

SCIENTIFIC OPINION

Flavouring Group Evaluation 57 (FGE.57)¹:

Consideration of two structurally related pulegone metabolites and one ester thereof evaluated by JECFA (55th meeting)

Scientific Opinion of the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)

(Question No EFSA-Q-2008-032H)

Adopted on 29 January 2009

PANEL MEMBERS

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SUMMARY

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) was asked to provide scientific advice to the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular the Panel was requested to consider the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. These flavouring substances are listed in the Register, which was adopted by Commission Decision 1999/217/EC and its consecutive amendments.

Six substances were evaluated by the JECFA in the group of pulegone and structurally related substances. Pulegone (JECFA-no: 753) and menthofuran (JECFA-no: 758) are in Annex III of

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Regulation (EC) No 1334/2008 of the European Parliament and of the Council (EC, 2008b) and accordingly cannot be used as flavouring substances in the EU. p-Mentha-1,4(8)-dien-3-one [FL-no: 07.127] is an alpha,beta-unsaturated ketone which was considered together with other alpha,beta-unsaturated ketones in FGE.213, in which it was concluded that additional genotoxicity data were required.

The Panel considered the remaining three substances in this FGE, isopulegol [FL-no: 02.067], isopulegone [FL-no: 07.067] and isopulegyl acetate [FL-no: 09.219]). However, as there are some data indicating that isopulegone might be partly isomerised to pulegone via a minor metabolic pathway (Gordon et al., 1987; McClanahan et al., 1988), the Panel also considered data on pulegone and the metabolically related menthofuran and took into account the SCF Opinion on pulegone and menthofuran (SCF, 2002g), later revised by the European Food Safety Authority (EFSA) based on additional data (EFSA, 2005i).

The Panel agrees with the application of the Procedure as performed by the JECFA for the three substances considered in this FGE until step B3. As no appropriate study could be identified to derive a No Observed Adverse Effect Level (NOAEL), the Panel concluded at step B4, contrary to the JECFA, that for all three substances [FL-no: 02.067, 07.067 and 09.219] additional toxicity data are required.

For the three substances [FL-no: 02.067, 07.067 and 09.219] evaluated through the Procedure use levels are needed to calculate the modified Theoretical Added Maximum Daily Intakes (mTAMDI) in order to identify those flavouring substances that need more refined exposure assessment and to finalise the evaluation.

In order to determine whether the conclusion for the three JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Specifications including purity and identity are available for one JECFA evaluated substance [FL-no: 07.067]. Information on composition of mixture is incomplete for the other two substances [FL-no: 02.067 and 09.219].

Thus, the Panel has reservations for all three substances. For two of the three substances the composition of the mixture has to be specified [FL-no: 02.067 and 09.219] and for all three substances [FL-no: 02.067, 07.067 and 09.219] additional toxicity data are required.

Key words: Pulegone, menthofuran, isopulegone, isopulegol, JECFA 55th meeting

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BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996) lays down a Procedure for the establishment of a list of flavouring substances, the use of which will be authorised to the exclusion of all other substances in the EU. In application of that Regulation, a Register of flavouring substances used in or on foodstuffs in the Member States was adopted by Commission Decision 1999/217/EC (EC, 1999a), as last amended by Commission Decision 2008/478/EC (EC, 2008a). Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.

Substances which are listed in the Register are to be evaluated according to the evaluation programme laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a), which is broadly based on the Opinion of the Scientific Committee on Food (SCF, 1999).

Commission Regulation (EC) No 1565/2000 lays down that substances that are contained in the Register and will be classified in the future by the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) so as to present no safety concern at current levels of intake will be considered by the European Food Safety Authority (EFSA), who may then decide that no further evaluation is necessary.

In the period 2000 – 2006, during its 55th, 57th, 59th, 61st, 63rd and 65th meetings, the JECFA evaluated about 900 substances which are in the EU register.

TERMS OF REFERENCE

EFSA is requested to consider the JECFA evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a). These flavouring substances are listed in the Register, which was adopted by Commission Decision 1999/217/EC (EC, 1999a) and its consecutive amendments.

ACKNOWLEDGEMENTS

European Food Safety Authority wishes to thank the members of the Working Groups on Flavourings and ac hoc experts for the preparation of this opinion: Ulla Beckman Sundh, Vibe Beltoft, Wilfried Bursch, Angelo Carere, Riccardo Crebelli, Karl-Heinz Engel, Henrik Frandsen, Jørn Gry, Rainer Gürtler, Frances Hill, Trine Husøy, John Christian Larsen, Catherine Leclercq, Pia Lund, Wim Mennes, Gerard Mulder, Karin Nørby, Gerard Pascal, Iona Pratt, Gerrit Speijers, Harriet Wallin.

ASSESSMENT

The approach used by EFSA for safety evaluation of flavouring substances is referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000a), hereafter named the “EFSA Procedure”. This Procedure is based on the Opinion of the Scientific Committee on Food (SCF, 1999), which has been derived from the evaluation procedure developed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1995; JECFA, 1996a; JECFA, 1997a; JECFA, 1999b), hereafter named the “JECFA Procedure”. The Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) compares the JECFA evaluation of structurally related substances with the result of a corresponding EFSA evaluation, focussing on specifications, intake estimations and toxicity data, especially genotoxicity data. The evaluations by EFSA will conclude whether the flavouring substances are of no safety concern at their estimated levels of intake, whether additional data are required or whether certain substances should not be evaluated through the EFSA Procedure.

The following issues are of special importance.

Intake

In its evaluation, the Panel as a default uses the Maximised Survey-derived Daily Intake (MSDI) approach to estimate the *per capita* intakes of the flavouring substances in Europe.

In its evaluation, the JECFA includes intake estimates based on the MSDI approach derived from both European and USA production figures. The highest of the two MSDI figures is used in the evaluation by the JECFA. It is noted that in several cases, only MSDI figures from the USA were available, meaning that certain flavouring substances have been evaluated by the JECFA only on the basis of these figures. For Register substances for which this is the case the Panel will need EU production figures in order to finalise the evaluation.

When the Panel examined the information provided by the European Flavour Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach. It is noted that the JECFA, at its 65th meeting, considered “how to improve the identification and assessment of flavouring agents, for which the MSDI estimates may be substantially lower than the dietary exposures that would be estimated from the anticipated average use levels in foods” (JECFA, 2006c).

In the absence of more accurate information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a modified Theoretical Added Maximum Daily Intake (mTAMDI) approach based on the normal use levels reported by Industry.

As information on use levels for the flavouring substances has not been requested by the JECFA or if it has not otherwise been provided to the Panel, it is not possible to estimate the daily intakes using the mTAMDI approach for the substances evaluated by the JECFA. The Panel will need information on use levels in order to finalise the evaluation.

Threshold Criterion of 1.5 Microgram/Person/Day (Step B5) Used by the JECFA

The JECFA uses the threshold of concern of 1.5 microgram/person/day as part of the evaluation procedure:

“The Committee noted that this value was based on a risk analysis of known carcinogens which involved several conservative assumptions. The use of this value was supported by additional information on developmental toxicity, neurotoxicity and immunotoxicity. In the judgement of the Committee, flavouring substances for which insufficient data are available for them to be evaluated using earlier steps in the Procedure, but for which the intake would not exceed 1.5 microgram per person per day would not be expected to present a safety concern. The Committee recommended that the Procedure for the Safety Evaluation of Flavouring Agents used at the forty-sixth meeting be amended to include the last step on the right-hand side of the original procedure (“Do the condition of use result in an intake greater than 1.5 microgram per day?”)” (JECFA, 1999b).

In line with the Opinion expressed by the Scientific Committee on Food (SCF, 1999), the Panel does not make use of this threshold criterion of 1.5 microgram per person per day.

Genotoxicity

As reflected in the Opinion of SCF (SCF, 1999), the Panel has in its evaluation focussed on a possible genotoxic potential of the flavouring substances or of structurally related substances. Generally, substances for which the Panel has concluded that there is an indication of genotoxic potential *in vitro*, will not be evaluated using the EFSA Procedure until further genotoxicity data are provided. Substances for which a genotoxic potential *in vivo* has been concluded, will not be evaluated through the Procedure.

Specifications

Regarding specifications, the Panel evaluation could lead to a different opinion than that of the JECFA, e.g. Panel requests additional information on isomerism.

Structural Relationship

In the consideration of the JECFA evaluated substances, the Panel will examine the structural relationship and metabolism features of the substances within the flavouring group and compare this with the corresponding FGE.

1. Presentation of the Substances in the JECFA Group of substances related to Pulegone

1.1. Description

1.1.1. JECFA Status

The JECFA Committee has evaluated a group of six flavouring substances consisting of pulegone and five structurally related substances. The six substances are pulegone, menthofuran, p-metha-1,4-(8)-dien-3-one, isopulegol, isopulegone and isopulegyl acetate (JECFA-no: 753, 758, 757, 755, 754 and 756).

1.1.2. EFSA Considerations

Pulegone (JECFA-no: 753) and menthofuran (JECFA-no: 758) are in Annex III of Regulation (EC) No 1334/2008 of the European Parliament and of the Council (EC, 2008b) and accordingly cannot be used as flavouring substances in the EU. p-Mentha-1,4(8)-dien-3-one [FL-no: 07.127] is an alpha,beta-unsaturated ketone which was considered together with other alpha,beta-unsaturated ketones in FGE.213, in which it was concluded that additional genotoxicity data were required before the substances could be evaluated using the Procedure. This consideration therefore only deals with three flavouring substances [FL-no: 02.067, 07.067 and 09.219].

There are some indication that, to a small extent, isopulegone may be isomerised to pulegone (Gordon et al., 1987; McClanahan et al., 1988). Therefore, the Panel considered it relevant to include data on pulegone and the metabolically related menthofuran. Accordingly, in the consideration of the three substances in this FGE (isopulegol [FL-no: 02.067], isopulegone [FL-no: 07.067] and isopulegyl acetate [FL-no: 09.219]) the Panel will take into account the SCF Opinion on pulegone and menthofuran (SCF, 2002g), later revised by EFSA based on additional data (EFSA, 2005i).

1.2. Stereoisomers

1.2.1. *JECFA Status*

All three substances [FL-no: 02.067, 07.067 and 09.219] in the group of the JECFA evaluated flavouring substances have one or more chiral centres.

1.2.2. *EFSA Considerations*

For two of the substances [FL-no: 02.067 and 09.219] the composition of the mixture has to be specified (see Table 1).

1.3. Specifications

1.3.1. *JECFA Status*

The JECFA specifications are available for all three substances (JECFA, 2000d) (see Table 1).

1.3.2. *EFSA Considerations*

Specifications are adequate for one substance [FL-no: 07.067]. Information on composition of mixture are requested for two substances [FL-no: 02.067 and 09.219] (see Section 1.2.2).

2. Intake Estimations

2.1. JECFA Status

For all three substances evaluated through the JECFA Procedure intake data are available for the EU.

2.2. EFSA Considerations

No comments.

3. Genotoxicity Data

3.1. Genotoxicity Studies – Text Taken² from the JECFA (JECFA, 2001b)

In vitro

Assays for genotoxicity have been performed with pulegone (JECFA no: 753) and menthofuran (JECFA no: 758). Pulegone did not induce reverse mutation in *Salmonella typhimurium* strain TA1537, TA1535, TA100, TA98 or TA97, with or without metabolic activation, at concentrations up to 800 microg/plate (Andersen & Jensen, 1984b). Neither substance was mutagenic in *S.*

² The text is taken verbatim from the indicated reference source, but text related to substances not included in the present FGE has been removed.

typhimurium strains TA100 and TA98 at concentrations up to 1000 microg/plate, with or without metabolic activation (Nelson & Dybing, 1998). In a study of the insecticidal properties of mint oils, concentrations of pulegone in excess of the LD₅₀ value for *Drosophila* larvae (0.17 microL) induced a slight increase in the frequency of wing mutations (mosaic spots) over that induced by control solutions (Franzios et al., 1997).

In vivo

No *in vivo* genotoxicity studies are available on isopulegol [FL-no: 02.067], isopulegone [FL-no: 07.067] and isopulegyl acetate [FL-no: 09.219].

Conclusion on genotoxicity

No conclusion was made by the JECFA with respect to genotoxicity.

For a summary of *in vitro* genotoxicity data considered by the JECFA, see Table 2.1.

3.2. EFSA Considerations

The Panel considered three substances in this FGE, isopulegol [FL-no: 02.067], isopulegone [FL-no: 07.067] and isopulegyl acetate [FL-no: 09.219]). However, as there are some data indicating that isopulegone might be partly isomerised to pulegone via a minor metabolic pathway (Gordon et al., 1987; McClanahan et al., 1988), the Panel also considered data on pulegone and the metabolically related menthofuran and took into account the SCF Opinion on pulegone and menthofuran (SCF, 2002g), later revised by EFSA based on additional data (EFSA, 2005i). In the EFSA Opinion (EFSA, 2005i) the following short-term studies on menthofuran and pulegone were mentioned:

Menthofuran was negative in the Ames assay using *S. typhimurium* strains TA97, TA98, TA100 or TA1535 at concentrations up to and including 667 microg/plate with or without metabolic activation by Syrian hamster or rat liver S9 preparations (NTP, 1999e).

Pulegone was negative in the Ames assay using *S. typhimurium* strains TA97 and TA1535 at concentrations up to and including 2167 microg/plate and against *S. typhimurium* strains TA98 and TA100 up to and including 3333 microg/plate with or without metabolic activation by Syrian hamster or rat liver S9 preparations (NTP, 2000e).

Pulegone was also negative against *E. coli* pKM101 and *S. typhimurium* strains TA98 and TA100 at concentrations up to and including 3500 microg/plate with or without metabolic activation by Syrian hamster or rat liver S9 preparations (NTP, 2000e).

The Panel also considered a genotoxicity study (NTP, 2000e), not included in the EFSA Opinion (EFSA, 2005i); a micronucleus test on mouse peripheral blood, performed within a subchronic toxicity NTP study, did not reveal treatment related increases in the incidence of micronucleated polychromatic erythrocytes. However, this test has to be considered largely inadequate due to limitations of the study protocol, especially for what concerns the low dosage achieved. Therefore, no definitive conclusions on the clastogenic potential of pulegone can be drawn from these findings, also taking into account the lack of an *in vitro* chromosome aberration test.

Overall, the Panel concluded that although no genotoxicity data were available for isopulegone, isopulegol and isopulegyl acetate [FL-no: 07.067, 02.067 and 09.219] and the genotoxicity data available for pulegone and menthofuran are incomplete, the data available do not preclude an evaluation of the three substances through the Procedure.

For a summary of additional genotoxicity data considered by EFSA, see Table 2.2.

4. Application of the Procedure

4.1. Application of the Procedure to Three Flavouring Substances Structurally Related to Pulegone by the JECFA (JECFA, 2001b):

Step 1

According to the JECFA, isopulegol [FL-no: 02.067] and isopulegyl acetate [FL-no: 09.219] are allocated to structural class I and isopulegone [FL-no: 07.067] is allocated to structural class II (Cramer et al., 1978).

Step 2

At the estimated levels of intake, none of the three flavouring substances would be expected to saturate the available metabolic detoxication pathways, but they are not completely metabolised to innocuous products. Since pulegone and related substances are metabolised, in part, to reactive metabolites, their evaluation proceeds via the B-side of the Procedure.

Step B3

The daily per capita intakes of all the substances in this group are below the threshold for human intake for each class (class I, 1800 µg; class II, 540 µg). Accordingly, evaluation of these substances proceeded to step B4.

Step B4

The lack of toxicity of pulegone at low levels of intake was demonstrated in a 90-day study in rats fed peppermint oil that contained 1.1 % pulegone. The No Observed Effect Level (NOEL) of 40 mg/kg body weight (bw) per day for nephropathy associated with hyaline droplets at a higher dose (Spindler & Madsen, 1992) corresponds to a NOEL of 0.44 mg/kg bw per day (26 mg/person per day) for pulegone. This NOEL is more than 10 000 times the intake of 0.033 µg/kg bw per day from use of pulegone as a flavouring agent. Since pulegone is metabolised to menthofuran [FL-no: 13.035], data on pulegone can be used to evaluate the safety of menthofuran, although the latter was about three times more hepatotoxic after single doses (Gordon et al., 1982). Isopulegone [FL-no: 07.067] was less hepatotoxic than pulegone after single doses. The NOEL of 0.44 mg/kg bw per day for pulegone in the 90-day study is more than 1000 times the daily intake of 0.4 µg/kg bw per day from use of menthofuran as a flavouring agent. Isopulegone [FL-no: 07.067], isopulegol [FL-no: 02.067] and isopulegyl acetate [FL-no: 09.219] are expected to be partly metabolised to menthofuran. Even if these compounds are assumed to be metabolised to menthofuran to the same extent as pulegone, however, the NOEL for pulegone is more than 10 000 times the daily intake from use of isopulegone and isopulegyl acetate and is more than 1000 times the daily intake from use of isopulegol as a flavouring agent.

In conclusion, the JECFA evaluated all three substances as to be of no safety concern at the estimated level of intake as a flavouring substance based on the MSDI approach.

The evaluations of the three flavourings are summarised in Table 3: Summary of Safety Evaluation of Three Flavourings Structurally Related to Pulegone (JECFA, 2001a).

4.2. EFSA Considerations

A No Observed Adverse Effect Level (NOAEL) could not be identified for isopulegone, isopulegol and isopulegyl acetate [FL-no: 02.067, 07.067 and 09.219] or for sufficiently structurally related

substances. Accordingly, the Panel concluded at step B4 (contrary to the JECFA) that further data are required for these three substances.

5. Conclusion

Six substances were evaluated by the JECFA in the group of pulegone and structurally related substances. Pulegone (JECFA-no: 753) and menthofuran (JECFA-no: 758) are in Annex III of Regulation (EC) No 1334/2008 of the European Parliament and of the Council (EC, 2008b) and accordingly cannot be used as a flavouring substance in the EU. p-Mentha-1,4(8)-dien-3-one [FL-no: 07.127] is an alpha,beta-unsaturated ketone which was considered together with other alpha,beta-unsaturated ketones in FGE.213, in which it was concluded that additional genotoxicity data were required.

The Panel considered the remaining three substances in this FGE, isopulegol [FL-no: 02.067], isopulegone [FL-no: 07.067] and isopulegyl acetate [FL-no: 09.219]). However, as there are some data indicating that isopulegone might be partly isomerised to pulegone via a minor metabolic pathway (Gordon et al., 1987; McClanahan et al., 1988), the Panel also considered data on pulegone and the metabolically related menthofuran and took into account the SCF Opinion on pulegone and menthofuran (SCF, 2002g), later revised by EFSA based on additional data (EFSA, 2005i).

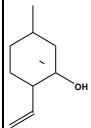
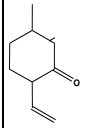
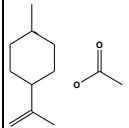
The Panel agrees with the application of the Procedure as performed by the JECFA for the three substances considered in this FGE until step B3. As no appropriate study could be identified to derive a NOAEL, the Panel concluded at step B4, contrary to the JECFA, that for all three substances [FL-no: 02.067, 07.067 and 09.219] additional toxicity data are required.

For the three substances [FL-no: 02.067, 07.067 and 09.219] evaluated through the Procedure use levels are needed to calculate the mTAMDI values in order to identify those flavouring substances that need more refined exposure assessment and to finalise the evaluation.

In order to determine whether the conclusion for the three JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Specifications including purity and identity are available for one JECFA evaluated substance [FL-no: 07.067]. Information on composition of mixture is incomplete for the other two substances [FL-no: 02.067 and 09.219].

Thus, the Panel has reservations for all three substances. For two of the three substances the composition of the mixture has to be specified [FL-no: 02.067 and 09.219] and for all three substances additional toxicity data are required [FL-no: 02.067, 07.067 and 09.219].

TABLE 1: SPECIFICATION SUMMARY FOR JECFA EVALUATED SUBSTANCES IN THE PRESENT GROUP (JECFA, 2000D)

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Three Flavourings Structurally Related to Pulegone								
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
02.067 755	Isopulegol		2962 2033 89-79-2	Liquid C ₁₀ H ₁₈ O 154.25	Slightly soluble Miscible	218 IR 95 %	1.468-1.477 0.904-0.913	Register name to be changed to 1R,2S,5R-isopulegone. According to the JECFA: Min. assay value is "95 % (total of isomers; <1 % citronellal)". Composition of mixture not specified.
07.067 754	Isopulegone		2964 2051 29606-79-9	Liquid C ₁₀ H ₁₆ O 152.24	Insoluble Miscible	208 MS 95 %	1.465-1.473 0.925-0.932	Register name to be changed to 2R,5S-isopulegone.
09.219 756	Isopulegyl acetate		2965 2067 57576-09-7	Liquid C ₁₂ H ₂₀ O ₂ 196.29	Insoluble Miscible	232 IR 95 %	1.454-1.457 0.929-0.936	Register name to be changed to 1R,2S,5R-isopulegyl acetate. According to the JECFA: Min. assay value is "95 % (total of isomers)". Composition of mixture not specified.

1) Solubility in water, if not otherwise stated.

2) Solubility in 95 % ethanol, if not otherwise stated.

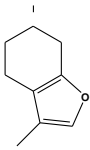
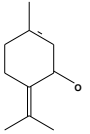
3) At 1013.25 hPa, if not otherwise stated.

4) At 20°C, if not otherwise stated.

5) At 25°C, if not otherwise stated.

TABLE 2: GENOTOXICITY DATA

Table 2.1: Genotoxicity Data (*in vitro* / *in vivo*) for Pulegone and Menthofuran (JECFA, 2001b)

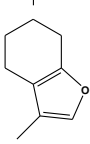
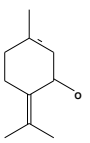
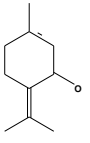
Table 2.1: Summary of Genotoxicity Data available for Three Flavoring Substances Structurally Related to Pulegone evaluated by the JECFA							
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
<i>In vitro</i>							
13.035 758	Menthofuran		Reverse mutation	<i>S. typhimurium</i> TA98, TA100	1000 mg/plate ^a	Negative	(Nelson & Dybing, 1998)
Not in Register 753	Pulegone		Reverse mutation	<i>S. typhimurium</i> TA97, TA98, TA100, TA1535, TA1537	≤ 800 mg/plate ^a	Negative	(Andersen & Jensen, 1984b)
			Reverse mutation	<i>S. typhimurium</i> TA98, TA1537	1000 mg/plate ^a	Negative	(Nelson & Dybing, 1998)
			Wing spot mutation	<i>D. melanogaster</i>	0.2 µL pulegone	^b Weakly positive	(Franzios et al., 1997)
			Wing spot mutation	<i>D. melanogaster</i>	^c 2.1 µL (9.8 µmol pulegone)	Negative	(Franzios et al., 1997)

^a With and without metabolic activation.

^b EFSA comment: Small single spots: Positive Total spots: Weakly positive.

^c EFSA comment: 2.1 µL essential oil of *Mentha pulegium* containing 75.7 % pulegone (9.8 µmol pulegone) & 10.1 % menthone.

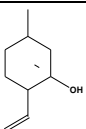
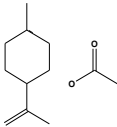
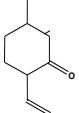
Table 2.2: Additional Genotoxicity Data for Pulegone and Menthofuran (EFSA, 2005i)

Table 2.2: Additional Genotoxicity data for Pulegone evaluated by EFSA							
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
<i>In vitro</i>							
13.035 758	Menthofuran		Reverse mutation	<i>S. typhimurium</i> TA97, TA98, TA100, TA1535	Up to 667 microg/plate	Negative ^a	(NTP, 1999e)
Not in Register 753	Pulegone		Reverse mutation	<i>S. typhimurium</i> TA97, TA1535	Up to 2167 microg/plate	Negative ^a	(NTP, 2000e)
			Reverse mutation	<i>S. typhimurium</i> TA98, TA100	Up to 3333 microg/plate	Negative ^a	(NTP, 2000e)
			Reverse mutation	<i>S. typhimurium</i> TA98, TA100	Up to 3500 microg/plate	Negative ^a	(NTP, 2000e)
			Reverse mutation	<i>E.coli pKM101</i>	Up to 3500 microg/plate	Negative ^a	(NTP, 2000e)
<i>In vivo</i>							
Not in Register 753	Pulegone		Micronucleus	B6C3F1 Mice	0 – 150 mg/kg	Negative	(NTP, 2000e)

^a With and without metabolic activation

TABLE 3: SUMMARY OF SAFETY EVALUATION TABLE

Table 3: Summary of Safety Evaluation of Three Substances Structurally Related to Pulegone (JECFA, 2001a)

Table 3: Summary of Safety Evaluation of Three JECFA-evaluated Substances Structurally Related to Pulegone (JECFA, 2001a)							
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI ($\mu\text{g}/\text{capita}/\text{day}$)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
02.067 755	Isopulegol		6.1 7	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No NOAEL on isopulegol or on a structurally related substance is available - Additional data required.	Composition of mixture to be specified. No NOAEL on isopulegol or on a structurally related substance is available - additional data required.
09.219 756	Isopulegyl acetate		0.97 1	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No NOAEL on isopulegyl acetate or on a structurally related substance is available - Additional data required.	Composition of mixture to be specified. No NOAEL on isopulegyl acetate or on a structurally related substance is available - additional data required.
07.067 754	Isopulegone		0.012 0.01	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No NOAEL on isopulegone or on a structurally related substance is available - Additional data required.	No NOAEL on isopulegone or on a structurally related substance is available - additional data required.

1) EU MSDI: Amount added to food as flavour in (kg/year) $\times 10E9 / (0.1 \times \text{population in Europe} (= 375 \times 10E6) \times 0.6 \times 365) = \mu\text{g}/\text{capita}/\text{day}$.

2) Thresholds of concern: Class I = 1800, Class II = 540, Class III = 90 $\mu\text{g}/\text{person}/\text{day}$.

3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

4) No safety concern based on intake calculated by the MSDI approach of the named compound.

5) Data must be available on the substance or closely related substances to perform a safety evaluation.

ND: not determined

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ABBREVIATIONS

CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CoE	Council of Europe
EC	European Commission
EFSA	The European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
ID	Identity
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LD ₅₀	Lethal Dose, 50%; Median lethal dose
MS	Mass spectrometry
MSDI	Maximum Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
No	Number
NOAEL	No observed adverse effect level
NOEL	No observed effect level
NTP	National Toxicology Program
SCF	Scientific Committee on Food
TAMDI	Theoretical Added Maximum Daily Intake
WHO	World Health Organisation