(b) (4)

Menthofuran

Toxicity monograph (with existing HCVs)

May 2018

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INTRODUCTION

(b) (4) was asked to produce a toxicity monograph of menthofuran (CAS RN[1] 494-90-6), focussing on the inhalation route of exposure, with inclusion of existing Health Criteria Values (HCVs) where available. Searches have also been performed on CAS RN 17957-94-7, the R-(+)-isomer of menthofuran, and CAS RN 80183-38-6, the S-(-)-isomer. Data on the inhalation of tobacco smoke containing the substance (if available) have not been included in this monograph.

EXPERTISE

(b) was founded[2] in 1961 to provide independent, high-quality research, information and advice on chemical toxicology to industry and governmental departments. Its risk assessors have been working together for many years (more than 40 years in some instances) and have a record of objectivity and scientific excellence. All senior and principal scientists in the current team are accredited and listed in the European (Eurotox) and UK Royal Society of Biology/British Toxicology Society Registers of Toxicologists and are thus bound by their specific codes of conduct.

TOXICITY DATA SEARCH CRITERIA[3]

Searches of the (b) (4) database (see Appendix for details) identified the EFSA (2005 and 2009) reviews, upon which this monograph is based. JECFA (2001) was consulted for additional information on the studies cited in EFSA (2005, 2009), as was SCF (2002). A subsequent search of the primary literature was restricted to (b) (4) and Toxline (the toxicity subset of Medline, via TOXNET) in an attempt to identify more recent data since the 2005 review. The remainder of the TOXNET system (which includes HSDB, GENETOX, DART, CCRIS, IRIS, ITER and CPDB) and eChemPortal was also consulted. Since the key reviews focussed on the use of menthofuran[4] in food and, as such, could not necessarily be relied upon to identify all critical local and systemic inhalation data, no date restriction was placed on searches in PubMed tailored to identify such information (and also cardiopulmonary data).

All searches were conducted in May 2018 using the CAS RNs and (in PubMed only) name and/or synonyms identified below, as appropriate.

The data summarised in this report refers to the unheated form unless otherwise stated.

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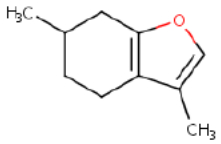
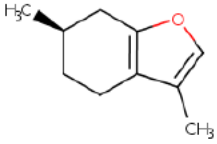
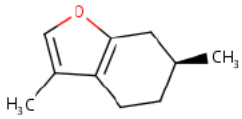
[1] Chemical Abstracts Service Registry Number.

[2] as the (b) (4)

[3] Disclaimer: searches are valid and complete as of the date of searching. (b) accepts no responsibility for the accuracy, completeness or sufficiency of any databases or searching platforms employed.

[4] EFSA/SCF ascribes CAS RN 494-90-6 to the (R)-(+)-isomer of menthofuran. This isomer is also identified by CAS RN 17957-94-7.

IDENTIFICATION, REACH STATUS AND EU CLASSIFICATION

Identifier			
Name	Menthofuran	Menthofuran, (+)-	Menthofuran, (-)-
Synonyms(s)	Menthofuranon (racemate, unspecified isomer) Benzofuran, 4,5,6,7-tetrahydro-3,6-dimethyl-	R-(+)-Menthofuran	S-(-)-Menthofuran
CAS RN	494-90-6	17957-94-7	80183-38-6
REACH registration number	Not registered	Not registered	Not registered
Molecular formula	$C_{10}H_{14}O$		
Molecular weight	150.22		
Structure			
Classification, according to EU CLP (EC 1272/2008)	**Harmonised classification:** None available	**Harmonised classification:** None available	**Harmonised classification:** None available
	REACH joint registrants: None available	**REACH joint registrants:** None available	**REACH joint registrants:** None available

ADME[5]

No relevant data were identified on the ADME of inhaled menthofuran[6] (or its isomers)

No data on the oral absorption or distribution of menthofuran were identified. Metabolic data on menthofuran are extensive, partly because it is a major metabolite of the related substance pulegone (e.g. EMA, 2014).

Menthofuran can undergo metabolism via a number of detoxification and bioactivation pathways. For example, it can undergo hydroxylation at the 7-*alpha* position, followed by glucuronidation (detoxification) and urinary excretion. Alternatively, a menthofuran double bond can be oxidised to form an unstable furanoepoxide intermediate, which breaks down to either 2-hydroxymenthofuran or a bioactive, electrophilic γ-ketoenal (8-pulegone aldehyde) (Peterson, 2013). This γ-ketoenal may also be formed directly from oxycarbonium ions

[5] Absorption, Distribution, Metabolism and Excretion.
[6] ADME predictions could be estimated on the basis of the structure and physic-chemical properties, if required.

formed in the oxidation of menthofuran (Nelson *et al.,* 1992; Thomassen *et al.*, 1992). Mintlactone and isomintlactone are formed as stable products of the γ-ketoenal, and by direct proton loss from oxycarbonium ions (Chen *et al.,* 2011). The γ-ketoenal can also be converted (detoxified) to 5-methyl-2-cyclohexenone or 4-methyl-2-cyclohexenone, the latter ultimately being converted to *para*-cresol and finally to benzoic acid (Madyastha and Raj, 1990, 1991, 1992, 1993).

Oxidative metabolites of menthofuran identified in rat and human liver microsomes and in rat liver slices exposed to cytotoxic concentrations of menthofuran include "monohydroxylation products of the furanyl and cyclohexyl groups, mintlactones and hydroxymintlactones, a reactive γ-ketoenal, and a glutathione conjugate" (Khojasteh *et al.,* 2010). An *in vivo* study, where rats received a single oral dose of 6 or 60 mg menthofuran/kg bw by gavage, identified three urinary sulphonic acid metabolites. Other urinary metabolites were attributed to further metabolism of mintlactones followed by hydroxylation and conjugation to form glucuronides (Chen *et al.*, 2003).

The reactive γ-ketoenal and furanoepoxide metabolites covalently bind to proteins and other cellular macromolecules, or are trapped by small-molecular scavengers such as glutathione. These metabolites are likely to be mainly responsible for menthofuran's liver toxicity (EMA, 2014; Gordon and Khojasteh, 2015; Khojasteh *et al*., 2010; Khojasteh *et al.*, 2012; Lassila *et al.,* 2016; Thomassen *et al.*, 1992). Menthofuran metabolites have been demonstrated to form adducts with the following rat liver proteins: serum albumin, mitochondrial acetaldehyde dehydrogenase, cytoplasmic malate dehydrogenase and subunit d of mitochondrial ATP synthase (Khojasteh *et al.*, 2012).

Studies with human CYP[7] enzymes and human liver microsomes indicate that menthofuran is metabolised by human liver CYP2E1, CYP1A2, CYP2C19 and CYP2A6 (Khojasteh *et al*., 1999). Menthofuran inhibits human CYP2A6 irreversibly, possibly by covalent adduction (Khojasteh *et al.,* 1998) and this may influence nicotine metabolism since nicotine is also primarily metabolised in humans by CYP2A6 (Kramlinger *et al.,* 2012).

TOXICOLOGY

LOCAL EFFECTS

Respiratory tract irritation

No relevant data were identified on menthofuran (or its isomers).

Skin irritation

No relevant data were identified on menthofuran (or its isomers).

Eye irritation

No relevant data were identified on menthofuran (or its isomers).

Other local effects

No relevant data were identified on menthofuran (or its isomers).

[7] Cytochrome P450 enzymes.

SENSITISATION AND INTOLERANCE

Respiratory tract sensitisation

No relevant data were identified on menthofuran (or its isomers).

Skin sensitisation

No relevant data were identified on menthofuran (or its isomers).

Oral allergy/intolerance

No relevant data were identified on menthofuran (or its isomers).

INHALATION TOXICITY STUDIES – SYSTEMIC EFFECTS

Single dose toxicity

No relevant data were identified on menthofuran (or its isomers).

Repeated dose toxicity

No relevant data were identified on menthofuran (or its isomers).

TOXICITY STUDIES – OTHER EXPOSURE ROUTES

Single dose toxicity

Expert-group opinion

No relevant data were identified on menthofuran (or its isomers).

Human

No relevant data were identified on menthofuran (or its isomers).

Non-human

No standard good-quality acute toxicity studies were identified for menthofuran (or its isomers).

Male albino rats [numbers unspecified] were administered 0, 100, 200 or 400 mg/kg bw R-(+)-menthofuran (CAS RN 17957-94-7) (as an aqueous suspension in 1% methylcellulose) by oral gavage. After 24 hours, the animals were killed and the levels of liver cytochrome P-450 and serum glutamate pyruvate transaminase (SGPT) were analysed. Statistically significant decreases in cytochrome P-450 levels (40% in the highest dose group) and statistically significant increases in SGPT levels (7.9-fold in the highest dose group) were seen in all treatment groups. The increase in SGPT activity was reported to have "a direct correlation with the extent of liver damage and toxicity" (Madyastha and Raj, 1994).

In male Swiss-Webster mice administered 100, 200 or 300 mg/kg bw R-(+)-menthofuran (CAS RN 17957-94-7) in corn oil by intraperitoneal injection, the 24-hour mortality was 2/15, 5/15 and 10/16 animals, respectively. Liver necrosis was observed in 10/13, 9/10 and 3/6 surviving animals, this effect having a dose-related increase in severity (Gordon *et al.*, 1982). In another [limited] study, hepatoxicity described as "severe midzonal hepatic necrosis" was reported in male Swiss-Webster mice administered 200 mg/kg bw R-(+)-menthofuran (CAS RN 17957-94-7) by intraperitoneal injection (Thomassen *et al.*, 1992).

Repeated dose toxicity

Expert-group opinion

No relevant data were identified on menthofuran (or its isomers).

Human

No relevant data were identified on menthofuran (or its isomers).

Non-human

No standard good-quality substance-specific data were identified for menthofuran (or its isomers).

In a toxicity screening test, groups of rats [sex and strain not specified] were given (R)-(+)-menthofuran (CAS RN 17957-94-7) in the diet for 14 days at 23 mg/kg bw/day. According to the SCF, "No effects were seen on body weight gain, food consumption, liver or kidney weights, nor on gross and histopathology of the liver and kidney" (Van Miller and Weaver, 1987).

In another limited study, (R)-(+)-menthofuran (CAS RN 17957-94-7) at 0 or 250 mg/kg bw/day was administered to male albino rats by oral gavage for three days. "A marked decrease (~59%) in hepatic microsomal cytochrome P-450 and 18.5-fold increase in SGPT value as compared to that of control animals" was noted at 72 hours. The study investigators concluded that the significant increase in SGPT level "suggest that menthofuran causes hepatotoxicity in rats" (Madyastha and Raj, 1994).

GENOTOXICITY

Expert-group opinions

In its evaluation of pulegone and menthofuran in flavourings and other food ingredients with flavouring properties, the EFSA CEF Panel[8] concluded that the newly available genotoxicity studies supported the existing [limited] data evaluated in the earlier SCF opinion of 2002. However, these studies did not meet the data requirements set out by the SCF (EFSA, 2005). Additional data on the genotoxic potential of menthofuran was not available for the most recent EFSA evaluation of two structurally-related pulegone metabolites and one ester, where data on pulegone and menthofuran were candidate substances (EFSA, 2009).

No conclusion was made by JECFA with respect to genotoxicity (JECFA, 2001).

Mammals (*in vivo*)

No relevant data were identified on menthofuran (or its isomers).

Mammalian cells (*in vitro*)

No relevant data were identified on menthofuran (or its isomers).

Micro-organisms

Menthofuran lacked mutagenic activity in a bacterial reverse mutation (Ames) assay using *Salmonella typhimurium* strains TA97, TA98, TA100 or TA1535 at concentrations of up to

[8] EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids.

75 µg/plate in the presence and absence of S9[9], and at concentrations up to 667 µg/plate in the presence, but not absence, of S9. Slight [cyto]toxicity and [cyto]toxicity was observed at the two highest doses tested, 333 and 667 µg/plate (NTP, 2002).

Two further [limited] assays also report a lack of mutagenic activity at menthofuran concentrations up to 10,000 µg/plate[10] in the presence and absence of S9. The first using strains TA100 and TA1535 (cited in EFSA, 2005) and the second using TA98 and TA100 (Nelson and Dybing, 1998).

Other

No relevant data were identified on menthofuran (or its isomers).

CARCINOGENICITY

No relevant data were identified on menthofuran (or its isomers).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

No relevant data were identified on menthofuran (or its isomers).

CARDIOPULMONARY EFFECTS[11]

No relevant data were identified on menthofuran (or its isomers).

OTHER TOXICITY CONSIDERATIONS

No relevant data were identified on menthofuran (or its isomers).

EXISTING HEALTH CRITERIA VALUES (HCVs)

No existing HCVs were identified for menthofuran (or its isomers).

In an evaluation of two structurally-related pulegone metabolites and one ester, EFSA noted that "only a limited database was available on (R)-(+)-pulegone and (R)-(+)-menthofuran (CAS RN 490-90-6[12]) and considered that these data were inadequate for the derivation of an ADI[13]" for either substance. However, both (R)-(+)-pulegone and (R)-(+)-menthofuran are in Annex III of Regulation (EC) No 1334/2008 of the European Parliament and of the Council (EC, 2008) and accordingly cannot be used as food flavouring substances in the EU (EFSA, 2009).

[9] Induced mammalian liver post-mitochondrial fraction used for metabolic activation.
[10] This concentration seems very high, considering cytoxicity was seen in the NTP studies at 333 and 667 µg/plate in the same Salmonella strains.
[11] Potential effects on the heart, blood vessels and/or respiratory tract.
[12] EFSA/SCF ascribes CAS RN 494-90-6 to the (R)-(+)-isomer of menthofuran. This isomer is also identified by CAS RN 17957-94-7.
[13] Acceptable Daily Intake

A safety factor of 200 was applied to a NOEL[14] of 20 mg/kg bw/day, reported in a 28-day dietary study in rats fed pulegone, resulting in a joint MDI[15] of 0.1 mg/kg bw for menthofuran and pulegone (Council of Europe, 2008).

JECFA concluded that menthofuran is of "no safety concern" at ('current') estimated levels of dietary intake as a food additive of 0.4 and 0.3 µg/person/day in the EU and US, respectively (JECFA, 2001).

REFERENCES

Chen LJ, Lebetkin EH and Burka LT (2003). Metabolism of (R)-(+)-menthofuran in Fischer-344 rats: identification 532 of sulfonic acid metabolites. Drug Metabolism and Disposition 31, 1208-1213.

Chen XW, Serag ES, Sneed KB and Zhou SF (2011). Herbal bioactivation, molecular targets and the toxicity relevance. Chemico-Biological Interactions 192, 161-176. [Cited in EMA, 2014.]

Council of Europe (2008). Natural sources of flavourings. Report No. 3. Council of Europe Publishing. Strasbourg. ISBN 978-92-871-6422-3.

EC (2008). European Commission Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. Official Journal of the European Communities 31.12.2008, L 354/34-50. [Cited in EFSA, 2009.]

EFSA (2005). European Food Safety Authority. Scientific Opinion of the Panel on Food Contact Material, Enzymes, Flavourings & Processing Aids (CEF) on a request from the Commission on Pulegone and Menthofuran in flavourings and other food ingredients with flavouring properties. EFSA Journal 298, 1-32. http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/298.pdf

EFSA (2009). European Food Safety Authority. Scientific Opinion of the Panel on Food Contact Material, Enzymes, Flavourings & Processing Aids (CEF) on a request from the Commission on Flavouring Group Evaluation 57: Consideration of two structurally related pulegone metabolites and one ester thereof evaluated by JECFA (55th meeting). The EFSA Journal ON-1079, 1-17. https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2009.1079

EMA (2014). European Medicines Agency. Committee on Herbal Medicinal Products (HMPC). Draft public statement on the use of herbal medicinal products containing pulegone and menthofuran. 24 November 2014. EMA/HMPC/138386/2005 Rev. 1. http://www.ema.europa.eu/docs/en_GB/document_library/Public_statement/2014/12/WC500179556.pdf

[14] No-Observed-Effect Level.

[15] Maximum Daily Intake – this has also been described as a Tolerated Daily Intake (TDI) by the EMA (2014).

Gordon P and Khojasteh SC (2015). A decades-long investigation of acute metabolism-based hepatotoxicity by herbal constituents: a case study of pennyroyal oil. Drug Metabolism Reviews 47, 12-20. [Cited in Zárybnický *et al.,* 2018.]

Gordon WP, Forte AJ, McMurtry RJ, Gal J and Nelson SD (1982). Hepatoxicity and pulmonary toxicity of pennyroyal oil and its constituent terpenes in the mouse. Toxicology and Applied Pharmacology 65, 413-424. [Cited in SCF, 2002.]

JECFA (2001). Safety evaluation of certain food additives and contaminants. Prepared by the fifty-fifth meeting of the Joint FAO/WHO Expert Committee on Food Additives. WHO Food Additives Series 46. http://www.inchem.org/documents/jecfa/jecmono/v46je01.htm

Khojasteh SC, Koenigs LL, Peter RM, Trager WF and Nelson SD (1998). (R)-(+)-Menthofuran is a potent mechanism-based inactivator of human liver cytochrome P450 2A6. Drug Metabolism and Disposition 26, 701-704.

Khojasteh SC, Chen W, Koenigs LL, Peter RM and Nelson SD (1999). Metabolism of (R)-(+)-pulegone and 566 (R)-(+)-menthofuran by human liver cytochrome P-450s: evidence for formation of a furan epoxide. Drug Metabolism and Disposition 27, 574-580.

Khojasteh SC, Oishi S and Nelson SD (2010). Metabolism and toxicity of menthofuran in rat liver slices and in 574 rats. Chemical Research in Toxicology 23, 1824-1832.

Khojasteh SC, Hartley DP, Ford KA, Uppal H, Oishi S and Nelson SD (2012). Characterization of rat liver proteins 572 adducted to reactive metabolites of menthofuran. Chemical Research in Toxicology 25, 2301-2309. [Cited in EMA, 2014.]

Kramlinger VM, von Weymarn LB and Murphy SE (2012). Inhibition and inactivation of cytochrome P450 2A6 and cytochrome P450 2A13 by menthofuran, β-nicotyrine and menthol. Chemico-Biological Interactions 197, 87-92. [Cited from abstract.]

Lassila T, Mattila S, Turpeinen M, Pelkonen O and Tolonen A (2016). Tandem mass spectrometric analysis of S- and N-linked glutathione conjugates of pulegone and menthofuran and identification of P450 enzymes mediating their formation. Rapid Communications in Mass Spectrometry 30, 917-926. [Cited in Zárybnický *et al.,* 2018.]

Madyastha KM and Raj CP (1990). Biotransformation of R-(+)-pulegone and menthofuran *in vitro*: Chemical basis for toxicity. Biochemical and Biophysical Research Communications 173, 1086-1092. [Cited in JECFA, 2001.]

Madyastha KM and Raj CP (1991). Evidence for the formation of a known toxin, *p*-cresol, from menthofuran. Biochemical and Biophysical Research Communications 177, 440-446. [Cited from abstract.]

Madyastha KM and Raj CP (1992). Metabolic fate of menthofuran in rats: Novel oxidative pathways. Drug Metabolism and Disposition 20, 295-301. [Cited in JECFA, 2001.]

Madyastha KM and Raj CP (1993). Studies on the metabolism of a monoterpene ketone, R-(+)-pulegone--a 582 hepatotoxin in rat: isolation and characterization of new metabolites. Xenobiotica 23, 509-518.

Madyastha KM and Raj CP (1994). Effects of menthofuran, a monoterpene furan on rat liver microsomal enzyme, *in vivo*. Toxicology 89, 119-125.

Nelson S and Dybing E (1998). Letter concerning pulegone and menthofuran. Unpublished data submitted by EFFA to SCF. [Cited in EFSA, 2009.]

Nelson SD, McClanahan RH, Thomassen D, Gordon WP and Knebel N (1992). Investigations of mechanisms of reactive metabolite formation from (R)-(+)-pulegone. Xenobiotica 22, 1157-1164. [Cited in EMA, 2014.]

NTP (2002). National Toxicology Program. Cellular and Genetic Toxicology Branch, Salmonella Testing Results No. A36119. Private communication to FEMA. Unpublished report. https://tools.niehs.nih.gov/cebs3/ntpViews/?activeTab=detail&studyNumber=002-02396-0001-0000-3

Peterson LA (2013). Reactive metabolites in the biotransformation of molecules containing a furan ring. Chemical Research in Toxicology 26, 6-25.

SCF (2002). EU Scientific Committee on Food. Opinion on pulegone and menthofuran. SCF/CS/FLAV/FLAVOUR/3 ADD2 Final. 25 July 2002. [Appended to EFSA, 2005].

Thomassen D, Knebel N, Slattery JT, McClanahan RH and Nelson SD (1992). Reactive intermediates in the oxidation of menthofuran by cytochromes P-450. Chemical Research in Toxicology 5, 123-130.

Van Miller JP and Weaver EV (1987). Fourteen-day dietary minimum toxicity screen (MTS) in albino rats. Unpublished report to FEMA. Bushy Run Research Center, Export, PA. [Cited in SCF, 2002.]

Zárybnický T, Boušová I, Ambrož M and Skálová L (2018). Hepatotoxicity of monoterpenes and sesquiterpenes. Archives of Toxicology 92, 1-13.

APPENDIX: The (b) (4) database and databank

(b) (4)

(b) (4) includes information from peer-reviewed toxicology and nutrition journals as well as secondary sources and websites. In addition to primary literature on the health effects of chemicals, (b) (4) covers official publications and evaluations issued by authoritative groups including:

- WHO/IPCS reports and evaluations (including CICADs and EHCs, and IARC, JECFA and JMPR monographs), and the WHO Air Quality and Drinking-Water Quality Guidelines
- OECD SIDS dossiers/SIARS
- IUCLID data sets
- EU Risk Assessment Reports
- EU expert committee opinions (including EU scientific committees, and EFSA scientific panels) and other reports from EU agencies and institutes etc (including ECHA, ECVAM, EMA and CPS&Q)
- ECETOC, HERA, Council of Europe and other pan-European programmes
- UK government agency (including Defra, EA, FSA, DoH, HSE, HPA, PSD and VMD) and advisory committee (e.g. COT, COM, COC, ACNFP, SACN, ACP, ACAF, VPC, VRC and ACRE) reports and evaluations
- Opinions from other UK organisations such as the Royal Society
- US agency reports and evaluations (EPA, ATSDR, FDA, NTP, OSHA, NCEA, CFSAN, CERHR, NIEHS, CDC, OEHHA and ACGIH)
- Health Canada evaluations
- BUA, DFG, BG Chemie and BfR reports and monographs
- Gezondheidsraad opinions, including those from its various committees such as DECOS
- RIVM reports
- Danish EPA reviews
- Reports and other information provided by Swedish governmental organisations, including the National Food Administration and the Swedish Chemicals Agency
- Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals
- Australian agency reviews including NICNAS Priority Existing Chemical Assessments, APMVA reports and (jointly with New Zealand) FSANZ assessments
- Japanese Chemical Industry Ecology-Toxicology & Information Center reports
- CIR, RIFM and other specialist industry groups
- (b)) Toxicity Profiles