 150 mg per kg bw. This is in the range of acute human toxicity (130–281 mg/kg bw). However, it is 3,750,000-times above the average dietary exposure (2 µg per day or 0.04 µg/kg bw) or at least 100-times above the exposure with therapeutic peppermint oil (1080 mg, containing maximally 3% pulegone, i.e. 33 mg or 0.55 mg/kg bw). As pharmacokinetic data are dose dependent, the study of Khojasteh *et al*. (2010) is not relevant for the safety assessment of dietary or therapeutic use of peppermint oil. **Proposal for revision** In a recent experimental study, several oxidative metabolites of menthofuran were characterized in rat and human liver microsomes and in rat liver slices exposed to very high cytotoxic concentrations of menthofuran of 150 mg per kg bw (Khojasteh *et al*., 2010).	Not endorsed High doses were used for the detection of hepatotoxic metabolites.
Schwabe lines 262-266	A novel approach based upon metabolomic technologies **Comment** Studies using the metabolomics approach have not been validated with regard to human toxicological risk assessment. The study should therefore not be used as a source of evidence in the public statement on pulegone.	Not endorsed. All evidence available are used for risk assessment.

Interested party	Comment and Rationale	Outcome
	Proposal for revision Lines 262-266 are deleted.	
Schwabe lines 266-269	Khojasteh *et al*. (2012) detected 10 rat liver proteins spots by an antiserum developed to detect protein adducts resulting from menthofuran bioactivation. Four of them were identified by LC- MS/MS analysis of tryptic peptides as serum albumin, mitochondrial acetaldehyde dehydrogenase, cytoplasmic malate dehydrogenase and subunit of mitochondrial ATP synthase. **Comment** The cited study by Khojasteh *et al*. (2012) was conducted by exposing the animals to pulegone concentrations of 5 ml per kg bw. This is about 20-times higher than the quantity causing acute toxicity in humans (130–281 mg/kg bw). It is at least 2000-times above the exposure with therapeutic peppermint oil (1080 mg, containing maximally 3% pulegone, i.e. 33 mg or 0.55 mg/kg bw). As pharmacokinetic data are dose dependent, the study of Khojasteh *et al*. (2012) does not deliver relevant information for the risk assessment of dietary or therapeutic use of peppermint oil. **Proposal for revision** Khojasteh *et al*. (2012) detected 10 rat liver proteins spots by an antiserum developed to detect protein adducts resulting from menthofuran bioactivation <u>after exposing the animals to about 20-times that of the human acute toxic dose</u>. Four of them were identified by LC-MS/MS analysis of tryptic peptides as serum albumin, mitochondrial acetaldehyde dehydrogenase, cytoplasmic malate dehydrogenase and subunit d of mitochondrial ATP synthase.	Not endorsed. High doses were used for the detection of protein targets of hepatotoxic.
Schwabe lines 270-274	The overall consensus on bioactivation of pulegone and menthofuran is that metabolic pathways leading to reactive metabolites have been elucidated to a considerable detail and the most probable hepatotoxic metabolite is derived from menthofuran, although	Reactive metabolites of this kind would most likely be disarmed by glutathione conjugation and thus be capable of binding

Interested party	Comment and Rationale	Outcome
	some additional toxic metabolites may contribute to hepatotoxicity. **Comment** Four metabolic pathways have been elucidated of which pathways 2 and 3 lead to detoxification of pulegone or menthofuran by binding to glucuronic acid and/or glutathione. This is the biological detoxification process by which the liver physiologically eliminates potentially harmful substances. Only if the detoxification process is exhausted or overloaded due to the high exposure, are the pathways leading to the formation of furane rings followed. This is supported experimentally by the human study published by Engel (2003). It is further in line with the fact that pharmacovigilance and epidemiological data do not indicate any human risk related to low exposures of pulegone. This is confirmed in the drafted HMPC public statement stating that at lower realistic exposure levels cellular protective mechanisms, trapping by glutathione and other scavengers of reactive metabolites, would constitute a practical threshold below which no genotoxicity would become manifest (see lines 373 - 375). This information is very important for the risk assessment and should therefore be added. **Proposal for revision** The overall consensus on bio activation of pulegone and menthofuran is that metabolic pathways only lead to reactive metabolites when the detoxification via conjugation to glucuronic acid or gluthatione is exhausted. Animal studies conducted at extremely high concentrations show that the most probable hepatotoxic metabolite is derived from menthofuran, although some additional toxic metabolites may contribute to hepatotoxicity.	to DNA only during prolonged and high exposure, when glutathione resources are depleted. *See #2 in the general considerations.*
2.4. Human toxicity		
AESGP Line 276	**Data from clinical trials do not reveal evidence for liver and/or kidney toxicity of peppermint oil** Peppermint oil (manufactured according to Ph.Eur.) as part of a fixed combination was	*See replies to #lines 47-51.*

Interested party	Comment and Rationale	Outcome
	investigated in randomised, placebo-controlled studies with regard to efficacy and tolerability in gastrointestinal discomfort. A systematic literature search was conducted and the marketing authorisation holder requested safety data from unpublished studies. Based on the data of all clinical trials and observational studies completed before October 2014, an analysis regarding the safety and tolerability was carried out. A total of 13 clinical trials and observational studies were identified. The data of 3144 patients and 58 healthy volunteers could be analysed. The combination product was effective and well tolerated in all studies with exposures of 180 mg to 270 mg per day. There was no serious adverse event in which an association with the use of the product was suspected. In the identified 7 double-blind, placebo-controlled studies, the number and type of adverse events in the verum group were similar to the placebo group. No abnormalities in terms of individual organs such as liver and bladder were observed (Madisch *et al*., 2015).	
ANME	Pennyroyal oil with a pulegone content of 62-97% pulegone was found toxic, with fatal liver toxicity observed with doses containing an equivalent of 90-150 mg/kg of pulegone for a person with 60 kg body weight. The problem with this description is that there is no clear description of the quality and composition of the essential oil. The mere observation of a case report would not allow attributing this case to pulegone as such – Pennyroyal or the preparation involved in this case could well have contained other contributing or causative substances. If these cases of adverse events with Pennyroyal were used for the limitation of mint and peppermint oil (which ultimately would correspond to discouraging the use of such preparations), the case report(s?) in question should be analysed in more detail, using the currently applicable CIOMS criteria for liver toxicity. It should be expected that regulatory action leading to a limitation of the access of the patient/consumer is based on actual observations, not mere hypotheses. If such observations exist, they should be elaborated in sufficient detail.	These cases of penny royal oil toxicities have not been decisive in developing the dPS.

Interested party	Comment and Rationale	Outcome
EUCOPE	**Clinical signals related to liver-damaging effects caused by peppermint oil do not exist** There are no clinical data available indicating damages of the liver in humans, which is regarded as the essential target for toxicological effects after consumption of peppermint oil. Only after intoxications with pennyroyal oil, resulting in an uptake of more than 90 mg/kg pulegone (i.e. 4.500 mg per person based on 50 kg body weight), moderate to severe damages of the liver could be found. This dose *exceeds the now suggested threshold by a factor of about 1250 (Anderson et al*. 1996).	This observation has been clearly stated in the PS (2.4.)
2.5. Subchronic and chronic toxicity and carcinogenicity of pulegone (NTP 2011)		
AESGP Lines 288-310	The proposed safety factor of 300 is inappropriate With reference to the NTP carcinogenicity study with pulegone, the HMPC draft Public Statement is misleading due to the wording "No NOAEL values could be determined, because hyaline glomerulopathy was seen also at the lowest dose of pulegone in female rats and in male and female mice. Thus the lowest LOAEL was 18.75 mg/kg bw". However, the lowest dose of pulegone administered to male and female mice as well as female rats was 37.5 mg/kg. A dose of 18.75 mg/kg was only given to male rats and the only findings in male rats treated with this dose which were significantly increased when compared to the control group were fatty changes of the liver and acinus atrophy in the pancreas. Both of these lesions are commonly found in aged rats and in fact were also observed in animals from the control group. At the same time the incidence of bile duct hyperplasia and of basophilic foci in the liver was even significantly reduced in male animals treated with 18.75 mg/kg, while definitively no hyaline glomerulopathy was diagnosed in these rats. On the basis of these results a NOAEL of 18.75 mg/kg has been reliably established for male rats as the most sensitive species and sex. An identical NOAEL also derives from the 3-month studies in rats and mice published by the NTP (NTP, 2011).	Endorsed The arguments have been considered in developing a new threshold. *See #4 in the general considerations*.

Interested party	Comment and Rationale	Outcome
	In view of these results and of the fact that peppermint oil has been used in foodstuff, confectionery and medicinal products for decades without any evidence of a specific toxic potential, we consider the proposed safety factor of 300 as inappropriate. All the more so as a safety factor of only 200 has previously been recommended for a foodstuff with lifelong exposure by the Committee of Experts on Flavouring Substances (CEFS) based on a 28-day toxicity study in rats (SPFA, 2005). For these reasons we would like to recommend a safety factor based on the FDA Guidance for Industry on Estimating the Maximum Safe Starting dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers (FDA, 2005). For the conversion of the NOAEL dose in rats to an equivalent dose in humans based on the body surface and assuming a human body weight of 60 kg, a factor of 6.2 has been established. In order to allow for variability in extrapolating from animal toxicity studies to humans, an additional default factor of 10 is recommended giving a final safety margin of 62 which is considered to be appropriate for the purposes of consumer protection, in particular as the use of peppermint oil in foodstuff, confectionery and medicinal products for decades did not provide any evidence for a specific toxic potential. Moreover, a Margin of Safety (MoS) of 100 is generally accepted by the WHO to conclude that a substance is safe for use in humans (SCCS 2012; WHO 1994, 1999, 2012).	Not endorsed *See #4 in the general considerations.*
ANME	We suggest to include information from the study of Escobar *et al*. (2015) – see comments on Introduction. Pulegone and menthofuran as pure substances may display toxicity different from that encountered in essential oils. A recent example was published by Escobar *et al.* (2015) [1]. This study examined the toxicity of the essential oil of *Minthostachys verticillata*, a plant related to peppermint featuring 64.7% pulegone in the essential oil. The authors report not only non-toxicity from a 90 day oral toxicity study in rats, but also the lack of mutagenicity through micronucleus and comet assays.	Not endorsed The recently published 3-month study in rats using peperina oil (Escobar *et al*., 2015) is considered inferior to the NTP 3-month study based on (i) dietary administration instead of oral gavage (more uncertain exposure); (ii) lack of complete histopathological evaluation (only three tissues examined). In addition, a different

Interested party	Comment and Rationale	Outcome
	The 90 day toxicity study involved doses up to 460 mg/kg body weight daily, with no toxicity observed. This includes the lack of liver toxicity. The micronucleus assay was performed in the same 90 day repeated dose toxicity study animal population as above. Oral doses up to 460 mg/kg b.w. did not lead to significant differences in micronucleus induction between dose groups (0, 70, 260 and 460 mg essential oil per kg b.w.). This non-mutagenicity was confirmed through a COMET assay, where again no DNA-altering effect was found after 90 day feeding of 0, 70, 260 and 460 mg/kg of the essential oil. The NOEL level derived from this study was 460 mg/kg of *Minthostachys* essential oil. As already mentioned, the essential oil contained 64.7% pulegone as the major constituent. The exposure to pulegone in this study corresponded to 297 mg pulegone per kilogram body weight in the rat. There is a clear discrepancy with respect to the previously described toxicity of pulegone (see Draft Public Statement): For pure pulegone, the LOEL had been defined with 20 mg/kg, less than 1/10th of the exposure in this study. As the Draft Public Statement, Escobar *et al*. (2015) cite the NTP studies: Pulegone has a hepatotoxic threshold of >10 mg/kg daily in rats, and of >75 mg/kg daily in mice. This dose was largely exceeded in the study of Escobar *et al*. (2015), with no toxicity encountered. Escobar *et al*. (2015) explain this discrepancy with the potential protective effect of other constituents, namely menthone. This latter constituent is also present in mint and peppermint oils.	rat strain (Wistar) was used in the Escobar study as compared with the NTP studies, which used F344 rats.
EUCOPE Page 10, lines 306-308	**The suggested safety factor is not appropriate** In our opinion the description of NOAEL and LOAEL, respectively, is imprecise and therefore misleading at this point. The lowest dose which has been administered to female mice and female rats was 37.5 mg/kg pulegone. A dose of 18.75 mg/kg was given only to male rats, resulting in mild toxic effects in terms of fatty changes of the liver, but no	Endorsed *See #4 in the general considerations.*

Interested party	Comment and Rationale	Outcome
	hyaline glomerulopathy was diagnosed. Based on these results a NOAEL of 18.75 mg/kg can be defined for the most sensitive species and sex. An identical NOAEL can also be derived from the subchronic toxicity study in male and female rats published by NTP (NTP, 2011), which is mentioned in the Draft Public Statement. For the reasons above the proposed safety factor of 300 seems to be inappropriate and too conservative, especially when compared to a safety factor of 200 for the foodstuff area with lifelong exposure as recommended by the Committee of Experts on Flavouring Substances (CEFS) based on a 28-days- toxicity study in rats (SPFA 2005). Taking into consideration the fact that peppermint oil has been used in foodstuff, luxury food and medicinal products for decades without any evidence of a specific toxic potential it seems to be indicated and sufficient in the light of consumer protection to use a factor of **62** instead of 300.	
Schwabe line 308	Thus the lowest LOAEL was 18.75 mg/kg bw. **Comment** This statement is misleading as the lowest dose of pulegone administered to male and female mice as well as female rats in the NTP study was 37.5 mg/kg bw. The dose of 18.75 mg/kg bw was given to male rats only. The sole findings in these animals compared to the control group were significantly increased fatty changes of the liver and acinus atrophy in the pancreas. In fact both these lesions are commonly found in aged rats and were also observed in animals in the control group. On the basis of these results the NOAEL of 18.75 mg/kg bw can be reliably established in male rats as the most sensitive species and sex.	Endorsed *See #4 in the general considerations.* The suggested changes have been taken into consideration.

Interested party	Comment and Rationale	Outcome
	Proposal for revision Thus the NOAEL is 18.75 mg/kg bw.	
AESGP Lines 288-294	**Carcinogenic effects in rats and mice at overt toxic and lethal doses only (NTP-studies) are of no biological relevance for men** Increased incidences of urinary bladder neoplasms in female rats were observed only at 150 mg/kg pulegone, which is well above the Maximum Tolerated Dose (MTD). At the Maximum Recommended Human Dose (MRHD) of 1080 mg for essential peppermint oils from mint plants (HMPC monograph max. 6 capsules each containing 0.2 ml = 1.2 ml = 1080 mg Peppermint oil per day), patients will be exposed to a maximum of 0.65 mg/kg pulegone on a 50 kg bw basis at the upper specification limit of 3% for pulegone (European Pharmacopoeia). This means that the exposure to pulegone at therapeutic use of peppermint oil is approximately 230-fold lower compared with the 150 mg/kg dose in rats. In the meantime, *in vitro* and *in vivo* mechanistic data support the hypothesis that the mode of action for pulegone-induced urothelial neoplasms in female rats is due to cytotoxicity and consequent regenerative cell proliferation ultimately leading to tumour formation. Following oral high level exposure to female rats, pulegone and its metabolites, especially piperitenone, are concentrated and excreted in the urine at cytotoxic levels. It is unlikely that humans could be exposed to the exceptionally high concentration of pulegone necessary to generate the high urinary concentrations required to produce urothelial cytotoxicity. Accordingly, cytotoxicity followed by regenerative cell proliferation is considered the mechanism of action (MOA) for pulegone-induced urothelial tumours in female rats (Da Rocha MS *et al*., 2012). The NTP cancerogenicity study revealed only a significant increase in benign but not malignant liver tumours in female mice. In male mice a significant increase in benign and malignant tumours could only be seen at a concentration of 75 mg/kg bw but not at	Endorsed *See #4 in the general considerations.* The suggested changes have been taken into consideration. MTD for male and female rats on the basis of the 3-month study was decided to be 75 and 150 mg/kg. Nephrotoxicity-based morbidity and mortality seen in these doses of the 2-year study came as a surprise, but in the opinion of the NTP scientists did not compromise the conclusions (see p 78 in the NTP report). Calculation does not take into consideration menthofuran, which is also a major constituent of peppermint oil. Mode of action of pulegone has been discussed in the PS 2.7. The evidence for liver carcinogenicity in male and female mice has been discussed in the original NTP report (pp 79-81) and also in the IARC monograph. The authors and assessors are of the opinion that hepatic tumours in mice as such are a real cancer

Interested party	Comment and Rationale	Outcome
	150 mg/kg bw. High-dose data in mice cannot be used as basis for hazard identification and cancer risk assessment because the incidence of hepatocellular adenoma and hepatoblastoma was not dose-dependent. Accordingly, there is no evidence of carcinogenicity in male and female mice (benign hepatocellular adenoma and hepatoblastoma). As the observed tumour incidences in rats and mice were not consistently dose-related and occurred only at overt toxic (lethal) doses of pulegone, these findings cannot be extrapolated to men at therapeutic doses of peppermint oil. Pulegone should not be listed as a carcinogen. Due to these experimental deficiencies (too high doses, lack of consistent dose-response relationships), this data is also not suitable as basis for any setting of exposure limits by regulatory bodies (Murray, 2012).	response. Although the conclusions of NTP and IARC have been challenged, a real concern is whether pulegone and menthofuran are genotoxic and thus genotoxic carcinogens.
EUCOPE Page 9 and 10, lines 288-294	**Carcinogenic potential** Data concerning the carcinogenic potential of pulegone is very heterogeneous and can thus only be evaluated in a limited way. Mild toxic changes observed in male rats treated with a dose of at least 18.75 mg/kg were not accompanied by carcinogenic effects. Female rats showed a significant increase of tumours of the urinary bladder, which can be related to a damage of the kidneys in form of hyaline glomerulopathy. More pronounced damages of the liver appeared using doses from 37.5 mg/kg (male rats) and 75 mg/kg (female rats and mice), respectively. The NTP cancerogenicity study revealed a significant increase only of benign but not malignant liver tumours in female rats. In male rats a significant increase of benign and malignant tumours could be observed at a dose of 75 mg/kg but not at 150 mg/kg. Therefore, these data can provide only limited indication for a carcinogenic potential.	Endorsed *See #4 in the general considerations.* The suggested changes have been taken into consideration.

Interested party	Comment and Rationale	Outcome
2.6. Genotoxicity of pulegone and menthofuran		
AESGP Lines 329-343	**Pulegone is not genotoxic in standard assays** Several studies have shown that pulegone is not mutagenic in S. typhimurium with and without metabolic activation. In a single study, pulegone was mutagenic in two bacterial strains with S9-activation (NTP, 2011). The NTP conclusion that pulegone is genotoxic is not supported by the data available especially without evidence that DNA adducts are formed (covalent binding). In vivo, no significant increase in the frequency of micronucleated erythrocytes in the peripheral blood of two 3-month mouse studies using pure pulegone or peperina oil was found (NTP, 2011; Escobar *et al*., 2015).	*See #2 in the general considerations.* Standard genotoxicity assays do not address tissue-specific genotoxicity when probably based on short-lived tissue-specific reactive metabolites. It is expected that micronucleus test would be negative in a case of such short-lived liver-specific metabolites.
	Absence of any toxicity and genotoxicity (comet assay and chromosomal damage) of the essential oil fraction in a 3-month study in rats In a recent study, Escobar *et al*. (2015) describe the results of a 90-day oral subchronic toxicity study in rats using the essential oil from *Minthostachys verticillata* (peperina) at a daily dose of 0, 1, 4 and 7 g per kg food. The high dose was considered the maximum feasible dose due to palatability. At a mean daily food consumption of 20 g/animal, doses of approximately 0, 110, 430 and 710 mg/kg peperina oil were examined on bodyweight basis. The main constituents are pulegone (64.6%) and menthone (23.9%). Assuming a concentration of 64.6% pulegone in the tested oil extract, this corresponds to doses of 70, 280 and 460 mg/kg bw pulegone. There were no treatment-related intercurrent deaths, adverse effects on general conditions or changes in body weight, food consumption and food conversion efficiency throughout the study in male and female rats. Subchronic administration of *Minthostachys verticillata* essential oil did not result in changes in organ weights of liver, kidney and intestine including histopathology. Other organs were not examined. Following repeat dosing (3 months) with *Minthostachys verticillata* essential oil, there was no evidence for genotoxicity as measured by micronucleus analysis in bone marrow and single cell gel	Genotoxicity assays of the study of Escobar *et al.* (2015) are conventional assays: the Ames test and the in-vivo Comet test in blood cells and chromosomal damage (micronuclei) in peripheral blood cells after in-vivo exposure of animals to the preparations.

Interested party	Comment and Rationale	Outcome
	electrophoresis (Comet assay) in blood cells. These results show that high level exposure of peperina oil did not result in any significant target organ toxicity, primary DNA-damage in peripheral cells and micronucleus induction in bone marrow. Due to the high content of pulegone (64.6%) in *Minthostachys verticillata* oil this finding contradicts the results which lead to the threshold values suggested in the Public Statement. Composition of the *Minthostachys verticillata* oil has a pronounced qualitative overlap in the terpene pattern with Peppermint oil (e.g. limonene, 1,8-cineole, menthone, isomenthone and pulegone are present in both essential oils, albeit at different concentrations), thus these results are at least partly transferrable to peppermint oil as well. In the NTP (TR-563) 3-month study in rats at doses of 9.3, 18.8, 37.5, 75 and 150 mg/kg pulegone (gavage), there were decreased body weights and increased relative liver and kidney weights at 75 and 150 mg/kg. Histopathological changes in the kidneys (hyaline glomerulopathy), liver (oval cell hyperplasia, bile duct hyperplasia, hepatocellular necrosis, portal fibrosis), bone marrow (hyperplasia) and forestomach (inflammation, hyperplasia, ulcer) were reported following high level exposure. The Argentinian and U.S. NTP studies are both 3-month studies in rats, but differ in study design regarding rat strains, substance tested (essential oil versus pulegone), administration (dietary admixture versus gavage) as well as maximum doses of pulegone (460 versus 150 mg/kg). Despite these differences, there is some evidence that the essential oil extract is less toxic compared with pure pulegone. The presence of protective mechanisms, e.g. radical scavengers in the herbal mixture, seems likely. Therefore, in terms of human risk assessment, toxicity data generated for pulegone cannot be extrapolated to complex herbal preparations such as extracts or essential oils. Following repeated dosing (3-month), there was no evidence for genotoxicity as measured	

Interested party	Comment and Rationale	Outcome
	by micronucleus analysis in bone marrow and single cell gel electrophoresis (Comet assay) in blood cells. The authors therefore concluded that high level exposure of peperina oil did not result in any significant target organ toxicity, primary DNA-damage in peripheral cells and micronucleus induction. **Peppermint oil is not genotoxic** In addition, a company conducted three genotoxicity studies with peppermint oil (EP grade) in 2012/2013, namely an in vitro bacterial reverse mutation study (Ames test), an in vitro mammalian cell gene mutation study (mouse lymphoma assay), and an in vivo mammalian study (rat bone marrow micronucleus test). This complete battery of GLP-compliant genotoxicity studies designed and conducted according to OECD and ICH guidelines have demonstrated that peppermint oil clearly shows no potential for genotoxic effects. The rat bone marrow micronucleus test was conducted at oral (gavage) doses up to 1350 mg/kg body weight/day, which corresponded to pulegone and menthofuran doses of respectively 12.2 and 50.0 mg/kg body weight/day. This study provided unequivocal evidence of a lack of genotoxicity for peppermint oil when administered orally. For the Ames test, experiments were carried out at dose levels up to 1500 µg/plate, corresponding to pulegone and menthofuran doses of respectively 13.5 and 55.5 µg/plate. In the mouse lymphoma assay, peppermint oil was tested as a solution at dose levels up to 5000 µg/ml (corresponding to pulegone and menthofuran concentrations of respectively 45 and 185 µg/ml). Overall, the weight of evidence showed that peppermint oil was not genotoxic. The reports will be submitted directly by the sponsoring company to the HMPC.	
ANME	Only recently a new test on mutagenicity using a preparation of mint oil (JHP Rödler, 95 % mint oil (partly dementholised) and 5% ethanol 96% was performed according to latest standards (OECD guidelines 474 and 489). The method used was an in vivo mammalian alkaline COMET assay and Micronucleus test [8]. JHP Rödler was dissolved in sesame oil. In a preliminary experiment the three dose levels 500 mg/kg, 1000 mg/kg and 2000 mg/kg were tested in rats for the determination of the	From the data provided no conclusion on pulegone exposure can be drawn. Ames and micronucleus tests may not be relevant for assessing genotoxicity of pulegone and menthofuran (see the dPS

Interested party	Comment and Rationale	Outcome
	maximum tolerated dose. 500 mg/kg b.w. did not cause toxicity, with 1000 mg/kg slight toxicity was observed, and 2000 mg/kg was fatal. In the main study ascending doses of 250, 500 and 1000 mg/kg per day of JHP Rödler were administered p.o. for three days. The negative control was sesame oil, a positive control group received ethyl methyl sulfonate. For the COMET assay liver and mucosa cells collected 48 hours after the first administration were examined. JHP Rödler did not increase DNA tail intensity at any dose compared to vehicle control. For the micronucleus assay erythrocytes were evaluated 48 hours post the first exposure. JHP Rödler did not increase the incidence of micronucleated polychromatic erythrocytes at any dose. JHP Rödler has also been examined in the AMES-test [9], however, due to the antimicrobial effects of mint oil the dose range with no interference from antibacterial effects on the Salmonella strains was a concentration of up to 100 µg/plate. No mutagenicity was detected under these conditions. In conclusion, we suggest that the results of these studies are mentioned in addition to the findings with pulegone, as they are clearly important for drawing conclusions on the potential carcinogenic risk (respectively its absence) in mint oil.	2.7).
EUCOPE	**No genotoxic potential of pulegone** Neither Ames test nor micronucleus assay could reveal any genotoxic potential of pulegone. Based on the available data the observed toxic effects are very probable due to cytotoxic properties of reactive metabolites and subsequent regenerative processes. According to this fact observed tumours aren't considered being relevant for human risk assessment as concluded in the Draft Public Statement.	*See above*

Interested party	Comment and Rationale	Outcome
2.7. Mode of action considerations		
ANME	We suggest the new studies cited herein are taken into account.	For the assessment of the studies, see above.
Schwabe lines 355-358	More appropriate tests to assess the potential genotoxicity of pulegone are probably the Comet assay or a transgenic gene mutation assay for both liver and bladder. Without such data it is not possible to conclude definitely on the genotoxic potential of pulegone and its metabolites. **Comment** Neither the Comet assay nor the transgenic gene mutation assay is a standard genotoxicity test. In the HMPC guidance document on the assessment of genotoxicity of herbal medicinal products (EMEA/HMPC/107079/2007) their performance is not foreseen. The results are therefore not validated with regard to the human risk assessment and / or regulatory procedures. Moreover the results of a Comet assay are publicly available. This was conducted with the essential oil from *Minthostachys verticillata* (peperina) containing 65% pulegone and 24% menthone (Escobar *et al*., 2015). The oil was administered to rats with the diet at doses of 0, 1, 4 and 7 g/kg feed for 90 days. Administration of the oil did not alter the weights of liver, kidney, and intestine, and no morphological or histopathological changes were observed in these tissues. Genotoxicity was tested by a bone marrow micronucleus test and a comet assay with peripheral blood cells. Peperina oil did not exert any genotoxic effect up to the highest concentration of 7 g/kg feed for 90 days. Thus, the highest dose corresponds to the very high daily intake of approx. 300 mg/kg pulegone. **Proposal for revision** Delete lines 355 to 358.	Not endorsed. All relevant studies should be considered. Standard genotoxicity assays such as Ames and micronucleus tests may not be relevant for assessing genotoxicity of pulegone and menthofuran (see the dPS, 2.7). The recently published 3-month study in rats using peperina oil (Escobar *et al*., 2015) is considered inferior to the NTP 3-month study based on (i) dietary administration instead of oral gavage (more uncertain exposure); (ii) lack of complete histopathological evaluation (only three tissues examined). In addition, a different rat strain (Wistar) was used in the Escobar *et al*. study as compared with the NTP studies, which used F344 rats.

Interested party	Comment and Rationale	Outcome
2.8. Relevance of experimental toxicities for human risk assessment		
ANME	This section outlines that the findings of carcinogenicity of pulegone are not likely transferable to humans and human risk assessment. This highly important conclusion is not reflected in the recommendations given in section 3. The proposed dose limitation for any medicinal product containing pulegone and menthofuran is still based on potential animal toxicity. If the liver toxicity and especially the carcinogenicity of pulegone is in fact not applicable to risk assessment in humans – as outlined in section 2.8 – there is no need for applying changes to the dosing of mint oil or peppermint oil, as would be the consequence of this Public Statement.	In toxicology, and especially when dealing with carcinogenicity and genotoxicity, toxicity studies in animals and mechanistic studies in-vitro constitute the background basis for the risk assessment. As is clear from the dPS, it is premature to decide that pulegone and methofuran are not genotoxicants, because definitive studies have not been performed. *See #2 in the general considerations.*
Schwabe line 406	In this study, the single pulegone dose administered was more similar to dietary exposure i.e. ~500 µg/kg bw. (Engel, 2003). However, the significance of this study in proving that at lower doses the conversion or pulegone to menthofuran is proportionally lower than in higher doses seems rather questionable. More definitive studies are needed. **Comment** The human study published by Engel (2003) uses doses of about 500 µg/kg bw, which is still 12500-times above the average dietary exposure (2 µg per day or 0.04 µg/kg bw), however. It is in the range of pulegone intake via therapeutic use of peppermint oil. It is in the range of pulegone intake via therapeutic use of peppermint oil intake. Moreover, peppermint oil contains far more menthol than pulegone. Menthol has been demonstrated to be a direct radical scavenger (Yang *et al.*, 2010) and can increase glutathione levels (Rozza *et al.*, 2014). Therefore, menthol likely increases detoxification capacity. **Proposal for revision** In this study, the pulegone exposure (500 µg/kg bw) was closer to the range of the	Not endorsed *See above*

Interested party	Comment and Rationale	Outcome
	therapeutic than dietary exposure (Engel, 2003).The significance of this study in proving that at lower doses the conversion or pulegone to menthofuran is proportionally lower than in higher doses is convincing. Only when the metabolic detoxification capacity is exhausted, are pathways leading to toxic metabolites followed. However more definitive studies are needed.	
2.9. Summary of weight-of-evidence toxicity risk assessment of pulegone and menthofuran		
ANME	The table needs updating with the results of the new studies presented herein.	New studies have been dealt with in appropriate sections of the PS.
Schwabe Table 1	Genotoxicity *in vitro:* Generally negative; few positive findings in the Ames test, which NTP considers significant, i.e. pulegone is genotoxic. The IARC working Group regards pulegone as non-genotoxic. **Current conclusion** Genotoxic potential cannot be evaluated. **Comment** The overall assessment of genotoxicity does not reflect the experimental test results and should therefore be modified. **Proposal for revision** Genotoxicity *in vitro*: Generally negative; two marginally positive findings in the Ames test, which NTP considers significant, i.e. pulegone is genotoxic. The IARC working Group regards pulegone as non-genotoxic **Current conclusion** Not genotoxic *in vitro.* Other information: Some evidence of non-linearity of metabolic activation and adduct	Not endorsed The dPS clearly considers non-linearity, although regards evidence not adequate.

Interested party	Comment and Rationale	Outcome
	formation **Comment** In the whole public statement there are no data on the non-linearity of metabolic activation and adduct formation. In contrast the public statement concludes that the hepatotoxicity is a mechanism with threshold. **Proposal for revision** The row is deleted.	
2.10. Determination of limit value		
AESGP	**Body weight of 50 kg not appropriate as basis for calculation** The HMPC is requested to reconsider its body weight (b.w.) 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 b.w. distribution frequent in those affected by severe conditions.	Even if the difference by using either 50 or 60 kg as a body weight is small, we'll decide in the end, which one to use. ICH Q3C uses 50 kg. Ultimately, the selection is dependent on the outcome of the coordination.

Interested party	Comment and Rationale	Outcome
	Table 1: Body weight (kg) statistics for adult subjects in all surveys of the EFSA Comprehensive database N: number of individuals in the database SD: standard deviation P5: 5th percentile P95: 95th percentile Source: EFSA 2012	
ANME	Defining a threshold of 3.5 mg/person/day of pulegone + menthofuran does not seem justified for peppermint oil and mint oil (see also Introduction). In both cases has the usual dosage scheme been clinically applied for decades without any hint to toxicity. Safety of the dose of essential oil compliant to the specifications of the European Pharmacopoeia has implicitly been confirmed through the German Commission E, the German Standard Marketing Authorisations and the HMPC monograph. The absence of mutagenic effects of mint oil was demonstrated through a state-of-the-art COMET assay and micronucleus test. The proposed limit of 3.5 mg/day would imply that none of the currently used preparations of peppermint oil and mint oil could be marketed in the present form/dosage, despite decades of experience with the undebated safety of such preparations. It would also lead to the discrepancy that food items containing mint/peppermint oil would still be	Not endorsed Argumentation is concerned with commercial or chemical-analytical facts, not scientific and toxicological assessment. Clinical or pharmacovigilance studies guarantee safety, at least not regarding carcinogenicity and genotoxicity. A modified NOAEL has been adopted, with a higher limit value.

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

Interested party	Comment and Rationale	Outcome
	available in the present form, whereas medicinal products would no longer be available with the argument of consumer safety. This would be hard to explain to the consumer. A limit definition should at least take the realities into account. With no hints to toxicity from peppermint/mint oil a proposed limit should be oriented at the regular dose scheme of peppermint oil used according to the well-established scheme. Peppermint oil contains up to 8.0 percent of menthofuran and 3.0 percent of pulegone. The sum of both is 11 percent, which at a daily dose of 1100 mg corresponds to an exposure of 121 mg per day. The established dose therefore exceeds the proposed limit by the factor of 34.6 – just based on hypothetical concerns. A dose reduction by the factor of 34.6 is impossible to achieve, as has been outlined in the comments to the Introduction. The definition of such a threshold would therefore come down to a *de facto* ban of mint oil and peppermint oil preparations as medicinal products.	
Schwabe line 430	The value of 20 mg/kg bw per day, based on the NTP chronic study, is taken as a LOAEL value. It is possible to use a safety factor of 300 (not 100, because of LOAEL was the lowest significant effect level). Consequently the acceptable exposure would be 0.07 mg/kg bw per day, which is close to the current ADI value of 0.1 mg/kg bw per day. The daily dose for an adult of 50 kg body weight would thus be 3.5 mg/person/day. **Comment** As discussed above the NOAEL of 18.75 mg/kg bw (rounded value of 20 mg/kg bw) can be reliably established in male rats as the most sensitive species and sex. For the conversion of the NOAEL dose in rats to an equivalent dose in humans based on the body surface and assuming a human body weight of 60 kg a factor of 6.2 has been established (FDA Guidance for Industry, 2005). In order to allow for variability in extrapolating from animal toxicity studies to humans an additional default factor of 10 is recommended, resulting in a safety margin of 62. The limit value for pulegone intake is therefore approx. 20 mg per day for a 60 kg person.	Partially endorsed A modified NOAEL has been adopted, with a higher limit value.

Interested party	Comment and Rationale	Outcome
	This is highly conservative compared to the risk assessment of the Committee of Experts on Flavouring Substances (CEFS). Based on a subacute toxicity study of only 28 days duration in rats, a safety factor of 200 has been recommended. This results in an ADI value of 0.1 mg/kg bw, which is considered as safe for foodstuffs upon lifelong exposure. Compared to that, medicinal products are ingested for a limited period of time and they must possess a positive risk/benefit ratio in order to be authorized. According to EFSA "a body weight of 70 kg should be used as default for the European adult population (aged above 18 years) as this is more realistic (EFSA, 2012). **Proposal for revision** The value of 18.75 mg/kg bw (rounded to 20 mg/kg bw) per day, based on the NTP chronic study, is taken as the NOAEL value of pulegone. It is possible to use a safety factor of 62 (FDA Guidance for Industry, 2005). Consequently the acceptable exposure is 0.3 mg/kg bw per day, which is close to the current Acceptable Daily Intake (ADI) value for foodstuffs of 0.1 mg/kg bw per day for lifelong exposure. The daily dose for an adult of 70 kg body weight (EFSA, 2012) would thus be approx. 20 mg/person/day.	
3.1. Toxicological conclusions		
Schwabe lines 459-462	As an interim recommendation, the HMPC suggests that an acceptable exposure limit is 0.07 mg/kg bw per day, which is close to the current ADI value of 0.1 mg/kg bw per day. This limit value should be reviewed when adequate genotoxicity studies are available and relevance of rodent tumours to human carcinogenicity has been assessed. **Comment** As discussed above the NOAEL of 18.75 mg/kg bw can be reliably established in male rats as the most sensitive species and sex. For the conversion of the NOAEL dose in rats to an equivalent dose in humans based on the body surface and assuming a human body weight of 60 kg a factor of 6.2 has been established (FDA Guidance for Industry, 2005). In order to allow for variability in extrapolating from animal toxicity studies to humans an	*See above*

Interested party	Comment and Rationale	Outcome
	additional default factor of 10 is recommended, resulting in a safety margin of 62. The safety threshold for pulegone intake with medicinal products is therefore approx.20 mg per person and day. The potential hepatotoxicity of menthofuran is much lower than that of pulegone (approx. half according to Thomassen *et al.*, 1988). This has to be considered with regard to human risk assessment. An adjustment factor on the safety assessment of menthofuran of 50% is proposed. Practically, the content of menthofuran in an herbal medicinal product can therefore be multiplied by the factor of 0.5 on a weight for weight basis with pulegone. **Proposal for revision** As an interim recommendation, the HMPC suggests that an acceptable exposure limit for pulegone is 0.3 mg/kg bw per day, which is close to the current ADI value for food stuffs of 0.1 mg/kg bw per day for lifelong exposure. The safety threshold for menthofuran is 0.6 mg/kg bw per day. This limit value should be reviewed when adequate genotoxicity studies are available and relevance of rodent tumours to human carcinogenicity has been assessed.	
3.2. Recommended limit values		
	Daily limit for oral use We regard parts of information outlined in the EMA/HMPC/138386/2005 Rev. 1 draft Public statement as not appropriate for the assessment of peppermint oil and mint oil, since it is specific to pulegone and/or menthofuran only. Based on the available non-clinical data on pulegone, limited toxicity data with peppermint oil and the presence of human safety data, there is no scientific rationale to justify an exposure limit of 3.5 mg/person/day (pulegone + menthofuran) (on a 50 kg bw basis) at the moment. Therefore, the available toxicity data on pulegone, menthofuran and peppermint oil should be re-discussed according to the Weight of Evidence (WOE)	*See #2-5 in the general considerations.*

Interested party	Comment and Rationale	Outcome
	approach and the potential mechanism of action (MOA). In conjunction with quantitative data on the natural daily intake of pulegone and menthofuran from food and other products, a new benefit/risk assessment should be made for the therapeutic use of the peppermint oil. For example, for the treatment of irritable bowel syndrome, the daily intake of 3 capsules each containing 182 mg peppermint oil would result in max. 16.4 mg pulegone (≤3.0%) and max. 43.7 mg menthofuran (1.0-8.0%) daily (the HMPC monograph recommends 1-2 capsules up to three times daily). **Due to the differences in potential toxicity of pulegone and menthofuran, a sum parameter cannot be established. Menthofuran should be taken into consideration to a lower extent only. Should a sum parameter nonetheless be established, for the above-mentioned reasons, in particular the safety factor, we propose a limit for the sum of pulegone and menthofuran of at least 60 mg/person daily.** In case a limit of 60 mg daily is not acceptable, a limit of 20 mg pulegone daily could be considered. This would be based on a NOAEL in male rats as the most sensitive animal species and sex, a safety factor of 62 and an average body weight of 70 kg in accordance with 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. A limit of 20 mg, however, would only be possible in case of careful selection of all batches of essential oil used as active substance and/or by a reduction of the approved dosage recommendation. If a limit value for the sum of pulegone and menthofuran is established, an adjustment factor for menthofuran is required. As the toxic potential of menthofuran is less than 50 % of that of pulegone (Thomassen *et al.,* 1988), it is suggested to multiply the content of menthofuran in a herbal medicinal product by a factor 0.5.	

Interested party	Comment and Rationale	Outcome
AESGP lines 467-469	**Duration of oral use of peppermint oil** A limitation of the use of medicinal products containing peppermint oil for two weeks is inappropriate and incompatible with the long-standing therapeutic use, especially in the treatment of gastrointestinal complaints such as irritable bowel syndrome. In clinical trials, the safety of peppermint oil for therapy intervals of 2 to 11 weeks and in one open study up to 6 months has been demonstrated (Grigoleit, 2005). Furthermore, such a limitation does not comply with existing marketing authorisations and the respective HMPC monograph which recommends a duration of use of 3 months based on clinical studies. **The established duration of use for peppermint oil of 3 months (intake for periods of no longer than 3 months per course) should not be modified.**	*See #2-4 in the general considerations.*
EUCOPE	**Daily intake and duration of oral use of peppermint oil** The daily limit of 3.5 mg/person/day is not in line with the recommendations of HMPC European Union Monograph on Peppermint oil (EMEA/HMPC/349466/2006). **Example:** For the treatment of irritable bowel syndrome the intake of three capsules daily, each containing 182 mg peppermint oil, would result in max. 16.4 mg pulegone (≤3.0%) and max. 43.7 mg menthofuran (1.0-8.0%) daily, resulting in approx. **60 mg**. We therefore propose a limit for the sum of pulegone and menthofuran of **at least 60 mg/person daily**. In case this value is not acceptable, a limit of 20 mg pulegone daily should be taken into consideration, which is based on a NOAEL in male rats as the most sensitive animal species and sex, a safety factor of 62 and an average body weight of 70 kg in accordance with 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. From our point of view the limitation of the use of herbal medicinal products containing peppermint oil for two weeks only is inappropriate and not in line with the safe	*See above*

Interested party	Comment and Rationale	Outcome
	therapeutical use during the last decades of functional gastro-intestinal disorders, which requires an administration for longer periods. The Public Statement should follow the recommendation of HMPC European Union Monograph on Peppermint oil (EMEA/HMPC/349466/2006): "The gastro-resistant capsules should be taken until symptoms resolve, usually within one or two weeks. At times when the symptoms are more persistent, the intake of gastro-resistant capsules can be continued for periods of no longer than 3 months per course." We therefore suggest to comply with the HMPC recommendations and propose an intake for periods of **no longer than 3 months per course**.	
AESGP Lines 494-497	**Cutaneous use** The public statement does not quote available data concerning absorption through the skin, but on the other hand does not differentiate between oral and cutaneous use. It refers to the use as "penetration enhancer". The analysis of the patent US 5128135 A (Percutaneous or trans-mucosal absorption enhancers, preparations containing the enhancers, and a method of preparing thereof) shows that the enhancing potency of pulegone is second weakest among the tested substances (Fig.1). A transdermal penetration study on rat skin (Narishetty, 2004) also points out pulegone as inferior to other tested substances, which makes it unlikely to be used in skin preparations in the cosmetic market. In the only patent US 6391324 B2 (Cosmetic skin care compositions containing pulegone) especially using pulegone as a "penetration enhancer" pulegone was shown to increase the absorption of glucose into keratinocytes in vitro. **Bath additives** Bath additives containing peppermint oil or mint oil can be purchased from retail outlets by the general public. Exposure to pulegone and menthofuran present in bath additives may occur by dermal absorption from water. For cosmetic bath additives the Scientific	Due to a lack of specific studies on dermal penetration of pulegone an assumption of "some penetration" was made.

Interested party	Comment and Rationale	Outcome
	Committee on Consumer Safety, SCCS, has assessed 2% peppermint oil as safe for use: "Preparation Essential oil. Toxicological data. Group 3 Recommended ingredients: This group consists of ingredients which, on basis of adequate data, do not present any health hazards, and which therefore may be safely used in cosmetic products for the purposes stated according to reported use levels. Maximum concentration 2 per cent". To assess the exposure, concentration parameters can be used to estimate the concentration of a substance in a medium that might come into contact with the body. The result obtained is not necessarily equal to the concentration of the substance in a bath additive, because a bath additive is usually diluted, undergoes evaporation, etc., before the substance of interest actually reaches the human body. Therefore, two factors are added to the equation: partition coefficient and rinse-off coefficient, which is shown at the following example of an exposure calculation for a rinse-off pharmaceutical product (bath additive): Intended use: 30 g bath additive in 100 l water (once daily use for maximal 15 minutes) Amount of product used (**G**): Concentration of peppermint oil in 30 g bath additive: 0.09 g Concentration of 11% substances (pulegone (3%) and menthofuran (8%) in 0.09 g peppermint oil): **9.9 mg** Rinse-off coefficient (**R**): **0.1** Partition coefficient (**P**): **0.1** Exposure relevant amount of pulegone and menthofuran (M): M = G x R x P M = 9.9 mg x 0.1 x 0.1 = **0.099 mg pulegone/menthofuran per person per day** taking into account an average of 70 kg bodyweight:	

Interested party	Comment and Rationale	Outcome
	exposure E = M/K = 1.414 µg pulegone + menthofuran per kg bw per day	
Schwabe lines 476-478	The intake (pulegone + menthofuran) of 3.5 mg/person/day (even if the limit represents the overall intake from all sources) can be accepted for herbal medicinal products as short-term intake (maximum 14 days). **Comment** The proposed limitation in the HMPC draft public statement (lines 467-469) for the use of medicinal products containing peppermint oil for two weeks is inappropriate. Moreover it is incompatible with the long-standing therapeutic use, especially in the treatment of gastrointestinal complaints such as irritable bowel syndrome (IBS). In clinical trials, the safety of peppermint oil for therapy intervals of 2 to 11 weeks and in one open study up to 6 months has been demonstrated (Grigoleit & Grigoleit, 2005). Furthermore such a limitation does not comply with existing marketing authorizations and the respective HMPC monograph which recommends a use of 3 months based on clinical studies. **Proposal for revision** The intake of pulegone and menthofuran of <u>20 mg/person/day</u> (even if the limit represents the overall intake from all sources) can be accepted as safe for herbal medicinal products as short to medium term intake (<u>maximum 3 months</u>).	*See above*
3.3. Proposals for regulatory actions		
AESGP Line 510	**Proposals for Regulatory Actions made by the HMPC** The analysis of data originating from the therapeutic application of peppermint oil does not reveal any drug associated adverse effects on the liver or kidney. The post-marketing experience shows that peppermint oil is safe and tolerable at therapeutic dosages. The state-of-the art pharmacovigilance practices complying with European legislation ensure that relevant safety information is collected, documented and properly assessed.	Not endorsed As stated above, pharmacovigilance is not a very sensitive tool to reveal increased incidences of common toxicities, including cancers. However, if genotoxicity and carcinogenicity

Interested party	Comment and Rationale	Outcome
	For example, the British MRHA can demonstrate the lowest incidence of bladder cancer, in spite of the very high use of peppermint oil in the UK. UK bladder cancer incidence rates are estimated to be the lowest in males in Europe, and 13th lowest in females. These data are broadly in line with Europe-specific data available elsewhere (Ferlay, 2012; 2013). It should be kept in mind that the United Kingdom at the same time is the largest single market for peppermint oil (US Department of Agriculture, 1972). For these reasons, no focused pharmacovigilance actions are required for the respective essential oils, and no increased awareness of the medical community is necessary. Thus the 2nd and 3rd bullet points of the HMPC draft can be deleted. With regard to the 4th bullet point, the recommended limit values and the limitation of use are considered incompatible with the long-standing therapeutic use of many medicinal products. At present, based on the available equivocal non-clinical data for pulegone, there is also no scientific rationale for a regulatory recommendation of 3.5 mg pulegone + menthofuran in herbal medicinal products containing peppermint oil or for a limited treatment duration of maximum 14 days. In this context, dietary (background) exposure to pulegone and menthofuran, genotoxic or non-genotoxic mechanism, mode of action, saturation of metabolic pathways and the existence of a threshold-dependent dose-relationship are of particular relevance. We would therefore like to ask the HMPC for a toxicological re-assessment of specific peppermint oils on the basis of data on pulegone, menthofuran and the specific peppermint oil in conjunction with relevant data on natural exposure and proven safety in humans. **Inspite of these comments, should any regulatory action still be considered necessary, AESGP would ask for a meeting to discuss the implications for existing products and the need for a transition period.**	findings of experimental studies were shown to be irrelevant for human risk assessment, the framework and boundaries for assessment of pulegone and menthofuran would be significantly different.

Interested party	Comment and Rationale	Outcome
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