

# PULEGONE

## 1. Exposure Data

Pulegone is a monoterpene ketone present in the leaves and flowering tops of several members of the mint family *Lamiaceae*. Two enantiomeric forms occur in nature, the *R*-(+)-enantiomer being the most abundant in the essential oils ([Hayes et al., 2007](#); [Barceloux, 2008](#)).

### 1.1 Identification of the agent

#### 1.1.1 Classification

##### (a) Nomenclature

Chem. Abstr. Serv. Reg. No: 89-82-7

IUPAC systematic name: (*R*)-5-Methyl-2-(1-methylethylidene) cyclohexanone

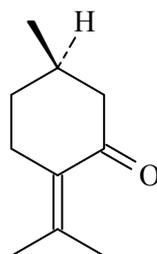
From [Scifinder \(2014\)](#).

##### (b) Description of origin

Pulegone is a major constituent of the volatile oils of European pennyroyal (*Mentha pulegium* L.) and American pennyroyal (*Hedeoma pulegioides* L.), where it comprises 85–97% (w/v) and about 30% (w/v) of the respective oil ([Guenther, 1949](#); [Smith & Levi, 1961](#); [Smith et al., 1963](#); [Von Hefendehl & Ziegler, 1975](#); [Farley & Howland, 1980](#)). The compound is also a minor component of several other edible mint (*Mentha*) species and their derived volatile oils, including peppermint (*Mentha piperita*) and spearmint (*Mentha spicata*) ([Virmani & Datta, 1968](#); [Turner &](#)

[Croteau, 2004](#)). It is found in different varieties of *M. piperita* oils at a range of 0.5% to 4.6%, and in *M. arvensis* oils at a range of 0.2% to 4.9%; oils in natural form contain lower concentrations of pulegone than those that have been partially dementholized ([Smith & Levi, 1961](#)). Pulegone is also found in various concentrations in Buchu leaf oils (*Barosma betulina* and *B. crenulata* with 3% and 50%, respectively) ([Kaiser et al., 1975](#)).

#### 1.1.2 Structural and molecular formulae and relative molecular mass



$C_{10}H_{16}O$

Relative molecular mass: 152.23

From [Farley & Howland \(1980\)](#), [Thomassen et al. \(1988\)](#), and [Da Rocha et al. \(2012\)](#).

#### 1.1.3 Chemical and physical properties of the pure substance

*Description:* Colourless oil with a strong pungent aromatic mint smell.

*Boiling point:* 224 °C

*Density:* 0.9346 g/mL at 25 °C

Optical activity ( $[\alpha]_{D}^{20}$ ): +22°

Solubility: Insoluble in water ([Farley & Howland, 1980](#); [O’Neil, 2013](#); [Sigma Aldrich, 2013](#)).

### 1.1.4 Analysis

Physical properties such as density and optical rotation are used to characterize essential oils. Gas chromatography with flame-ionization detection has been the standard method of analysis for essential oil composition. [Petraakis et al. \(2009\)](#) have developed a direct and rapid method to quantify pulegone using Fourier transform mid-infrared spectroscopy, which showed equivalent results to those obtained when using gas chromatography.

## 1.2 Production and use

### 1.2.1 Production

According to the United Nations Commodity Trade Statistics Database ([United Nations Comtrade, 2013](#)), 3.4–3.7 tonnes of essential oils of mint other than peppermint were imported by the China, Germany, Japan, Singapore, and USA and in recent years. No separate data were available for spearmint, peppermint, or pennyroyal oil from this source.

### 1.2.2 Use

Aerial parts [leaves and flowering tops] of plant species containing pulegone have been used as a traditional remedy, as flavouring, as spice, and for brewing teas. Pennyroyal oil has been used as a traditional medicine. It is also used to flavour alcoholic beverages, baked goods, candies, ice creams, as a fragrance component of detergents, cosmetics and oral hygiene products, and as an insect repellent ([Karousou et al., 2007](#); [Da Rocha et al., 2012](#)).

### (a) Medicinal indications

The aerial parts of both American and European pennyroyal plants have traditionally been used internally as a tea for non-ulcer dyspepsia, primary dysmenorrhoea, secondary amenorrhoea and oligomenorrhoea, as abortifacient, and as a diaphoretic. Pennyroyal oils have been used for these same medicinal indications ([Hoppe, 1975](#); [List & Hörhammer, 1976](#); [Foster & Duke, 1990](#); [Skenderi, 2003](#)). Today, recorded uses for *Mentha piperita* and *Mentha pulegium* L. are for common cold, headache, and as diuretic, spasmolytic, anti-convulsive, anti-emetic, heart stimulant, and sedative ([Karousou et al., 2007](#)). Peppermint oil is used for the treatment of the symptoms of inflammatory bowel syndrome ([Cappello et al., 2007](#)).

### (b) Dosage

No typical dose was found in the literature for pulegone. According to literature reports on levels of pulegone present in *M. piperita* oils, two capsules of 225 mg of oil taken twice per day could contain an amount of pulegone of between 4.5 and 41.4 mg (0.5–4.6%) ([Smith & Levi, 1961](#)). According to the European Union, the highest recommended daily dose is 1.2 mL of peppermint oil; 1080 mg of peppermint oil contains a maximum of 140 mg of pulegone, a daily intake of 2.3 mg/kg body weight (bw) for a person of 60 kg ([EMEA, 2005](#)).

## 1.3 Occurrence and exposure

### 1.3.1 Occurrence

Pulegone is naturally found in plants of the *Lamiaceae* (or *Labiatae*) family. The amount of pulegone in the various oils varies depending on several factors such as origin of the plant, yearly weather conditions, harvest date, plant age, fertilization, location and planting time ([Farley & Howland, 1980](#); [Weglarz & Zalecki, 1985](#); [Murray et al., 1988](#); [Voirin et al., 1990](#);

[Marotti et al., 1994](#); [Kumar et al., 2000](#); [Misra & Srivastava, 2000](#); [Dolzhenko et al., 2010](#)).

### 1.3.2 Consumer exposure

In addition to the use in medication, humans are exposed to pulegone as a constituent of the essential oil in flavourings, confectionery, and cosmetics ([Karousou et al., 2007](#); [Barceloux, 2008](#)). According to the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the estimated intake of pulegone per capita is 2 µg per day and 0.04 µg/kg bw per day for Europe, and 12 µg per day and 0.03 µg/kg bw per day for the USA ([IPCS, 2001](#)).

### 1.3.3 Occupational exposure

No data were available to the Working Group. Workers from the flavouring, confectionary, and cosmetic industries are probably exposed to pulegone.

## 1.4 Regulations and guidelines

Limits in the use of pulegone in food products have been issued for different applications. According to regulation (EC) 1334/2008, the use of pulegone in food and beverages has limits of: 100 mg/kg for mint/peppermint containing alcoholic beverages; 20 mg/kg for mint/peppermint containing non-alcoholic beverages; 2000 mg/kg for “micro breath freshening confectionery”; 350 mg/kg for chewing gum; and 250 mg/kg for mint/peppermint containing confectionery, except the “micro breath.” As a pure ingredient, pulegone may not be added to foodstuffs. According to the Committee of Experts on Flavoring Substances (CEFS), provisional consumption limits were established for pulegone at 20 mg/kg in food and beverages ([European Commission, 2002, 2008](#)).

In the USA, pulegone is not authorized as a synthetic flavouring substance ([DHHS-FDA,](#)

[2012](#)). According to the Cosmetic Ingredient Review Expert Panel, the concentration of pulegone in cosmetic formulations should not exceed 1% ([Nair, 2001](#)).

## 2. Cancer in Humans

No data were available to the Working Group.

## 3. Cancer in Experimental Animals

### 3.1 Mouse

See [Table 3.1](#)

In one study of oral administration, groups of 50 male and 50 female B6C3F<sub>1</sub> mice (age, 6–7 weeks) were given pulegone at a dose of 0 (corn oil only, 10 mL/kg bw), 37.5, 75, or 150 mg/kg body weight (bw) by gavage, 5 days per week for 105 weeks. The purity of pulegone was approximately 96%. Survival in all dosed groups was similar to that in the vehicle-control group. Mean body weights of males and females at 150 mg/kg bw were lower than those in the vehicle-control group after weeks 25 and 33, respectively.

In males, the incidence of hepatoblastoma was significantly higher in the group at the intermediate dose. The incidences of hepatocellular adenoma, and hepatocellular adenoma or carcinoma (combined) were also significantly higher in the group at the intermediate dose. The incidences of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) showed a significant positive trend. [The Working Group noted that the lower average body weight in the group at the highest dose may have reduced the incidences of liver tumours ([Haseman et al., 1997](#)).] The incidence of liver clear cell foci was significantly higher in all dosed groups; the incidence of eosinophilic liver

**Table 3.1 Studies of carcinogenicity with pulegone in mice and rats**

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance <sup>a</sup>	Comments
Mouse, B6C3F <sub>1</sub> (M, F) 105 wk <a href="#">NTP (2011)</a>	0 (control), 37.5, 75, 150 mg/kg bw, by gavage in corn oil, 5 days per wk, for 105 wk 50 M and 50 F/group (age, 5–6 wk)	Hepatoblastoma: 1/50, 3/50, 7/50*, 2/50 (M); 0/50, 1/50, 2/50, 2/50 (F) Hepatocellular adenoma: 22/50, 31/50, 35/50*, 28/50 (M); 13/49**, 15/50, 13/50, 27/50*** (F) Hepatocellular carcinoma: 13/50, 11/50, 18/50, 15/50 (M); 5/50, 1/50, 4/50, 8/50 (F) Hepatocellular adenoma or carcinoma (combined): 29/50, 36/50, 42/50*, 35/50 (M); 17/49**, 15/50, 15/50, 32/50*** (F) Hepatocellular adenoma or carcinoma or hepatoblastoma (combined): 29/50*, 37/50, 42/50**, 36/50 (M); 17/49***, 15/50, 15/50, 33/50**** (F) Osteoma or osteosarcoma (combined <sup>b</sup> ): 0/49, 0/50, 3/50*, 1/50 (F)	* <i>P</i> = 0.040  * <i>P</i> = 0.008 ** <i>P</i> < 0.001 (trend) *** <i>P</i> < 0.002  * <i>P</i> = 0.004 ** <i>P</i> = 0.001 (trend) *** <i>P</i> = 0.002  * <i>P</i> = 0.038 (trend) ** <i>P</i> = 0.004 *** <i>P</i> < 0.001 (trend) **** <i>P</i> < 0.001 # Greater than historical controls	Purity, 96% (approximate) Body weight of group at 150 mg/kg bw was 10% less than that of the vehicle-control group after week 25 for males and after week 33 for females
Rat, F344 (M, F) 104 wk <a href="#">NTP (2011)</a>	0 (control), 18.75 (M only), 37.5, 75 or 150 (F only) mg/kg bw, by gavage in corn oil, 5 days per wk, for 104 wk Dosing of males at the highest dose (75 mg/kg bw) and females at the highest dose (150 mg/kg bw) was stopped after wk 60 because of high morbidity and mortality. Surviving animals at these doses were treated with corn oil only from wk 60 to end of study. 50 M and 50 F/group (age, 6–7 wk)	Urinary bladder papilloma: 0/50, 0/49, 1/50 (2%), 3/47* (F) Urinary bladder carcinoma: 0/50, 0/49, 0/50, 2/47 (F) Urinary bladder papilloma or carcinoma (combined) <sup>c</sup> : 0/50, 0/49, 1/50, 5/47** (F)	* <i>P</i> = 0.044          ** <i>P</i> = 0.005	Purity, 96% (approximate) Mean body weight of males and females at 75 mg/kg bw was 10% less than that of the vehicle-control group after week 13 and 21 respectively; and mean body weight in females at 150 mg/kg bw was 12% less than that in the vehicle-control group after week 9. No treatment-related increases in tumour incidences were found in males.

<sup>a</sup> Poly-3 test was used for all the statistical analyses in this table

<sup>b</sup> Historical incidence for 2-year gavage studies with corn oil vehicle-control groups: 1/248 (0.4% ± 0.9%); range 0–2%; for all routes: 8/1498 (0.5% ± 1.0%), range 0–4%

<sup>c</sup> Historical incidence for 2-year gavage studies with corn oil vehicle-control groups: 0/200; for all routes, 0/1347

bw, body weight; F, female; M, male; wk, week

cell foci was significantly higher in the groups receiving the intermediate and highest doses; and the incidence of mixed liver cell foci was significantly higher in the group at the highest dose. The incidence of forestomach squamous cell hyperplasia was significantly higher in the group at the highest dose.

In females, the incidence of hepatocellular adenoma was significantly higher in the group at the highest dose. The incidence of hepatocellular adenoma or carcinoma (combined) was significantly higher in the group at the highest dose and had a significant positive trend. The incidence of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) was significantly higher in the group at the highest dose and had a significant positive trend. The incidence of liver clear cell foci was significantly higher in all dosed groups; the incidence of eosinophilic liver cell foci was significantly higher in the high-dose group; and the incidence of mixed liver cell foci was significantly higher in the groups receiving the intermediate and highest doses. The incidence of osteoma or osteosarcoma (combined) was increased in the group at the intermediate dose (3 out of 50) compared with historical controls (historical incidence for gavage studies was 1 out of 248 (0.4% ± 0.9%); range, 0–2%). The incidence of forestomach squamous cell hyperplasia was significantly higher in the groups receiving the intermediate and highest doses ([NTP, 2011](#)).

### 3.2 Rat

See [Table 3.1](#)

In one study of oral administration, groups of 50 male and 50 female F344/N rats (age, 6–7 weeks) were given pulegone at 0 (corn oil only, 5 mL/kg bw), 18.75 (males only), 37.5, 75, or 150 (females only) mg/kg bw by gavage, 5 days per week for up to 104 weeks. Due to excessive morbidity and mortality, males at 75 mg/kg bw and females at 150 mg/kg bw were not given pulegone after week 60 (stop-exposure); these groups

were given the corn-oil vehicle until the end of the study. Survival of males at 37.5 mg/kg bw was significantly lower than that of the vehicle controls; only two males in the group receiving 75 mg/kg bw and stop-exposure survived to the end of the study, and no females in the group receiving 150 mg/kg bw and stop-exposure. Compared with those of the rats in the vehicle-control group, mean body weights of males in the group receiving 75 mg/kg bw and stop-exposure, and of females in the group receiving 75 mg/kg bw, and of females receiving 150 mg/kg bw and stop-exposure were lower after weeks 13, 21 and 9, respectively.

In females, the incidence of urinary bladder papilloma was significantly higher in the group at the highest dose. The incidence of urinary bladder papilloma or carcinoma (combined) was significantly higher in the group at the highest dose (150 mg/kg bw with stop-exposure). [The Working Group noted that no urinary bladder papillomas or carcinoma were observed in 1347 control animals from previous studies (all routes of exposure) by the National Toxicology Program (NTP).] In males, no treatment-related increases in tumour incidences were found ([NTP, 2011](#)).

## 4. Mechanistic and Other Relevant Data

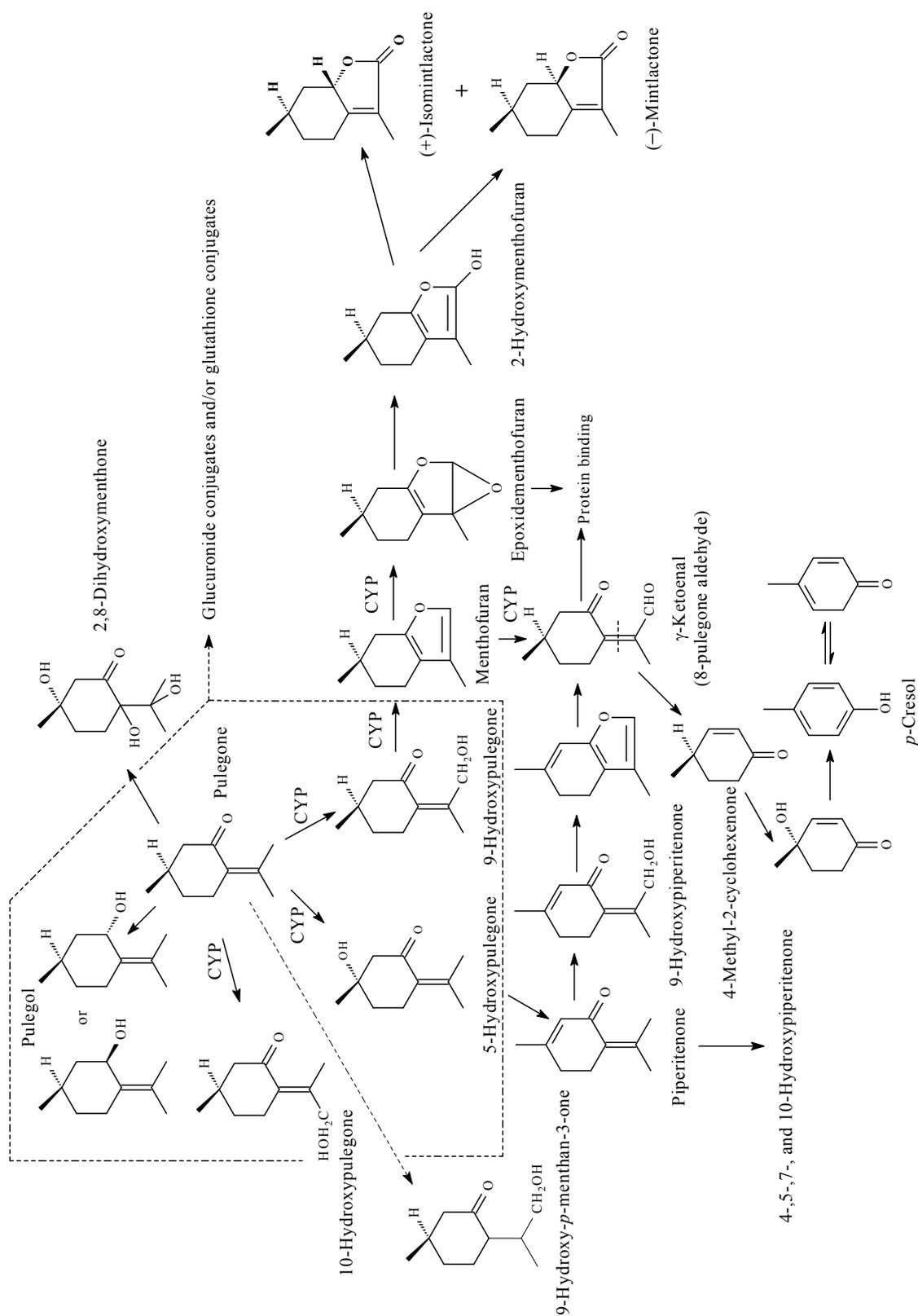
### 4.1 Absorption, distribution, metabolism, and excretion

[Fig. 4.1](#) describes the metabolism of pulegone in humans and rodents.

#### 4.1.1 Humans

A haematological post-mortem examination of a woman who ingested pennyroyal extract used as an abortifacient, indicated that both serum concentrations of pulegone at 18 ng/mL and menthofuran at 1 ng/mL possibly resulted

Fig. 4.1 Metabolism of pulegone in humans and rodents



Most of the metabolites of pulegone are derived from menthofuran and piperitone. A  $\gamma$ -ketoenal is generated as a major electrophilic metabolite from both pulegone and menthofuran. CYP, cytochrome P450. Adapted from [Chen \*et al.\* \(2011\)](#) with permission from Elsevier

in fatal poisoning. Serum samples were analysed for both metabolites at 26 hours post mortem, 72 hours after ingestion ([Anderson et al., 1996](#)). In another case, serum was found to contain menthofuran at 40 ng/mL, with no detectable pulegone, 10 hours after ingestion ([Anderson et al., 1996](#)). Menthofuran is considered the major proximate toxic metabolite; however, pulegone oxidation produces other metabolites that may also be toxic (see [Fig. 4.1](#); [Anderson et al., 1996](#)).

Pulegone is metabolized by multiple human liver CYPs to menthofuran, a toxic metabolite of pulegone ([Khojasteh-Bakht et al., 1999](#)). In a study by [Khojasteh-Bakht et al. \(1999\)](#), pulegone (200  $\mu$ M) was separately incubated with individual human CYPs, namely CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4. It was found that CYP2E1, CYP1A2, and CYP2C19 metabolized the oxidation of pulegone to menthofuran with the following  $K_m$  and  $V_{max}$ : CYP2E:  $K_m$ , 29  $\mu$ M;  $V_{max}$ , 8.4 nmol/min per nmol protein; CYP1A2:  $K_m$ , 94  $\mu$ M;  $V_{max}$ , 2.4 nmol/min per nmol protein; and for CYP2C19:  $K_m$ , 31  $\mu$ M; and  $V_{max}$ , 1.5 nmol/min per nmol protein.

Menthofuran was metabolized by the same human liver CYPs involved in the metabolism of pulegone except for the addition of CYP2A6 ([Khojasteh-Bakht et al., 1999](#)).

After oral administration of (*R*)-(+)-pulegone at 0.5 mg/kg bw or (*S*)-(-)-pulegone at 1 mg/kg bw to six volunteers, six metabolites were identified in the urine. The major metabolite of (*R*)-(+)-pulegone was 10-hydroxypulegone ([Engel, 2003](#)). Although 10-hydroxypulegone was shown to convert to menthofuran *in vitro*, menthofuran and its metabolites were found in relative small amounts in the urine. Alternatively, pulegone may be reduced to menthone, a product that is detected in small amounts in the urine. It might be possible that pulegone is also reduced at carbonyl group first; however, no trace of pulegol was found in the urine. Consequently, it was also proposed that pulegol can be reduced to menthol

or rearranged under conditions found in the human body, forming  $\alpha,\alpha,4$ -trimethyl-1-cyclohexene-1-methanol (3-*p*-menthen-8-ol) as a minor metabolite ([Engel, 2003](#)). It was also deduced that the reduction of the carbonyl group in menthone leads to the formation of menthol, while oxidation at C-5 also yields the menthone metabolite. The formation of  $\alpha,\alpha,4$ -trimethyl-1-cyclohexene-1-methanol from pulegol occurs at a body pH of 6.5. However, the formation of the same metabolite from pulegol as an artefact during enzymatic hydrolysis with sulfatase and glucuronidase cannot be totally excluded ([Engel, 2003](#)). The major metabolite, 9-hydroxy-*p*-menthan-3-one, is formed through the oxidation of 10-hydroxypulegone via the reduction of the exocyclic double bond ([Engel, 2003](#)). Although the results of this study suggested that menthofuran is not a relevant metabolite in humans, menthofuran was found to be present in the serum of two individuals, hours after ingestion of a large amount of pennyroyal oil in a study by [Anderson et al. \(1996\)](#). Additionally, it should be noted that toxicity seems to be stereospecific, in that for (*R*)-(+)-pulegone both reduction steps (to menthone and from 10-hydroxypulegone to 9-hydroxy-*p*-menthan-3-one) are much less favoured by the conformation of the terpene, as opposed to the (*S*)-(-)-pulegone enantiomer ([Engel, 2003](#)). Subsequently, 10-hydroxypulegone is accumulated and further oxidation at the hydroxyl group leads to pulegon-10 aldehyde, a toxic metabolite.

In summary, from a general perspective, minimal data existed on the excretion of pulegone in humans.

#### 4.1.2 Experimental systems

The majority of experimental studies used the toxic stereoisomer (*R*)-(+)-pulegone, which is the natural component of pennyroyal oil, but the (*S*)-(-)-isomer is also metabolized in the same manner ([Speijers, 2001](#)). A total of approximately

14 phase I metabolites exist in rats *in vivo*, with approximately 10 identified phase II metabolites (Thomassen *et al.*, 1991; Chen *et al.*, 2001; Zhou *et al.*, 2005). Observed metabolites account for only 3% of total radiolabel typically excreted in bile, with glucuronide conjugates and minimal glutathione conjugates being found in highest quantities (Speijers, 2001). The most common biliary metabolites observed were the glucuronide conjugates of hydroxylated pulegone and pulegol (Speijers, 2001).

The metabolism of pulegone involves three major metabolic pathways: (i) hydroxylation to give monohydroxylated pulegones at C-5 or methyl (9- or 10-), followed by conjugation with glucuronic acid or with glutathione; the conjugates being further metabolized; (ii) reduction of the carbon-carbon double bond that leads to the formation of menthofuran; and (iii) the formation of piperitenone after 5-hydroxylation, followed by dehydration (see Fig. 4.1) (Thomassen *et al.*, 1990; Speijers, 2001; Chen *et al.*, 2011). Most of the metabolites of pulegone are derived from menthofuran and piperitenone, and 4-methyl-2-cyclohexenone is one of these metabolites. A  $\gamma$ -ketoenal is generated as a major electrophilic metabolite from both pulegone and menthofuran (Thomassen *et al.*, 1992; Speijers, 2001). This reactive enonal may be derived directly from incipient oxycarbonium ions formed in the oxidation of menthofuran by cytochrome P450 (CYP), or from an epoxyfuran intermediate (Thomassen *et al.*, 1992). Mintlactones are formed as stable products of the  $\gamma$ -ketoenal, but also may be formed by direct proton loss from an incipient oxycarbonium ion (Chen *et al.*, 2011).

As shown experimentally, pulegone is specifically metabolized to menthofuran and *para*-mentha-1,4(8)-dien-3-one, commonly known as piperitenone (Speijers, 2001). In subsequent reactions, the tertiary ring carbon (C-5) is hydroxylated to obtain 5-hydroxypulegone (Speijers, 2001). This product is then dehydrated to piperitenone, which is further metabolized in

terms of ring- and side-chain oxidation to obtain numerous hydroxylated by-products (Speijers, 2001). For the predominant pathway, the isopropylidene substituent of pulegone is subjected to regiospecific allylic oxidation to yield 9-hydroxypulegone, which forms menthofuran cyclically (Gordon *et al.*, 1987; Madyastha & Raj, 1993). As a minor pathway, it is presumed that the exocyclic alkene of pulegone is oxidized (with the assumption of an epoxide intermediate) to yield 2,8-dihydroxymenthone, (Speijers, 2001). Additionally, pulegone is reduced to pulegol which is then rearranged to isopulegone with the aid of a supposed free-radical intermediate (Gordon *et al.*, 1987; Speijers, 2001).

When pulegone is converted to menthofuran, it undergoes a reaction where an oxycarbonium ion is created via CYP-mediated oxidation of menthofuran, generating an intermediate,  $\gamma$ -ketoenal (one of the primary reactive metabolites), but also another intermediate epoxyfuran (Chen *et al.*, 2011). Additionally, *p*-cresol is also generated via pulegone metabolism and also depletes glutathione with minor hepatotoxic effects (Chen *et al.*, 2011).

In addition to cyclizing to obtain menthofuran, 9-hydroxypulegone can also be oxidized in a secondary detoxication pathway to 9-carboxypulegone, also called 5-methyl-2-(1-methyl-1-carboxyethylidene) cyclohexanone (Speijers, 2001). This product is then partially cyclized to hydroxylactone or is assumed to be oxidized and hydrated to hydroxyacids that are eliminated through urine (Speijers, 2001). Studies of oral administration in rats have shown piperitenone is hydroxylated: metabolites found in the urine were isolated and found to be hydroxylated at the 4, 5, 7, 9, and 10 positions (Speijers, 2001). 9-Piperitenone can be further converted to an analogous furan metabolite and to the  $\gamma$ -ketoenal (Chen *et al.*, 2011).

According to studies in experimental animals treated orally, gastrointestinally absorbed pulegone is excreted in the urine within 24 hours.

Additional studies have demonstrated that pulegone is excreted and eliminated at 6–24% in faecal matter, and a small amount in expired air ([NTP, 2011](#)).

## 4.2 Genetic and related effects

### 4.2.1 Humans

No data were available to the Working Group.

### 4.2.2 Experimental systems

A limited number of studies of genotoxicity have been conducted with pulegone ([Table 4.1](#)).

#### (a) Mutation

##### (i) Bacteria

Pulegone was not mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, or TA1537, with or without metabolic activation ([Andersen & Jensen, 1984](#)).

Three additional assays for gene mutation in bacteria were conducted and results were mixed ([NTP, 2011](#)). In the first two studies, pulegone was not mutagenic with or without metabolic activation. Bacterial strains tested in the first study included *S. typhimurium* TA97, TA98, TA100, and TA1535, with and without metabolic activation (10% or 30% S9 from Syrian hamster or Sprague-Dawley rat liver). Strains tested in the second study included *S. typhimurium* strains TA98 and TA100, and *Escherichia coli* strain WP2 *uvrA*/pKM101, with and without metabolic activation (10% S9 from rat liver S9). The third study also tested pulegone in *S. typhimurium* and *E. coli*; results were positive in *S. typhimurium* strain TA98 and *E. coli* strain WP2 *uvrA*/pKM101 in the presence of metabolic activation. [There was no explanation for the discrepancy between the multiple assays for mutagenicity.]

##### (ii) *Drosophila melanogaster*

Pulegone was reported to be weakly mutagenic in the *Drosophila melanogaster* somatic mutation and recombination test. However, a sample of pennyroyal oil that was reported to contain pulegone at 75.7% was not mutagenic in this assay ([Franzios et al., 1997](#)).

#### (b) Cytogenetic effects

In B6C3F<sub>1</sub> mice given pulegone at doses up to 150 mg/kg bw per day by gavage for 3 months, there was no increase in the frequency of micronucleus formation in peripheral blood erythrocytes ([NTP, 2011](#)).

## 4.3 Other mechanistic data

### 4.3.1 Humans

In a review of published reports, excessive consumption of pennyroyal oil has been shown to induce moderate to severe toxicity ([Anderson et al., 1996](#); [Speijers, 2001](#); [NTP, 2011](#)). Consumption of amounts greater than 15 mL or approximately 250 mg/kg bw may result in death ([Anderson et al., 1996](#); [Speijers, 2001](#); [NTP, 2011](#)). Adverse physiological reactions following excessive consumption lead to massive centrilobular hepatic necrosis, pulmonary oedema, internal bleeding, and body weight loss ([Anderson et al., 1996](#); [Speijers, 2001](#)).

Eighteen cases of hepatotoxicity were described in people who had ingested 10 mL or more of pennyroyal oil ([Anderson et al., 1996](#); [NTP, 2011](#)). Symptoms of toxicity included coma, seizures, liver, and kidney effects, while consumption of less than 10 mL of pennyroyal oil resulted in gastritis and mild toxicity of the central nervous system ([Anderson et al., 1996](#); [NTP, 2011](#)). There was no clear correlation between dose and toxicological effect ([NTP, 2011](#)).

Functionally, reactive metabolites of pulegone and menthofuran bind to cellular proteins.

**Table 4.1 Genetic and related effects of pulegone**

Test system	Results <sup>a</sup>		Dose or concentration (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
<i>Salmonella typhimurium</i> TA100, TA1535, TA1537, TA98, TA97, reverse mutation	–	–	800 µg/plate	<a href="#">Andersen &amp; Jensen (1984)</a>
<i>Salmonella typhimurium</i> TA100, TA1535, TA98, TA97, reverse mutation	–	– <sup>b</sup>	2167 µg/plate	<a href="#">NTP (2011)</a>
<i>Salmonella typhimurium</i> TA100, TA98, <i>Escherichia coli</i> WP2, <i>uvrA</i> pKM101	–	– <sup>b</sup>	3500 µg/plate	<a href="#">NTP (2011)</a>
<i>Salmonella typhimurium</i> TA98, <i>Escherichia coli</i> WP2, <i>uvrA</i> pKM101	–	+ <sup>c</sup>	500 µg/plate	<a href="#">NTP (2011)</a>
<i>Salmonella typhimurium</i> TA100	–	– <sup>c</sup>	1500 µg/plate	<a href="#">NTP (2011)</a>
<i>Drosophila melanogaster</i> , somatic mutation (and recombination)	–	NT	2.1 µL, applied to filter paper <sup>d</sup>	<a href="#">Franzios et al. (1997)</a>
<i>Drosophila melanogaster</i> , somatic mutation (and recombination)	+	NT	0.2 µL, applied to filter paper <sup>d</sup>	<a href="#">Franzios et al. (1997)</a>
Micronucleus formation in peripheral blood lymphocytes, B6C3F <sub>1</sub> male and female mice in vivo	–	NT	150 mg/kg bw per day by gavage, for 3 months	<a href="#">NTP (2011)</a>

<sup>a</sup> +, positive; –, negative

<sup>b</sup> 10% or 30% S9 from Syrian hamster or Sprague-Dawley rat liver

<sup>c</sup> 10% S9 from Sprague-Dawley rat liver

<sup>d</sup> Larval feeding for 18 hours

HID, highest ineffective dose; LED, lowest effective dose; NT, not tested

Reactive metabolites of pulegone deplete hepatic glutathione concentrations, whereas menthofuran, only slightly diminishes these concentrations. On a molecular level, diminished concentrations of pulegone-induced glutathione result from the generation of electrophilically metabolites that form covalent adducts with glutathione ([Anderson et al., 1996](#)). Thus, *N*-acetylcysteine serves as a protectant against pennyroyal oil poisoning within the first few hours after ingestion and may also protect cells from further damage in late-stage injuries ([Anderson et al., 1996](#)).

[Bakerink et al. \(1996\)](#) have reported two cases of infant death following the ingestion of mint tea containing pulegone. The male patient (aged 6 months) was given mint tea rich in pulegone along with two crushed aspirin tablets. Presentation of this case indicated hepatic fulmination with cerebral oedema and necrosis, and the patient died with a serum concentration of menthofuran of 10 ng/mL. Most importantly, characteristic hepatotoxicity findings included hepatomegaly, poor perfusion, and dark blood from the nasogastric tube and rectum. The second case presented with hepatic dysfunction and severe epileptic encephalopathy and had serum concentrations of pulegone at 25 ng/mL, and menthofuran at 41 ng/mL. Overall, findings were similar for both cases, suggesting that pulegone intake is associated with marked hepatotoxicity ([Bakerink et al., 1996](#)).

#### 4.3.2 Experimental systems

Many studies in experimental animal have observed that *p*-cresol, a pulegone metabolite, induces diminished hepatic function, increased liver and kidney weight, gastrointestinal and nasal epithelial irritation, and atrophy of female reproductive organs ([Chen et al., 2011](#)).

Female Fischer rats (age, 6 weeks) were given pulegone orally at a dose of 0, 75, or 150 mg/kg bw per day, 5 days per week, for 4 or 6 weeks.

Urinary bladders from treated rats showed superficial cell layer necrosis and exfoliation in both treated groups, and a significant increase in the incidence of cellular proliferation in the group at the highest dose (150 mg/kg bw) ([Da Rocha et al., 2012](#)). Examination of urine collected during week 6 of treatment revealed the presence of pulegone, piperitone, piperitenone, and menthofuran. Piperitenone was concentrated in the urine at cytotoxic levels in rats treated with pulegone at the highest dose.

#### 4.4 Susceptibility

No relevant data were available to the Working Group.

#### 4.5 Mechanistic considerations

Studies in humans and rodents indicated that the metabolism of pulegone to menthofuran generates electrophilic species that can bind to proteins. This may result in chronic regenerative cell proliferation that may be related to the carcinogenicity in the liver and urinary bladder that is observed in experimental animals (see Section 3).

### 5. Summary of Data Reported

#### 5.1 Exposure data

Pulegone is present as a major constituent in pennyroyal oils, and to a minor extent in the oil of several other species of mint. Pulegone is also found in Buchu leaf oils. Pennyroyal oil has been used as flavouring in confectionery, as a spice, and in brewing teas. Pennyroyal leaves and oil are used in traditional medicine applications, for the treatment of dyspepsia and menstrual disorders, and as a diaphoretic. Pennyroyal oil has also been used as a fragrance in foods and

in cosmetics, and as a flea repellent. Given the wide range of uses of mint, there is a possibility of exposure to pulegone on a daily basis. Limits to the use of pulegone have been issued for foods and beverages. Synthetic pulegone is not authorized as a flavouring substance in the USA or Europe.

## 5.2 Human carcinogenicity data

No data were available to the Working Group.

## 5.3 Animal carcinogenicity data

Pulegone was tested for carcinogenicity after oral administration in one study in mice and one study in rats.

In male and female mice given pulegone by gavage, there was a significant increase in the incidences of hepatocellular adenoma, and hepatocellular adenoma and carcinoma (combined) in males and females, and of hepatoblastoma in males. In female mice, the incidence of osteoma or osteosarcoma (combined) was higher than that in historical controls.

In female rats given pulegone by gavage, there was an increase in the incidence of urinary bladder papilloma and of urinary bladder papilloma or carcinoma (combined). In males, there were no treatment-related increases in tumour incidences.

## 5.4 Mechanistic and other relevant data

Pulegone is readily absorbed in humans. It is metabolized in humans and rodents to isomers of hydroxypulegone, predominantly by hepatic oxidation at the 5-, 9-, and 10-positions. In rodents, 9-hydroxypulegone is further oxidized to menthofuran, which is converted to a reactive epoxide and a reactive aldehyde ( $\gamma$ -ketoenal). 5-Hydroxypulegone is converted to piperitenone,

which is then hydroxylated at the 9-position and further converted to an analogous furan metabolite and to the  $\gamma$ -ketoenal. Further metabolism of the  $\gamma$ -ketoenal produces 4-methyl-2-cyclohexenone and *p*-cresol.

Pulegone was not mutagenic in standard bacterial assays, either with or without exogenous metabolic activation.

Studies in humans and rodents indicated that some of the pulegone metabolites deplete hepatic levels of glutathione and can bind to cellular proteins. This may result in chronic regenerative cell proliferation, which may be related to the carcinogenicity observed in the liver and urinary bladder in experimental animals.

## 6. Evaluation

### 6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of pulegone.

### 6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of pulegone.

### 6.3 Overall evaluation

Pulegone is *possibly carcinogenic to humans* (Group 2B).

## References

- Andersen PH & Jensen NJ (1984). Mutagenic investigation of peppermint oil in the Salmonella/mammalian-microsome test. *Mutat Res*, 138:17–20. doi:[10.1016/0165-1218\(84\)90080-6](https://doi.org/10.1016/0165-1218(84)90080-6) PMID:[6387476](https://pubmed.ncbi.nlm.nih.gov/6387476/)
- Anderson IB, Mullen WH, Meeker JE *et al.* (1996). Pennyroyal toxicity: measurement of toxic metabolite levels in two cases and review of the literature. *Ann Intern Med*, 124:726–734. doi:[10.7326/0003-4819-124-8-199604150-00004](https://doi.org/10.7326/0003-4819-124-8-199604150-00004) PMID:[8633832](https://pubmed.ncbi.nlm.nih.gov/8633832/)

- Bakerink JA, Gospe SM Jr, Dimand RJ, Eldridge MW (1996). Multiple organ failure after ingestion of pennyroyal oil from herbal tea in two infants. *Pediatrics*, 98:944–947. PMID:[8909490](#)
- Barceloux DG (2008). Pennyroyal and Pulegone (*Mentha pulegium* L.). In: *Medical Toxicology of Natural Substances: Foods, Fungi, Medicinal Herbs, Plants and Venomous Animals*. Hoboken, NJ: John Wiley & Sons, Inc.; pp. 563–567.
- Cappello G, Spezzaferro M, Grossi L, Manzoli L, Marzio L (2007). Peppermint oil (Mintoil) in the treatment of irritable bowel syndrome: a prospective double blind placebo-controlled randomized trial. *Dig Liver Dis*, 39(6):530–6. doi:[10.1016/j.dld.2007.02.006](#) PMID:[17420159](#)
- Chen LJ, Lebetkin EH, Burka LT (2001). Metabolism of (R)-(+)-pulegone in F344 rats. *Drug Metab Dispos*, 29:1567–1577. PMID:[11717176](#)
- Chen XW, Serag ES, Sneed KB, Zhou SF (2011). Herbal bioactivation, molecular targets and the toxicity relevance. *Chem Biol Interact*, 192:161–176. doi:[10.1016/j.cbi.2011.03.016](#) PMID:[21459083](#)
- Da Rocha MS, Dodmane PR, Arnold LL, Pennington KL, Anwar MM, Adams BR *et al.* (2012). Mode of action of pulegone on the urinary bladder of F344 rats. *Toxicol Sci*, 128(1):1–8. doi:[10.1093/toxsci/kfs135](#) PMID:[22499580](#)
- DHHS-FDA (2012). Synthetic flavouring substances and adjuvants. 21CFR182.60. Department of Health and Human Services, Food and Drug Administration. Available from: <http://www.gpo.gov/fdsys/granule/CFR-2011-title21-vol3/CFR-2011-title21-vol3-sec182-60/content-detail.html>. Accessed 18 June 2014.
- Dolzhenko Y, Berteau CM, Occhipinti A, Bossi S, Maffei ME (2010). UV-B modulates the interplay between terpenoids and flavonoids in peppermint (*Mentha x piperita* L.). *J Photochem Photobiol B*, 100(2):67–75. doi:[10.1016/j.jphotobiol.2010.05.003](#) PMID:[20627615](#)
- EMA; European Medicines Agency (2005). Public Statement on the use of herbal medicinal products containing pulegone and menthofuran. EMA/HMPC/138386/2005. London, United Kingdom: Committee on Herbal Medicinal products (HMPC). Available from: [http://www.ema.europa.eu/docs/en\\_GB/document\\_library/Scientific\\_guideline/2010/04/WC500089958.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2010/04/WC500089958.pdf). Accessed 18 June 2014.
- Engel W (2003). *In vivo* studies on the metabolism of the monoterpene pulegone in humans using the metabolism of ingestion-correlated amounts (MICA) approach: explanation for the toxicity differences between (S)-(-)- and (R)-(+)-pulegone. *J Agric Food Chem*, 51:6589–6597. doi:[10.1021/jf034702u](#) PMID:[14558782](#)
- European Commission (2002). Opinion of the Scientific Committee on Food on pulegone and menthofuran. Brussels, Belgium: Scientific Committee on Food. Available from: [http://ec.europa.eu/food/fs/sc/scf/out133\\_en.pdf](http://ec.europa.eu/food/fs/sc/scf/out133_en.pdf). Accessed 18 June 2014.
- European Commission (2008). 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. *Off J Eur Union L*, 354:34
- Farley DR & Howland V (1980). The natural variation of the pulegone content in various oils of peppermint. *J Sci Food Agric*, 31(11):1143–51. doi:[10.1002/jsfa.2740311104](#)
- Foster S, Duke J (1990). A field guide to medicinal plants. Boston (MA): Houghton Mifflin Co.; p. 190.
- Franzios G, Mirotsoy M, Hatzia Apostolou E *et al.* (1997). Insecticidal and Genotoxic activities of mint essential oils. *J Agric Food Chem*, 45:2690–2694. doi:[10.1021/jf960685f](#)
- Gordon WP, Huitric AC, Seth CL *et al.* (1987). The metabolism of the abortifacient terpene, (R)-(+)-pulegone, to a proximate toxin, menthofuran. *Drug Metab Dispos*, 15:589–594. PMID:[2891472](#)
- Guenther E (1949). *The Essential Oils*. New York (NY): D Van Nostrand Company Inc.
- Haseman JK, Young E, Eustis SL, Hailey JR (1997). Body weight-tumor incidence correlations in long-term rodent carcinogenicity studies. *Toxicol Pathol*, 25:256–263. doi:[10.1177/019262339702500302](#) PMID:[9210256](#)
- Hayes JR, Stavanja MS, Lawrence BM (2007). Biological and toxicological properties of mint oils and their major isolates: Safety assessment. In: Lawrence BM, editor. *Mint: The Genus Mentha*. CRC Press, Taylor & Francis Group; pp. 462–477.
- Hoppe HA (1975). *Drogenkunde*, 8<sup>th</sup> Ed. Berlin: Walter de Gruyter; Vol 1, p. 764.
- IPCS (2001). WHO food additives series 46: Pulegone and related substances. Available from: <http://www.inchem.org/documents/jecfa/jecmono/v46je10.htm#46101200>. Accessed 19 June 2014.
- Kaiser R, Lamparsky D, Schudel P (1975). Analysis of buchu leaf oil. *J Agric Food Chem*, 23(5):943–50. doi:[10.1021/jf60201a021](#)
- Karousou R, Balta M, Hanlidou E, Kokkini S (2007). “Mints”, smells and traditional uses in Thessaloniki (Greece) and other Mediterranean countries. *J Ethnopharmacol*, 109(2):248–57. doi:[10.1016/j.jep.2006.07.022](#) PMID:[16962274](#)
- Khojasteh-Bakht SC, Chen W, Koenigs LL *et al.* (1999). Metabolism of (R)-(+)-pulegone and (R)-(+)-menthofuran by human liver cytochrome P-450s: evidence for formation of a furan epoxide. *Drug Metab Dispos*, 27:574–580. PMID:[10220485](#)
- Kumar S, Bahl JR, Bansal RP, Kukreja AK, Garg SN, Naqvi AA (2000). Profits of Indian menthol mint *Mentha*

- arvensis cultivars at different stages of crop growth in northern plains *J Med Arom Plant Sci*, 22:774–786.
- List PH, Hörhammer L, editors (1976). Hagers handbook of pharmaceutical practice. 4<sup>th</sup> Ed. Berlin: Springer-Verlag; Vol 5, p. 20.
- Madyastha KM & Raj CP (1993). Studies on the metabolism of a monoterpene ketone, R-(+)-pulegone—a hepatotoxin in rat: isolation and characterization of new metabolites. *Xenobiotica*, 23(5):509–18. doi:[10.3109/00498259309059391](https://doi.org/10.3109/00498259309059391) PMID:[8342298](https://pubmed.ncbi.nlm.nih.gov/8342298/)
- Marotti M, Piccaglia R, Giovanelli E, Deans SG, Eaglesham E (1994). Effects of planting time and mineral fertilization on peppermint (*Mentha piperita* L.) essential oil composition and its biological activity. *Flavour Fragrance J*, 9(3):125–9. doi:[10.1002/ffj.2730090307](https://doi.org/10.1002/ffj.2730090307)
- Misra A & Srivastava NK (2000). Influence of water stress on Japanese mint. *J Herbs Spices Med Plants*, 7(1):51–8. doi:[10.1300/J044v07n01\\_07](https://doi.org/10.1300/J044v07n01_07)
- Murray MJ, Marble P, Lincoln D, Hefendehl FW (1988). Peppermint oil quality differences and the reason for them. In: Lawrence BM, Mookherjee BD, Willis BJ, editors. Flavor and Fragrances: A World Perspective. Amsterdam, the Netherlands: Elsevier Press; pp. 189–210.
- Nair B (2001). Final report on the safety assessment of Mentha Piperita (Peppermint) Oil, Mentha Piperita (Peppermint) Leaf Extract, Mentha Piperita (Peppermint) Leaf, and Mentha Piperita (Peppermint) Leaf Water. *Int J Toxicol*, 20(4):Suppl 3: 61–73. doi:[10.1080/10915810152630747](https://doi.org/10.1080/10915810152630747) PMID:[11766133](https://pubmed.ncbi.nlm.nih.gov/11766133/)
- NTP; National Toxicology Program (2011). Toxicology and carcinogenesis studies of pulegone (CAS No. 89–82–7) in F344/N rats and B6C3F1 mice (gavage studies). *Natl Toxicol Program Tech Rep Ser*, 563:1–201. PMID:[21921962](https://pubmed.ncbi.nlm.nih.gov/21921962/)
- O’Neil MJ (2013). An Encyclopedia of Chemicals, Drugs, and Biologicals. 15<sup>th</sup> Ed. Whitehouse (NJ): Merck Sharp & Dohme Corp., RSC Publishing.
- Petrakis EA, Kimbaris AC, Pappas CS, Tarantilis PA, Polissiou MG (2009). Quantitative determination of pulegone in pennyroyal oil by FT-IR spectroscopy. *J Agric Food Chem*, 57(21):10044–8. doi:[10.1021/jf9026052](https://doi.org/10.1021/jf9026052) PMID:[19817373](https://pubmed.ncbi.nlm.nih.gov/19817373/)
- Scifinder (2014). CAS Database: Pulegone. American Chemical Society. Available from: <https://scifinder.cas.org>
- SigmaAldrich (2013). Pulegone. MSDS Analytical Standard. Available from: <http://www.sigmaaldrich.com/catalog/product/aldrich/w296309?lang=en&region=US>. Accessed 19 June 2014.
- Skenderi G (2003). Herbal Vade Mecum. Rutherford (NJ): Herbacy Press; p. 287.
- Smith DM & Levi L (1961). Essential oils, treatment of compositional data for the characterization of essential oils. Determination of geographical origins of peppermint oils by gas chromatographic analysis. *J Agric Food Chem*, 9(3):230–44. doi:[10.1021/jf60115a017](https://doi.org/10.1021/jf60115a017)
- Smith DM, Skakum W, Levi L (1963). Determination of botanical and geographical origin of spearmint oils by gas chromatographic and ultraviolet analysis. *J Agric Food Chem*, 11(3):268–76. doi:[10.1021/jf60127a010](https://doi.org/10.1021/jf60127a010)
- Speijers GJA (2001). WHO Food Additives Series 46: Pulegone and related substances. Bilthoven, the Netherlands: National Institute of Public Health and the Environment, Section on Public Health, Centre for Substances and Risk Assessment. Available from: <http://www.inchem.org/documents/jecfa/jecmono/v46je10.htm>. Accessed 22 July 2014.
- Thomassen D, Knebel N, Slattery JT *et al.* (1992). Reactive intermediates in the oxidation of menthofuran by cytochromes P-450. *Chem Res Toxicol*, 5:123–130. doi:[10.1021/tx00025a021](https://doi.org/10.1021/tx00025a021) PMID:[1581528](https://pubmed.ncbi.nlm.nih.gov/1581528/)
- Thomassen D, Pearson PG, Slattery JT, Nelson SD (1991). Partial characterization of biliary metabolites of pulegone by tandem mass spectrometry. Detection of glucuronide, glutathione, and glutathionyl glucuronide conjugates. *Drug Metab Dispos*, 19(5):997–1003. PMID:[1686249](https://pubmed.ncbi.nlm.nih.gov/1686249/)
- Thomassen D, Slattery JT, Nelson SD (1988). Contribution of menthofuran to the hepatotoxicity of pulegone: assessment based on matched area under the curve and on matched time course. *J Pharmacol Exp Ther*, 244(3):825–9. PMID:[3252034](https://pubmed.ncbi.nlm.nih.gov/3252034/)
- Thomassen D, Slattery JT, Nelson SD (1990). Menthofuran-dependent and independent aspects of pulegone hepatotoxicity: roles of glutathione. *J Pharmacol Exp Ther*, 253:567–572. PMID:[2338648](https://pubmed.ncbi.nlm.nih.gov/2338648/)
- Turner GW & Croteau R (2004). Organization of monoterpene biosynthesis in Mentha. Immunocytochemical localizations of geranyl diphosphate synthase, limonene-6-hydroxylase, isopiperitenol dehydrogenase, and pulegone reductase. *Plant Physiol*, 136(4):4215–27. doi:[10.1104/pp.104.050229](https://doi.org/10.1104/pp.104.050229) PMID:[15542490](https://pubmed.ncbi.nlm.nih.gov/15542490/)
- United Nations Comtrade (2013). United Nations Comtrade Database. Available from: <http://comtrade.un.org/>, accessed 17 December 2014.
- Virmani OP & Datta SC (1968). Oil of spearmint *Perfume Essential Oil Rec*, 59:351–362.
- Voirin B, Brun N, Bayet C (1990). Effects of day length on the monoterpene composition of leaves of Mentha × piperita. *Phytochemistry*, 29(3):749–55. doi:[10.1016/0031-9422\(90\)80012-6](https://doi.org/10.1016/0031-9422(90)80012-6)
- Von Hefendehl FW & Ziegler E (1975). Analysis of peppermint oil. *Deut Lebens-Ritnds*, 8:287–290.
- Weglarz Z & Zalecki R (1985). Investigations of dependence of the crop season of peppermint (*Mentha piperita* L.) herb upon the crop itself and the quality of the raw material. *Herba Pol*, 31:175–180.
- Zhou S, Chan E, Duan W *et al.* (2005). Drug bioactivation, covalent binding to target proteins and toxicity relevance. *Drug Metab Rev*, 37:41–213. PMID:[15747500](https://pubmed.ncbi.nlm.nih.gov/15747500/)