Integrated Laboratory Systems

Pulegone
[89-82-7]

and One Of Its Metabolites

Menthofuran
[494-90-6]

Review of Toxicological Literature

Prepared for

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EXECUTIVE SUMMARY

The nomination of pulegone and menthofuran for testing is based on the potential for human exposure and the absence of carcinogenicity data.

Pulegone and menthofuran are available from suppliers of laboratory test chemicals while pennyroyal oil with pulegone as a major constituent is commonly available in health food stores. Pulegone can be synthetically produced but no data were found concerning the synthetic production process; pulegone can also be produced by shoot cultures of *Mentha piperita* grown in fermenters. No data were found on production or import volumes.

Pulegone is a major constituent of several essential oils (e.g., peppermint, pennyroyal) used for flavoring foods and drinks. Pennyroyal oil, which has been reported to contain 75-85% pulegone, has also been used as a fragrance agent and as an herbal medicine to induce menstruation and abortion.

A number of plant species contain pulegone and menthofuran, including numerous species of mints such as peppermint and spearmint, the pennyroyals, and mountain mints. Menthofuran is present in peppermint and in *M. aquatica* and its hybrids.

Exposure to pulegone and menthofuran is primarily through ingestion of food products (e.g., frozen dairy dessert, candy, baked goods, gelatins, and puddings) and of alcoholic and nonalcoholic beverages flavored with spearmint oil, peppermint oil, or synthetic pulegone. Pulegone was not detected in meat products, processed fruit, confectioner frosting, jams, or jellies.

Pulegone was authorized in the U.S. as a synthetic flavoring substance under 21 CFR 172.515. Pennyroyal oil was voluntarily canceled for re-registration as a pesticide.

A number of case reports provide evidence that ingestion of pennyroyal oil is associated with acute poisoning. Moderate to severe toxicity to the nervous system, liver, and kidneys with a duration of hours to days was reported; death occurred in some cases. Ingestion of less than 10 mL was generally associated with gastritis and mild central nervous system toxicity, but the effects reported after ingestion of different amounts were variable and depended on emetics or other treatments given.

In experimental animals, pulegone was metabolically transformed to menthofuran and other metabolites with formation of glucuronide and glutathione conjugates. *In vitro* studies with rat and mouse liver microsomes showed that menthofuran is a major metabolite of pulegone with metabolism dependent on phenobarbital-induced cytochrome P-450 isozymes and influenced by exposure to substances that induce or inhibit these isozymes. Pulegone or metabolites were shown to covalently bind to tissue proteins. Ketone and organic acid metabolites were found in the urine of rats orally administered menthofuran, while studies of menthofuran metabolism using mouse liver microsomes showed the formation of a -ketoenal and mint lactone.

Acute lethal doses are available for pulegone. An LD_{50} of 1709 mg/kg (11.23 mmol/kg) was reported for mice given pulegone by the subcutaneous (s.c.) route, while rats exposed by the intraperitoneal (i.p.) route had an LD_{50} of 150 mg/kg (0.985 mmol/kg). An absolute lethal dose of 330 mg/kg (2.17 mmol/kg) was found in dogs intravenously (i.v.) administered pulegone. No lethal values for menthofuran were found.

Most toxicity data are from acute exposure of experimental animals to relatively high doses of pulegone or menthofuran. The primary toxic response was liver toxicity. Rats orally administered pulegone once or once daily for five or six days showed a reduction in hepatic cytochrome P-450 and an increase in serum glutamate pyruvate transaminase (SGPT). Mice treated with pulegone i.p. showed hepatic necrosis in addition

to an elevation of SGPT or alanine transferase (ALT) 24 hours after dosing. Similarly, menthofuran administered i.p. to mice also produced hepatic necrosis and increases in plasma levels of SGPT or ALT. Rats given pulegone i.p. developed a decrease in hepatic cytochrome P-450, an increase in ALT, and liver necrosis. Acute hepatic and lung damage in male mice (Swiss-Webster and BALB/c) was seen 24 hours after pennyroyal oil was administered acutely i.p. Pennyroyal oil topically applied to a dog resulted in seizures and death of the dog within 30 hours; pulegone was identified in a liver specimen.

Animal experiments also measured the effect of pretreatment with chemicals that influence cytochrome P-450 on the hepatotoxicity of pulegone or menthofuran. Pretreatment of mice or rats with inducers of cytochrome P-450 (e.g., phenobarbital, diethyl maleate) enhanced the hepatotoxicity of pulegone or menthofuran. In contrast, pretreatment of mice or rats with chemicals that inhibit cytochrome P-450 (e.g., cobaltous chloride, piperonyl butoxide) reduced hepatotoxicity.

Short-term toxicity studies suggested that pulegone may have neurological effects. Histopathological changes in the white matter of the cerebellum were observed in rats given oral doses of pulegone for 28 days.

Only very limited *in vitro* genotoxicity data on pulegone were located. Pulegone did not induce mutations in *Salmonella* strains TA1537, TA98, TA1535, and TA100, with and without metabolic activation. Pulegone, but not pennyroyal oil, was weakly mutagenic in *Drosphila melanogaster* larvae in the wing spot test.

Pulegone was shown to have antihistaminic activity on guinea pig ileum, based on its ability to inhibit histamine-induced contractions *in vitro.*

No data were found on chronic exposure, teratogenicity and embryotoxicity, and carcinogenicity.

Pulegone inhibited the growth of three bacterial species (*Escherichia coli*, *Staphylococcus aureus, Pseudomonas aeruginosa*) and three fungal species (*Candida albicans*,*Aspergillus niger*,*Mucor mucedo*) in an agar diffusion test system.

Pulegone demonstrated insecticidal activity based on lethality to *D. melanogaster*, as did mint oil extracted from the species *M. pulegium* (European pennyroyal)*,* found to contain 75.7% pulegone.

Treatment of human epidermis *in vitro* with pulegone increased the electrical conductivity, which suggests that pulegone can open new polar pathways across the stratum corneum.

Menthone and carvone, both structurally similar to pulegone, induced a conversion of rat cytochrome P-450 to cytochrome P-420 and no loss of heme in microsomes from rats pretreated with phenobarbital. When these two compounds were orally administered to rats for three days, there was no significant decrease in cytochrome P-450 levels in liver microsomes.

A study with 16 compounds structurally similar to pulegone indicated that a particular structural feature, -isopropylidene ketone with a methyl positioned *para* to the isopropylidene group, is necessary for the *in vitro* destruction of cytochrome P-450.

Isopulegone, another terpene identified in pennyroyal oil, was also found to be a hepato- and pulmonary toxicant to mice after administration by i.p. The enantiomer of pulegone, *S*-(-)-pulegone, produced no lung damage and was one-third less hepatotoxic than its congener.

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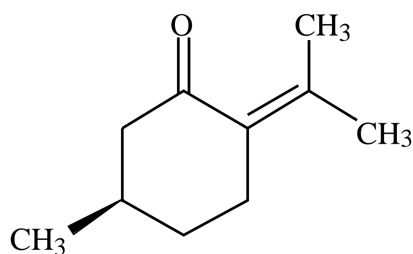
1.0 BASIS FOR NOMINATION TO THE ICCEC

The nomination of pulegone and menthofuran for testing is based on the potential for human exposure and the absence of carcinogenicity data.

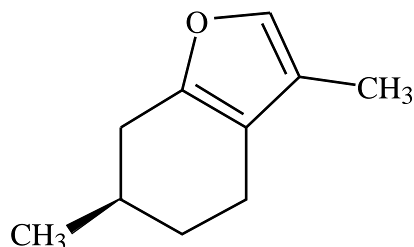
2.0 PROPERTIES

2.1 Chemical Identification

Pulegone
[89-82-7]



Menthofuran
[494-90-6]



Pulegone ($C_{10}H_{16}O$, mol. wt. = 152.23) is also called:

Cyclohexanone, 5-methyl-2-(1-methylethylidene)-, *R*- (9CI)
(+)-*R*-Pulegone
(+)-Pulegone
d-Pulegone
Pulegon
p-Menth-4(8)-en-3-one, *R*-(+)- (8CI)
(1*R*)-(+)-*p*-Menth-4(8)-en-3-one

Unless otherwise specified, pulegone will refer to the dextro enantiomer.

Menthofuran ($C_{10}H_{14}O$, mol. wt. = 150.21) is also called:

Benzofuran, 4,5,6,7-tetrahydro-3,6-dimethyl-
Benzofuran, 3,6-dimethyl-1,4,5,6-tetrahydro-
Benzofuran, 3,6-dimethyl-4,5,6,7-tetrahydro-
(*R*)-3,6-Dimethyl-4,5,6,7-tetrahydrobenzofuran
(*R*)-3,9-Epoxy-*p*-mentha-3,8-diene
p-Mentha-3,8-diene, 3,9-epoxy-
(+)-Menthofuran

2.2 Physical-Chemical Properties

2.2.1 Pulegone

Property	Information	Reference
Boiling Point	224 °C at 760 mm Hg	HODOC (1997)
Solubility	Soluble in: carbon tetrachloride Miscible in: ethanol, diethyl ether, chloroform Insoluble in: water	HODOC (1997)
Density	0.9346 g/cm^3 at 45 °C	HODOC (1997)
Optical Rotary Power	23.4° at 20 °C; 589.3 nm	HODOC (1997)
Physical State	oil	Budavari (1996)
UV Peak	253 nm in methanol	HODOC (1997)

2.2.2 Menthofuran

Property	Information	Reference
Melting Point	-17 °C	Beilstein (1997)
Boiling Point	196 °C at 760 mm Hg	Beilstein (1997)
Density	0.972 g/cm^3 at 15 °C	HODOC (1997)
UV Peak	219 nm in ethanol	HODOC (1997)

Oil of pennyroyal–European is a volatile oil from *Mentha pulegium* (Grundschober, 1979), containing about 85% (62-97%) pulegone (Budavari, 1996). Oil of pennyroyal–American is the volatile oil from *Hedeoma pulegioides* leaves and flowering tops, which contains about 30% (range not provided) pulegone. Pennyroyal oil is also derived from *M. longifolia.*

2.3 Commercial Availability

Pulegone and menthofuran are available from U.S. suppliers of flavor chemicals, i.e., from Aldrich Chemical Co. (Aldrich, 1996). Pennyroyal oil is commonly available in health food stores (Anderson et al., 1996).

3.0 PRODUCTION PROCESSES

Pulegone can be produced synthetically (Grundschober, 1979) but no information was found concerning the synthetic production process. Pulegone can be produced also by shoot cultures of *M. piperita* grown in fermenters (Hilton et al., 1995). Oils containing pulegone are derived from many plant species, including *Hedeoma pulegioides* (American Pennyroyal) (Budavari, 1996), species of the genus *Bystropogon* (evergreen shrubs) (Economou and Nahrstedt, 1991), and species of the genus *Mentha* (e.g., European Pennyroyal [*M. pulegium*], cornmint, Biblical mint) (ARS, 1997; Kokkini, 1991). Essential (i.e., volatile) plant oils preexist in the plant tissues or are produced by the reaction of certain constituents when tissues contact

water; most oils are obtained from plants by steam distillation (Martin and Cook, 1961). Menthofuran is found in essential oils of mint plants (Sang, 1982; Kokkini, 1991).

4.0 PRODUCTION AND IMPORT VOLUMES

No data were found.

5.0 USES

Several essential oils containing pulegone (e.g., peppermint oil, pennyroyal oil) are used for flavoring foods and drinks (Grundschober, 1979). Pennyroyal oil has also been used as a fragrance agent and as an herbal medicine proposed to induce menstruation and abortion (Nelson et al., 1992).

6.0 ENVIRONMENTAL OCCURRENCE

Many plants contain pulegone and menthofuran, including numerous species of mints, such as peppermint, spearmint, the pennyroyals, and mountain mints (Bicchi and Frattini, 1980; Sang, 1982; Pino et al., 1996; ARS, 1997). No information was found regarding pulegone or menthofuran in food processing wastes.

7.0 HUMAN EXPOSURE

Exposure to pulegone is primarily through ingestion of food products and of beverages flavored with oils or synthetic pulegone (Grundschober, 1979). Average levels of pulegone for various whole food product categories in the U.S. were reported as 9.07 ppm for nonalcoholic beverages, 10.5 ppm for alcoholic beverages, 28.0 ppm for frozen dairy dessert, 27.4 ppm for candy, 35.4 ppm for baked goods, and 27.3 ppm for gelatins and puddings (Grundschober, 1979). A survey of food flavorings in the United Kingdom (U.K.) reported pulegone contents of 18.2% in essential oil of buchu leaves, 6.2% in essential oil of mint blend, 73.3% in essential oil of pennyroyal, and 6.6% in essential oil of peppermint/cornmint (MAFF, 1994). Mint products and herbal teas available in the U.K. were reported to contain peppermint oil with pulegone content ranging from 0.2 to 2.9% (w/w) (2,000 to 29,000 ppm) (MAFF, 1996a). Other pulegone-containing products available in the U.K. include mint-flavored sugar, chocolate, and gum-based confectionery products (0-119 ppm pulegone), peppermint and peppermint-containing teas (0-27 ppm pulegone), and mint liqueur (5 ppm pulegone) (MAFF, 1996b). Pulegone was not detected in meat products, processed fruit, confectioner frosting, or mint jams or jellies.

Exposure to menthofuran is primarily through ingestion of beverages flavored with peppermint oil (Grundschober, 1979).

8.0 REGULATORY STATUS

Pulegone is authorized in the U.S. as a synthetic flavoring substance under 21 CFR 172.515 (Grundschober, 1979), and American and European pennyroyal oils are authorized as natural flavorings (21 CFR 172.510). Pennyroyal oil was voluntarily canceled for re-registration as a pesticide (U.S. EPA, 1997). An FDA regulatory letter was written to a manufacturer of a product proposed for use in the treatment of corns, calluses, and warts with a warning that the product containing pennyroyal oil and other essential oils was mislabeled since it would require a new drug application as a wart remover drug (21 CFR 358.101) (U.S. FDA, 1992).

Some European countries have regulated the use of pulegone (Grundschober, 1979). Levels of pulegone in natural (unspecified) flavorings are limited to 20 ppm in Spain, Switzerland (draft), the Netherlands (draft), and the U.K. In the U.K., a provisional limit of synthetic pulegone not to be exceeded in food is 125 ppm. In Germany, the use of pennyroyal oil is prohibited, but the use of pulegone is not limited.

9.0 TOXICOLOGICAL DATA

Summary: A number of case reports provide evidence that ingestion of pennyroyal oil is associated with acute poisoning. Moderate to severe toxicity to the nervous system, liver, and kidneys with a duration of hours to days was reported; death occurred in some cases. Ingestion of less than 10 mL was generally associated with gastritis and mild central nervous system toxicity, but the effects reported after ingestion of different amounts were variable and depended on emetics or other treatments given.

In experimental animals, pulegone was metabolically transformed to menthofuran and other metabolites with formation of glucuronide and glutathione conjugates. *In vitro* studies with rat and mouse liver microsomes showed that menthofuran is a major metabolite of pulegone with metabolism dependent on phenobarbital-induced cytochrome P-450 isozymes and influenced by exposure to substances that induce or inhibit these isozymes. Pulegone or metabolites were shown to bind covalently to tissue proteins. Ketone and organic acid metabolites were found in the urine of rats orally administered menthofuran, while studies of menthofuran metabolism using mouse liver microsomes showed the formation of a -ketoenal and mint lactone.

Acute lethal doses are available for pulegone but not menthofuran. An LD_{50} of 1709 mg/kg (11.23 mmol/kg) was reported for mice given pulegone by the subcutaneous (s.c.) route, while rats exposed by the intraperitoneal (i.p.) route had an LD_{50} of 150 mg/kg (0.985 mmol/kg). An LD_{Lo} of 330 mg/kg (2.17 mmol/kg) was found for dogs intravenously (i.v.) administered pulegone.

Most toxicity data are from acute exposure of experimental animals to relatively high doses of pulegone or menthofuran. The primary toxic response was liver toxicity. Rats orally administered 100-400 mg/kg (0.657-2.63 mmol/kg) pulegone once or once daily for five or six days showed a reduction in hepatic cytochrome P-450 and an increase in serum glutamate pyruvate transaminase (SGPT). Mice given 300-500 mg/kg (1.97-3.28 mmol/kg) pulegone i.p. showed hepatic necrosis in addition to an elevation of SGPT or alanine transferase (ALT) 24 hours after dosing. Acute hepatic and lung damage in male mice (Swiss-Webster and BALB/c) was seen 24 hours after pennyroyal

oil was given as a single i.p. dose of 400-800 mg/kg. Rats given 300 mg/kg (1.97 mmol/kg) pulegone i.p. also developed a decrease in hepatic cytochrome P-450, an increase in ALT, and liver necrosis. Pennyroyal oil (60 mL) topically applied to a dog resulted in seizures and death within 30 hours; pulegone was identified in a liver specimen. The necropsy showed histopathologic damage including massive hepatocellular necrosis, lung congestion, hemorrhage, and edema. Menthofuran (100-300 mg/kg; 0.666-2.00 mmol)/kg) administered i.p. to mice also produced hepatic necrosis and increases in the plasma levels of SGPT or ALT.

Animal experiments also measured the effect of pretreatment with chemicals that influence levels of cytochrome P-450 on the hepatotoxicity of pulegone or menthofuran. Pretreatment of mice or rats with inducers of cytochrome P-450 (e.g., phenobarbital, diethyl maleate) resulted in an increase in SGPT or ALT and increased hepatic necrosis in animals subsequently treated with pulegone or menthofuran, compared to groups that were not pretreated. In contrast, pretreatment of mice or rats with chemicals that inhibit cytochrome P-450 (e.g., cobaltous chloride, piperonyl butoxide) reduced hepatotoxicity compared to groups not pretreated.

Short-term toxicity studies suggested that pulegone may have neurological effects. Histopathological changes in the white matter of the cerebellum were observed in rats given oral doses (80 or 160 mg/kg/day; 0.53 or 1.05 mmol)/kg/day) of pulegone for 28 days.

Only very limited *in vitro* genotoxicity data on pulegone were located. Pulegone did not induce mutations in *Salmonella* strains TA1537, TA98, TA1535, and TA100, with and without metabolic activation. Pulegone but not pennyroyal oil was weakly mutagenic in *Drosophila melanogaster* in the wing spot test.

Pulegone was shown to have antihistaminic activity on the guinea pig ileum, based on its ability to inhibit histamine-induced contractions *in vitro.*

No data were found on chronic exposure, teratogenicity and embryotoxicity, and carcinogenicity.

Pulegone inhibited the growth of three bacterial species (*Escherichia coli*, *Staphylococcus aureus, Pseudomonas aeruginosa*) and three fungal species (*Candida albicans*,*Aspergillus niger*,*Mucor mucedo*) in an agar diffusion test system.

Pulegone demonstrated insecticidal activity based on lethality to *D. melanogaster*, as did mint oil found to contain 75.7% pulegone.

Treatment of human epidermis *in vitro* with pulegone induce an increase in electrical conductivity, suggesting that pulegone can open new polar pathways across the stratum corneum.

9.1 General Toxicology

9.1.1 Human Data

All available published information concerns case reports associated with ingestion of pennyroyal oil. A review of published reports of pennyroyal poisoning (1869-1996) identified 18 previous cases in which there was moderate to severe toxicity from ingestion of at least 10 mL (about two teaspoonsful) of pennyroyal oil (Anderson et al., 1996). The toxic symptoms included coma, seizures, and hepatic and renal effects. Ingestion of less than 10 mL was generally associated with gastritis and mild central nervous system toxicity. However, the severity of the

toxic effects was not always related strictly to dose and depended on the use of emetics or other treatments. For example, in two cases reported before 1905, coma and seizures were associated with ingestion of 5 mL pennyroyal oil; two other cases reportedly survived after ingestion of 30 mL.

A woman (age unspecified) experienced delirium 4 hours after ingestion of 7 g (0.25 fluid oz) pennyroyal oil (Schimmel Berichte, 1914; cited by Grundschober, 1979). She was completely recovered the following day.

Three cases were reported on by CDC (1978). One 21-year-old woman seen in an emergency room with symptoms of nausea, dizziness, and paresthesia of the fingers claimed to have ingested one-quarter fluid ounce (7.1 g) of pennyroyal oil in four gelatin capsules. The symptoms subsided after two hours in the emergency room. A 24-year-old woman admitted to an emergency room said she felt dizzy, nauseated, with a burning throat sensation, one hour after ingesting one-quarter of a fluid ounce of pennyroyal oil. She was observed overnight and discharged the following day. An 18-year-old woman entered an emergency room in a semi-comatose state two hours after ingesting one fluid ounce of pennyroyal oil. After seven days in the hospital, she died from cardiopulmonary arrest despite resuscitation attempts. The autopsy revealed hepatic necrosis, fluid in the peritoneal cavity, bilateral pulmonary congestion, and edematous kidneys.

In another case report, a 22-year-old woman was admitted to an emergency room with complaints of dizziness, two hours after ingestion of approximately 10 mL of pennyroyal oil (Sullivan et al., 1979). She did not report other symptoms and blood chemistry test results were normal; she was discharged two days after admission.

Two cases identified at one poison control center during a two-year period were medically evaluated, including quantitative analyses of pulegone and menthofuran in sera and qualitative detection of protein-bound adducts of metabolites in the liver of one case (Anderson et al., 1996). One fatal case involved ingestion of a pennyroyal herbal extract in combination with black cohosh root. Post-mortem serum levels of pulegone (18 ng/mL) and menthofuran (1 ng/mL) determined 72 hours after ingestion did not match the predictions of experimental animal models, which predicted a higher menthofuran level relative to the pulegone level. Another patient who had ingested an unknown amount of pennyroyal oil was treated with a substance (*N*-acetylcysteine) believed to protect against pulegone toxicity based on experimental animal studies. Evidence of pennyroyal oil toxicity was minimal; a menthofuran level of 40 ng/mL was measured in the serum 10 hours after ingestion.

Hepatic and neurologic damage occurred in two infants who ingested mint tea later found to contain pennyroyal oil (Bakerink et al., 1996). The sera of both infants were analyzed for

pulegone and menthofuran. Both pulegone (25 ng/mL) and menthofuran (41 mg/mL) were detected in the serum of the one infant, who developed hepatic dysfunction and a severe epileptic encephalopathy. In the other infant, who died from fulminant liver failure with cerebral edema, only menthofuran (10 ng/mL) was identified in the serum.

9.1.2 Chemical Disposition, Metabolism, and Toxicokinetics

In vitro incubation studies with pulegone and male BALB/c mouse liver microsomes identified menthofuran as a major metabolite (Gordon et al., 1987; Nelson et al., 1992). The *in vitro* rate of formation of menthofuran was significantly increased by pretreatment of mice with phenobarbital but was decreased by pretreatment of mice with inhibitors of cytochrome P-450 (piperonyl butoxide, cobaltous chloride, -naphthoflavone) (Gordon et al., 1987). These results suggested to the investigators that menthofuran formation is mediated by a cytochrome P-450 of the phenobarbital class.

Menthofuran metabolism in mouse liver microsomes *in vitro* involved cytochrome P-450 oxidation, more likely through an electrophilic -ketoenal intermediate, to give the diastereometric mintlactones (-)-mintlactone and (+)-mintlactone (Nelson et al., 1992). The intermediate also formed covalent adducts with nucleophilic groups on proteins.

Covalent binding of pulegone and/or a metabolite was quantitated in liver, lung, muscle, and kidney cellular protein from male Swiss-Webster mice, three or seven hours after intraperitoneal (i.p.) administration of 280 mg/kg (1.84 mmol/kg) of ^{14}C-labeled pulegone (McClanahan et al., 1989). The binding was greatest in liver protein at both times after dosing, and was increased by pretreatment with phenobarbital. In contrast, pretreatment with piperonyl butoxide, an inhibitor of P-450 metabolism, reduced binding in all tissues except muscle.

When liver microsomes (isolated from male IISc rats pretreated with phenobarbital) were incubated with pulegone in the presence or absence of reduced nicotinamide-adenine dinucleotide phosphate (NADPH) or oxygen, both microsomal and purified cytochrome P-450 were significantly destroyed (Madyastha et al., 1985; Moorthy et al., 1991a). Microsomes from rats pretreated with 3-methylcholanthrene also exhibited increased destruction of cytochrome P-450 compared to microsomes from untreated rats, but the effect was not as great as from phenobarbital pretreatment (Madyastha et al., 1985). Microsomal heme loss accompanied the loss of enzyme.

An *in vitro* study found that a metabolite of pulegone covalently bound to cell macromolecules (Madyastha and Moorthy, 1989). Covalent binding of radiolabel from *R*-(+)-[^{14}C]pulegone to rat microsomal protein was observed in assay mixtures with NADPH. Involvement of cytochrome P-450 in activation was suggested by increased binding in assays with microsomes from rats pre-treated with phenobarbital, and inhibition of binding by addition to the assay mixture of piperonyl butoxide or antibodies to cytochrome P-450. A related study reported

that menthofuran is the major metabolite of pulegone resulting from incubation with phenobarbital-induced rat liver microsomes in the presence of NADPH (Madyastha and Raj, 1990).

An *in vivo* study of pulegone and menthofuran in rats showed that pulegone, but not menthofuran, depleted glutathione (GSH) in plasma and liver tissue (Thomassen et al., 1990). Inhibition of cytochrome P-450 prevented the GSH depletion by pulegone, suggesting to the investigators that a reactive metabolite other than menthofuran was involved.

Rat biliary metabolites of pulegone were characterized by selective derivatization and analyses of radiolabeled conjugates (Thomassen et al., 1991). Bile samples were taken from rats (male Sprague-Dawley) after i.p. administration of 250 mg/kg (1.64 mmol/kg) of equimolar mixtures of $^{2}H_{3}$- and ^{14}C-labeled pulegone; samples were collected for 8.5 hours after dosing. Results indicated formation of glucuronide and GSH conjugates and bioactivation of pulegone by at least three distinct pathways for formation of different GSH conjugates.

A toxicokinetic study in male Sprague-Dawley rats after i.p. administration of pulegone or menthofuran found that the peak menthofuran concentration in plasma formed *in vivo* after pulegone dosing was 4.5 times higher and occurred much earlier than after administration of only menthofuran (Thomassen et al., 1988). The difference was attributed to slow menthofuran absorption. Different doses of pulegone (150 mg/kg; 0.985 mmol/kg) and menthofuran (100 mg/kg; 0.666 mmol/kg) were determined in preliminary studies to yield comparable area under the plasma concentration vs. time curve of metabolically generated and synthetic menthofuran (98% pure [α]*D* enantiomer).

When pulegone or menthofuran were administered orally to male IISc rats at doses of 250 mg/kg/day for four days or 200 mg/kg/day for 3 days, respectively, various metabolites were detected in urine (Madyastha and Raj, 1992; 1993). These metabolites included *p*-cresol; ketones (5-methyl-2-cyclohexen-1-one, 3-methylcyclohexanone, and 4-hydroxy-4-methyl-2-cyclohexen-1-one); organic acids (geranic acid, neronic acid, benzoic acid, 2-[2 -keto-4 -methylcyclohexyl]propionic acid); and 3-methylcyclohexanol. An unsaturated aldehyde was formed when menthofuran was incubated with phenobarbital-induced rat liver microsomes in the presence of NADPH and oxygen.

Menthofuran incubated with hepatic microsomes from mice, rats, and humans formed a reactive -ketoenal metabolite that accounted for 70% of the total binding to microsomal proteins (Thomassen et al., 1992).

9.1.3 Acute Exposure

Acute lethal values for pulegone are presented in **Table 1**; acute lethal values for menthofuran were not located. Acute exposure studies of pulegone and menthofuran are summarized below and in **Table 2**.

Table 1. Acute Lethal Values for Pulegone

Route	Species (sex and strain)	LD_{50} or LD_{Lo}	Reference
s.c.	mouse (sex and strain n.p.)	LD_{50}: 1709 mg/kg (11.23 mmol/kg)	Wenzel and Ross (1957; cited by Grundschober, 1979)
i.p.	rat (sex and strain n.p.)	LD_{50}: 150 mg/kg (0.985 mmol/kg)	Plazak and Doull (1969; cited by Grundschober, 1979)
i.v.	dog (sex and strain n.p.)	LD_{Lo}: 330 mg/kg (2.17 mmol/kg)	Caujolle et al., (1953; cited by Grundschober, 1979; RTECS, 1997)

Abbreviations: i.p. = intraperitoneal; i.v. = intravenous; n.p. = not provided; s.c. = subcutaneous

9.1.3.1 Oral Administration

Acute exposure of rats to pulegone resulted in dose-related hepatotoxicity. Pulegone administered once at 100, 200, 300, or 400 mg/kg (0.66, 1.31, 1.97, or 2.63 mmol/kg) by gastric intubation to male IISc rats caused a significant decrease in hepatic cytochrome P-450 at 24 hours after treatment for all dose groups except 100 mg/kg (0.66 mmol/kg) (Moorthy et al., 1989). There was a significant increase in serum glutamate pyruvate transaminase (SGPT) and a significant decrease in heme in rats dosed with 200 or 300 mg/kg (1.31 or 1.97 mmol/kg).

Menthofuran induced a dose-related increase in hepatotoxicity in male IISc rats after gastric intubation of a single dose of 100, 200, 250, 300, or 400 mg/kg (0.67, 1.33, 1.66, 2.00, or 2.66 mmol/kg), as indicated by an increase in SGPT among all dosed rats at 24 hours after treatment (Madyastha and Raj, 1994). Doses of 200 mg/kg (1.33 mmol/kg) or greater were also associated with a significant decrease in hepatic cytochrome P-450. Rats treated once with 250 mg/kg (1.66 mmol/kg) also showed significant decreases in glucose-6-phosphatase and aminopyrine *N*-demethylase, but cytochrome b_5 and NADPH-cytochrome *c* reductase activities were not altered.

9.1.3.2 Intraperitoneal Injection

Hepatic necrosis was observed in male Swiss-Webster and BALB/c mice 24 hours after an i.p. injection of pulegone at 200, 300, 400, or 500 mg/kg (1.31, 1.97, 2.63, or 3.28 mmol/kg) (Gordon et al., 1982). Bronchiolar necrosis occurred in BALB/c mice given 400 mg/kg (2.63 mmol/kg), but lung necrosis was not mentioned as a result of pulegone treatment in Swiss-Webster mice. An increase in plasma GPT and a depletion of hepatic GSH were reported for mice treated

Table 2. Acute Exposure Studies of Pulegone and Menthofuran

Species Strain, Age	Number and Sex of Animals	Chemical Form	Dose	Exposure/ Observation Period	Results/Comments	Reference
9.1.3.1 Oral Administration						
rat (IISc; 3-4 mo.)	exposed: Group I, 9M; Group II, 9M; Group III, 9M; Group IV, ≥12M controls: 9M	pulegone, purity n.p.	I 100 mg/kg (0.66 mmol/kg) II 200 mg/kg (1.31 mmol/kg) III 300 mg/kg (1.97 mmol/kg) IV 400 mg/kg (2.63 mmol/kg) controls 1% methyl cellulose	Dosed once, sacrificed 24 hr later	There was a significant dose-related decreases in hepatic cytochrome P-450 and heme in Groups II-IV. There was a significant increase in serum glutamate pyruvate transaminase (SGPT) in Groups II-III; SGPT levels were not reported for Group IV.	Moorthy et al. (1989)
rat (IISc; 3-4 mo.)	exposed: Group I, n.p.; Group II, n.p.; Group III, ≥ 12 M; Group IV, n.p.; Group V, n.p. controls: 3M	menthofuran, purity >99%	I 100 mg/kg (0.67 mmol/kg) II 200 mg/kg (1.33 mmol/kg) III 250 mg/kg (1.66 mmol/kg) IV 300 mg/kg (2.00 mmol/kg) V 400 mg/kg (2.66 mmol/kg) controls 1% methyl cellulose	Dosed once; sacrificed 24 hr later	There was a significant increase in SGPT in all dose groups, and a significant decrease in hepatic cytochrome P-450 in groups II-V. Animals treated once with 250 mg/kg also showed significant decreases in glucose-6-phosphatase and aminopyrine N-demethylase, but cytochrome b_5 and NADPH-cytochrome *c* reductase activities were not altered.	Madyastha and Raj (1994)
9.1.3.2 Intraperitoneal Injection						
mice (Swiss-Webster and BALB/c; age n.p.)	exposed: Group I, 10M; Group II, 19M; Group III, 16M; Group IV, 5M; Group V, 10M; Group VI, 10M controls: n.p.	pulegone, purity n.p.	I 200 mg/kg (1.31 mmol/kg) II 300 mg/kg (1.97 mmol/kg) III 400 mg/kg (2.63 mmol/kg) IV 500 mg/kg (3.28 mmol/kg) V 100 mg/kg (0.66 mmol/kg) after diethyl maleate (300 mg/kg) pretreatment 30 minutes prior VI 100 mg/kg	Dosed once; sacrificed 0-24 hr later	Hepatic necrosis was observed in Groups II-IV. Bronchiolar necrosis was observed in Group III. Elevation of plasma GPT and depletion of hepatic glutathione (GSH) was reported in Group II. Group V also displayed an increase in plasma GPT compared to Group VI. Bronchiolar necrosis occurred in BALB/c mice in Group III, but lung necrosis was not mentioned as a result of pulegone treatment in Swiss-Webster mice.	Gordon et al. (1982)

Abbreviations: M = male; n.p. = not provided

Table 2. Acute Exposure Studies of Pulegone and Menthofuran (continued)

Species Strain, Age	Number and Sex of Animals	Chemical Form	Dose	Exposure/ Observation Period	Results/Comments	Reference
mice (BALB/c, adult)	exposed: Group I, 10M; Group II, 10M; Group III, 10M; Group IV, 7M; Group V, 7M; Group VI, 7M; Group VII, 10M; Group VIII, 10M; Group IX, 10M; Group X, 10M	pulegone, purity n.p. pulegone-d_6, purity n.p.	I pulegone 300 mg/kg (1.97 mmol/kg) II pulegone-d_6 300 mg/kg (1.97 mmol/kg) III pulegone-d_6 400 mg/kg (2.63 mmol/kg) IV pulegone-d_6 500 mg/kg (3.28 mmol/kg) V pulegone-d_6 600 mg/kg (3.94 mmol/kg) VI pulegone-d_6 800 mg/kg (5.26 mmol/kg) VII pulegone 300 mg/kg after phenobarbital (80 mg/kg) pretreatment 24 -72 hr prior VIII pulegone 300 mg/kg after -naphtho-flavone (80 mg/kg) pretreatment 24 or 48 hr prior IX pulegone 300 mg/kg after cobaltous chloride pretreatment (30 mg/kg) 0.5 hr prior X pulegone 300 mg/kg after piperonyl butoxide pretreatment (1360 mg/kg) 0.5 hr prior controls I for II-X	Dosed once; sacrificed 24 hr later	There was a dose-related increase in hepatotoxicity in Groups II-VI, as measured by hepatic blood and plasma concentrations of alanine aminotransferase (ALT). The toxicity was reduced compared to that of pulegone, and a significant isotope effect was noted. The hepatoxicity of the highest dose of pulegone labeled with deuterium in the allylic methyl groups (VI) was about that seen with an unlabeled pulegone dose (I). Oxidation of an allylic methyl group, slowed by the heavier deuterium atoms, apparently is required to generate the hepatotoxic metabolite. Pretreatment with -naphthoflavone or inhibitors of hepatic cytochrome P-450 metabolism (cobaltous chloride, piperonyl butoxide) inhibited pulegone-induced hepatotoxicity. The authors stated that -naphthoflavone induces cytochrome P-450 isozymes that metabolize pulegone or its metabolites to nontoxic products, or it represses the isozymes involved in the formation of toxic metabolites. Pretreatment with an inducer of hepatic cytochrome P-450 (phenobarbital) enhanced the extent of hepatoxicity. Concurrent metabolic experiments identified menthofuran as a proximate toxic metabolite.	Gordon et al. (1987)
mice (ddY; 7 wk)	exposed: Groups I-V (M, # n.p) controls: M (# n.p.)	pulegone, purity n.p.	I 300 mg/kg (1.97 mmol/kg) II 400 mg/kg (2.63 mmol/kg) III 400 mg/kg after SKF-525A (25 mg/kg) pretreatment 1 hr prior IV 400 mg/kg after metyrapone (180 mg/kg) pretreatment 0.5 hr prior V 400 mg/kg co-administered with piperonyl butoxide (400 mg/kg)	Dosed once; Group I sacrificed 1, 2, and 4 days later; remaining Groups sacrificed 2 days after treatment	There was increased hepatotoxicity in Group I, as indicated by significantly increased SGPT and centrilobular liver necrosis. Group III mice had significantly less necrosis and no change in SGPT, and neither effect was seen in Groups IV-V.	Mizutani et al. (1987)
male (Swiss-Webster; 43-45 days)	exposed: Group I, 7M; Group II, 12M; Group III, 7M	pulegone, purity n.p.	I 280 mg/kg (1.84 mmol/kg) II 280 mg/kg after treatment with 80 mg/kg phenobarbital 24-84 hr prior III 280 mg/kg after treatment with 1500 mg/kg piperonyl butoxide 0.5 hr prior controls I for II-III	Dosed once; sacrificed 24 hr later	Group II had significantly increased plasma ALT levels, but no significant change in overall hepatic necrosis. Group III showed significantly lower plasma ALT levels and no hepatic necrosis.	McClanahan et al. (1989)

Abbreviations: M = male; n.p. = not provided

Table 2. Acute Exposure Studies of Pulegone and Menthofuran (continued)

Species Strain, Age	Number and Sex of Animals	Chemical Form	Dose	Exposure/ Observation Period	Results/Comments	Reference
mice (Swiss-Webster and BALB/c; age n.p.)	exposed: control:	pennyroyal oil containing pulegone; % and purity n.p.	400-800 mg/kg	Dosed once; sacrificed 24 hr later	Acute liver and lung damage was induced.	Gordon et al. (1982)
mice (Swiss-Webster and BALB/c; age n.p.)	exposed: Group I, 15M; Group II, 15M; Group III, 16M; Group IV, 10M; Group V, 10M	menthofuran, purity n.p.	I 100 mg/kg (0.66 mmol/kg) II 200 mg/kg (1.33 mmol/kg) III 300 mg/kg (2.00 mmol/kg) IV 50 mg/kg (0.33 mmol/kg) after treatment with diethyl maleate (300 mg/kg) 30 minutes prior V 50 mg/kg	Dosed once; sacrificed 0-24 hr later	Hepatic necrosis was observed in Groups I-III. Elevation of plasma plasma GPT and depletion of hepatic GSH was detected in Group II. Group IV had no increase in plasma GPT compared to Group V.	Gordon et al. (1982)
mice (Swiss-Webster; 43-45 days)	exposed: Group I, 7M; Group II, 7M; Group III, 8M; Group IV, 7M; Group V, M (# n.p.)	menthofuran, purity n.p.	I 200 mg/kg (1.33 mmol/kg) II 200 mg/kg after treatment with piperonyl butoxide (1500 mg/kg) 0.5 hr prior III 125 mg/kg (0.832 mmol/kg) IV 125 mg/kg after treatment with phenobarbital (80 mg/kg) 24-84 hr prior V 200 mg/kg after treatment with phenobarbital (80 mg/kg) 24-84 hr prior controls I for II; III for IV	Dosed once; sacrificed 24 hr later	Group II showed significantly lower plasma ALT and less hepatic necrosis. Group IV had significantly increased ALT, and a significant increase in hepatic necrosis. Group V treatments were lethal.	Thomassen et al. (1992)
rat (Sprague-Dawley; adult)	exposed: Group I, 9M; Group II, 13M	pulegone, 98% pure menthofuran, 98% pure	I pulegone 4 x 75 mg/kg (4 x 0.49 mmol/kg) at 0, 180, 400, and 650 min II menthofuran 110 mg/kg (0.732 mmol/kg)	Dosed 4 times (I) or once (II); sacrificed 30 hr later	Group I had significantly more plasma ALT and hepatic necrosis than Group II. The two groups of rats were matched with respect to the plasma time course of generated and synthetic menthofuran; the multiple-dosing regimen for pulegone mimicked the concentration-time profile for synthetic menthofuran, as demonstrated by the lack of a difference in C_{max} and the area under the curve (AUC).	Thomassen et al. (1988)

Abbreviations: M = male; n.p. = not provided

Table 2. Acute Exposure Studies of Pulegone and Menthofuran (continued)

Species Strain, Age	Number and Sex of Animals	Chemical Form	Dose	Exposure/ Observation Period	Results/Comments	Reference
rats (IISc; 3-4 mo.)	exposed: Groups I-V, 12M per group	pulegone, purity n.p.	I 300 mg/kg (1.97 mmol/kg) II 300 mg/kg after treatment with phenobarbital 80 mg/kg/day for 4 days prior III 300 mg/kg after treatment with 3-methylcholanthrene 40 mg/kg/day for 4 days prior IV 300 mg/kg after treatment with diethyl maleate 300 mg/kg 0.5 hr prior V 300 mg/kg after treatment with piperonyl butoxide 500 mg/kg 0.5 hr prior controls I for II-V	Dosed once; sacrificed 24 hr later	Hepatic cytochrome P-450, heme, aminopyrine *N*-demethylase, and glucose-6-phosphatase levels were significantly lower, and SGPT significantly higher, in Groups II and IV. These indicators of hepatotoxicity were unaffected in Groups III and V.	Moorthy et al. (1989)
rat (Sprague-Dawley; age n.p.)	exposed: Group I, 6M; Group II, 6M; Group III, 4M; Group IV, 4M; Group V, M (# n.p.)	pulegone, purity n.p. menthofuran, purity n.p.	I pulegone 150 mg/kg (0.985 mmol/kg) II pulegone 150 mg/kg after treatment with buthionine sulfoximine (6 mmol/kg) 5 hr prior III menthofuran 125 mg/kg (0.832 mmol/kg) IV menthofuran 125 mg/kg after treatment with buthionine sulfoximine (6 mmol/kg) 5 hr prior V pulegone 150 mg/kg after treatment with piperonyl butoxide (1.36 g/kg) 0.5 hr prior controls I for II and V; III for IV	Dosed once; sacrificed 1 hr after pulegone treatment and 4 hr after menthofuran treatment	Blood samples obtained throughout; ALT levels and hepatic necrosis were significantly increased in Group II. These parameters were unaffected in Group IV. Group V showed very low levels of ALT and no liver necrosis.	Thomassen et al. (1990)
rat (strain IISc; age n.p.)	exposed: Group I, 30M controls: M (# n.p.)	pulegone, purity n.p.	I 300 mg/kg (1.97 mmol/kg) controls coconut oil	Dosed once; sacrificed at 0, 6, 12, 18, and 24 hr later	Significant decreases of hepatic cytochrome P-450, heme, aminopyrine *N*-demethylase, and glucose-6-phosphatase at 24 hours after treatment. ALT levels steadily increased over the 24 hour post-treatment period. Microscopic examination of liver tissue showed that the liver endoplasmic reticulum was first affected, followed by other degenerative changes that ultimately caused cell death.	Moorthy et al. (1991b)

Abbreviations: M = male; n.p. = not provided

Table 2. Acute Exposure Studies of Pulegone and Menthofuran (continued)

Species Strain, Age	Number and Sex of Animals	Chemical Form	Dose	Exposure/ Observation Period	Results/Comments	Reference
rat (IISc; 3-4 mo.)	exposed: Groups I-III, 9M in each Group	menthofuran, purity >99%	I 250 mg/kg (1.66 mmol/kg) II 250 mg/kg after treatment with phenobarbital (80 mg/kg/day for 4 days prior III 250 mg/kg after treatment with 3-methylcholanthrene (40 mg/kg/day for 4 days prior controls I for II-III	Dosed once; sacrificed 4 hr later	In Group II, there was a significant increase in SGPT and a significant decrease in hepatic cytochrome P-450, aminopyrine *N*-demethylase, and glucose-6-phosphatase. In Group III, SGPT and aminopyrine N-demethylase were unchanged, but hepatic cytochrome P-450 was increased and glucose-6-phosphatase was decreased.	Madyastha and Raj (1994)
9.1.3.3 Dermal Application						
dog (breed, age, n.p.)	one (sex n.p.)	Pennyroyal oil % pulegone & purity n.p.	60 mL, applied topically	Dosed once; observed for 30 hr	Treatment resulted in seizures and death within 30 hours; pulegone identified in the liver. The necropsy showed histopathologic damage including massive hepatocellular necrosis, lung congestion, hemorrhage, and edema.	Sudekum et al. (1992)

Abbreviations: M = male; n.p. = not provided

with 300 mg/kg (1.97 mmol/kg); these endpoints were not presented for mice treated with higher doses. Pretreatment of mice with diethyl maleate (300 mg/kg), which depleted GSH levels to 30% of control values, 30 minutes prior to injection of 100 mg/kg (0.67 mmol/kg) pulegone resulted in a greater increase in plasma GPT and hepatoxicity compared to mice treated with pulegone only.

Administration of a single i.p. treatment of deuterated pulegone ($[^2H_6]$pulegone) at 300, 400, 500, 600, or 800 mg/kg (1.97, 2.63, 3.28, 3.94, or 5.26 mmol/kg) to adult BALB/c male mice induced a dose-related increase in hepatotoxicity (Gordon et al., 1987). The toxicity was reduced compared to that of pulegone, and a significant isotope effect was noted. The hepatoxicity of the highest dose of pulegone labeled with deuterium in the allylic methyl groups was about that seen with an unlabeled pulegone dose of 300 mg/kg (1.97 mmol/kg) i.p. Oxidation of an allylic methyl group, slowed by the heavier deuterium atoms, apparently is required to generate the hepatotoxic metabolite. Hepatotoxicity was measured by hepatic blood and plasma concentrations of ALT. Pretreatment of mice with -naphthoflavone (80 mg/kg, 24 or 48 hr prior) or inhibitors of hepatic cytochrome P-450 metabolism (cobaltous chloride at 30 mg/kg, 0.5 hr prior; piperonyl butoxide at 1360 mg/kg, 0.5 hr prior) inhibited pulegone-induced hepatotoxicity. The authors stated that -naphthoflavone induces cytochrome P-450 isozymes that metabolize pulegone or its metabolites to nontoxic products, or it represses the isozymes involved in the formation of toxic metabolites. Pretreatment with an inducer of hepatic cytochrome P-450 metabolism (phenobarbital at 80 mg/kg, 24-72 hr prior) enhanced the extent of hepatotoxicity. Concurrent metabolic experiments identified menthofuran as a proximate toxic metabolite.

Pulegone induced hepatoxicity in male ddY mice given one i.p. dose of 300 mg/kg (1.97 mmol/kg) (Mizutani et al., 1987). The damage was evident from significantly increased SGPT and centrilobular necrosis of hepatocytes, 1 to 4 days after treatment. The cytochrome P-450 inhibitor SKF-525A (25 mg/kg) administered one hour before treatment with pulegone at 400 mg/kg (2.63 mmol/kg) reduced the extent of necrosis and prevented an increase in SGPT. Two other cytochrome P-450 inhibitors, metyrapone (180 mg/kg, 0.5 hour prior) and piperonyl butoxide (400 mg/kg, coadministered), completely prevented the hepatotoxic effects.

Male Swiss-Webster mice pretreated with 80 mg/kg phenobarbital, 24-84 hours before a single i.p. dose of pulegone (280 mg/kg; 1.84 mmol/kg), had significantly increased plasma ALT levels but no change in the level of hepatic necrosis 24 hours following administration of pulegone (McClanahan et al., 1989). Pretreatment of mice with piperonyl butoxide (1500 mg/kg) 0.5 hour before i.p. administration of pulegone (280 mg/kg; 1.84 mmol/kg) resulted in significantly lower plasma ALT levels and prevented pulegone-induced hepatic necrosis.

Acute liver and lung damage was observed in male Swiss-Webster and BALB/c mice 24 hours after a single i.p. treatment with pennyroyal oil at 400 to 800 mg/kg (Gordon et al., 1982). The pulegone concentration in the sample of pennyroyal oil tested was not provided.

The acute toxicity of menthofuran was also tested. Male Swiss-Webster and BALB/c mice injected i.p. with menthofuran at 100, 200, or 300 mg/kg (0.66, 1.33, or 2.00 mmol/kg) showed hepatic necrosis within 24 hours after treatment (Gordon et al., 1982). A significant increase in plasma GPT and a decrease in hepatic GSH was reported for mice treated with 200 mg/kg (1.33 mmol/kg) menthofuran; these measurements were not reported for mice administered any other dose. Pretreatment with diethyl maleate (300 mg/kg) 30 minutes prior to injection of menthofuran (50 mg/kg; 0.33 mmol/kg) did not increase hepatoxicity as measured by plasma GPT levels.

The hepatotoxicity of menthofuran (200 mg/kg; 1.33 mmol/kg) in male Swiss-Webster mice was decreased by pretreatment with 1500 mg/kg piperonyl butoxide 0.5 hour before treatment with menthofuran (Thomassen et al., 1992). Pretreatment of mice with phenobarbital (80 mg/kg) 24 to 84 hours prior to administration of menthofuran resulted in increased mortality at 200 mg/kg (1.33 mmol/kg) menthofuran and increased hepatotoxicity at 125 mg/kg (0.832 mmol/kg) menthofuran. Hepatotoxicity was measured by plasma ALT levels and the extent of hepatic necrosis.

A study with adult male Sprague-Dawley rats suggested that the hepatotoxicity observed after an i.p. dose of pulegone was not solely attributable to the disposition of menthofuran (Thomassen et al., 1988). Rats given i.p. doses of pulegone (4 x 75 mg/kg; 4 x 0.49 mmol/kg) were found to have significantly more plasma ALT and hepatocellular necrosis than rats administered a single i.p. dose of synthetic menthofuran (110 mg/kg; 0.732 mmol/kg). The two groups of rats were matched with respect to the plasma time course of metabolically generated and synthetic menthofuran; the multiple-dosing regimen for pulegone mimicked the concentration-time profile for synthetic menthofuran, as demonstrated by the lack of a difference in C_{max} and the area under the curve (AUC).

Hepatic cytochrome P-450, heme, aminopyrine *N*-demethylase, and glucose-6-phosphatase levels were significantly lower, and SGPT significantly higher, in male IISc rats pretreated with 80 mg/kg/day phenobarbitone for four days before given an i.p. dose of 300 mg/kg (1.97 mmol/kg) pulegone (Moorthy et al., 1989). Similar observations were made in rats pretreated with 300 mg/kg diethyl maleate. Levels of SGPT and aminopyrine *N*-demethylase were not altered in rats pretreated with 3-methylcholanthrene 0.5 hour or for four days prior to dosing with pulegone, or in rats pretreated with piperonyl butoxide (500 mg/kg) 0.5 hours before pulegone treatment. These results are consistent with the hypothesis that the reactive metabolite of pulegone is formed by a phenobarbital-induced cytochrome P-450.

Further study of the metabolism and toxicity of pulegone revealed the formation of a reactive intermediate in addition to menthofuran (Thomassen et al., 1990). The hepatotoxicity of pulegone (i.p. 150 mg/kg; 0.985 mmol/kg) in male Sprague-Dawley rats, as indicated by increased plasma ALT and hepatocellular necrosis, was enhanced 10-fold five hours after administration of a substance known to deplete GSH (buthionine sulfoximine [BSO], 6 mmol/kg). Hepatoxicity induced by synthetic menthofuran (125 mg/kg; 0.832 mmol/kg) was unchanged by pretreatment with BSO. Pretreatment of rats with piperonyl butoxide (1.36 g/kg 0.5 hour) prior to treatment with pulegone at 150 mg/kg (0.985 mmol/kg) prevented pulegone-induced hepatotoxicity.

Significant decreases of hepatic cytochrome P-450, heme, aminopyrine *N*-demethylase, and glucose-6-phosphatase and a significant increase in ALT were measured in male rats (strain IISc) 24 hours after a single i.p. dose of 300 mg/kg (1.97 mmol/kg) pulegone (Moorthy et al., 1991b). Microscopic examination of liver tissue showed that the liver endoplasmic reticulum was first affected by treatment with pulegone, followed by other degenerative changes that ultimately caused cell death.

Pretreatment of male IISc rats with phenobarbital (80 mg/kg/day) four days prior to treatment with menthofuran (250 mg/kg; 1.66 mmol/kg) resulted in a significant increase in SGPT and a significant decrease in hepatic cytochrome P-450, aminopyrine *N*-demethylase, and glucose-6-phosphatase compared to treatment with menthofuran alone (Madyastha and Raj, 1994). Pretreatment with 40 mg/kg/day 3-methylcholanthrene for 4 days did not alter the extent of hepatotoxicity induced by menthofuran.

9.1.3.3 Dermal Application

Pennyroyal oil (60 mL) topically applied to a dog resulted in seizures and death within 30 hours; pulegone (concentration not provided) was identified in the pennyroyal oil and in the liver (Sudekum et al., 1992). The necropsy showed histopathologic damage including massive hepatocellular necrosis, lung congestion, hemorrhage, and edema.

9.1.4 Short-Term and Subchronic Exposure

The studies described in this section are presented in **Table 3**.

When an oral dose of pulegone at 400 mg/kg/day (2.63 mmol/kg) was administered to rats for 5 days, a significant decrease in hepatic cytochrome P-450 and heme was observed (Moorthy et al., 1989).

A gradual decrease in levels of hepatic cytochrome P-450 occurred in male IISc rats dosed with pulegone at 400 mg/kg/day (2.63 mmol/kg/day) by gastric intubation for six days, with an initial reduction of 32% after 24 hours (Madyastha et al., 1985). The activities of cytochrome b5 and NADPH-cytochrome *c* reductase were unchanged.

A 28-day study with rats (strain n.p.) treated by gavage with pulegone at 80 or 160 mg/kg/day (0.53 or 1.05 mmol/kg/day) reported atonia, decreased blood creatinine content, lower terminal body weight, and histopathological changes in the liver and the white matter of the cerebellum (Thorup et al., 1983). The lowest dose tested, 20 mg/kg/day (0.13 mmol/kg/day), did

Table 3. Short-Term and Subchronic Exposure Studies of Pulegone

Species Strain, Age	Number and Sex of Animals	Chemical Form	Dose	Exposure/ Observation Period	Results/Comments	Reference
rat (IISc; 3-4 mo.)	3M	pulegone, purity n.p.	400 mg/kg (2.63 mmol/kg)	Dosed once daily for 5 days and sacrificed 12-72 hr later	Treatment induced a significant decrease in hepatic cytochrome P-450 and heme	Moorthy et al. (1989)
rat (IISc; age n.p.)	exposed: M (# n.p.) controls: M (# n.p.)	pulegone, purity n.p.	400 mg/kg/day (2.63 mmol/kg/day) control: 1% methyl cellulose	Dosed once daily for 6 days, sacrificed 6, 12, 18, 24, 48, 72, 96, 120, and 144 hr later	There was a gradual decrease in hepatic cytochrome P-450, with an initial reduction of 32% after 24 hr. The activities of cytochrome b5 and NADPH-cytochrome *c* reductase were unchanged.	Madyastha et al. (1985)
rat (strain, age n.p.)	exposed: 10 M; 10F controls: 10 M; 10F	pulegone, purity 99%	20, 80, or 160 mg/kg/day; in soybean oil by gavage	Dosed for 28 days.	At 80 and 160 mg/kg/day (0.53 and 1.05 mmol/kg/day), pulegone induced atonia, decreased blood creatinine, lowered terminal body weight, and resulted in histopathological changes in the liver and white matter of the cerebellum. The NOEL (no observed effect level) was 20 mg/kg/day.	Thorup et al. (1983)
rat (strain, age n.p.)	exposed: 10 M; 10F controls: 10 M; 10F	pulegone, purity n.p.	20, 80, or 160 mg/kg/day (0.13, 0.53, or 1.05 mmol/kg/day)	Dosed for 28 days.	At 80 and 160 mg/kg/day (0.53 and 1.05 mmol/kg/day), cyst-like spaces were observed in the cerebellum. The NOEL was 20 mg/kg/day	Olsen and Thorup (1984)
rat (IISc; 3-4 mo.)	3M	menthofuran, purity >99%	250 mg/kg (1.66 mmol/kg)	Dosed once daily for 3 days and sacrificed 72 hr later	Treatment induced higher levels of SGPT and lower levels of hepatic cytochrome P-450, compared to mice treated once.	Madyastha and Raj (1994)

Abbreviations: M = males; F=females; n.p. = not provided

not produce these effects. In a related study, dose-related cyst-like spaces were observed in the white matter of the cerebellum of rats treated orally with pulegone at 80 or 160 mg/kg/day (0.53 or 1.05 mmol/kg/day) for 28 days, while the lowest dose, 20 mg/kg/day (0.13 mmol/kg/day), did not produce this effect (Olsen and Thorup, 1984). The same encephalopathy was observed in rats administered 40 or 100 mg/kg bw/day peppermint oil, but not 10 mg/kg bw/day, for 28 days. The concentration of pulegone or menthofuran in the peppermint oil was not given.

An oral menthofuran dose of 250 mg/kg/day (1.66mmol/kg) to rats for 3 days resulted in an increase in SGPT and a decrease in hepatic cytochrome P-450, compared to rats treated once with 250 mg/kg (Madyastha and Raj, 1994).

9.1.5 Chronic Exposure

No data were found.

9.2 Teratogenicity and Embryotoxicity

No data were found.

9.3 Carcinogenicity

No data were found.

9.4 Genotoxicity

The studies described in this section are presented in **Table 4**.

9.4.1 Prokaryotic Systems

Pulegone was not mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, and TA1537 at 6.4, 32, 160, and 800 µg (0.04, 0.21, 1.05, 5.26 mmol) per plate, with and without S9 metabolic activation (Andersen and Jensen, 1984).

9.4.2 Lower Eukaryotic Systems

Pulegone was reported to be weakly genotoxic in *Drosophila melanogaster* at a dose of 0.2 µL (1 µmol), based on the presence of small single spots in the wing spot test (Franzios et al., 1997). However, pennyroyal oil reported to contain 75.7% pulegone was not mutagenic in the same assay at a dose of 2.1 µL (9.8 µmol pulegone).

9.5 Immunotoxicity

No data were found.

Table 4. Genotoxicity Studies of Pulegone

Test System	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
9.4.1 Prokaryotic Systems							
Salmonella typhimurium strains TA97, TA98, TA100, TA1535, and TA1537	*his* reverse gene mutations	-/+	pulegone, purity 99%	6.4, 32, 160, 800 µg/plate (0.04, 0.21, 1.05, 5.26 µmol/plate)	negative/negative		Andersen and Jensen (1984)
9.4.2 Lower Eukaryotic Systems							
Drosophila melanogaster	wing spot	NA	pulegone, purity n.p.	0.2 µL (186.9 µg=1 µmol)	weakly positive	Small single spots induced that may indicate somatic mutations and/or recombination.	Franzios et al. (1997)
			pennyroyal oil, 75.7% pulegone, purity n.p.	2.1 µL (9.8 µmol pulegone)	negative		

Abbreviations: NA=not applicable; n.p. = not provided

9.6 Other Data

Pulegone (10 µL; 61 µmol per plate; purity not provided) inhibited the growth of three bacterial species (*Escherichia coli*, *Staphylococcus aureus, Pseudomonas aeruginosa*) and three fungal species (*Candida albicans*, *Aspergillus niger*, *Mucor mucedo*) in an agar diffusion test system (Economou and Nahrstedt, 1991).

Pulegone demonstrated insecticidal activity in *D. melanogaster* (Franzios et al., 1997), as did mint oil extracted from the species *M. pulegium,* which contained 75.7% pulegone. The toxicity of pulegone to *D. melanogaster* was reduced in the presence of another mint oil component (menthone).

Treatment of human epidermis *in vitro* with pulegone increased the electrical conductivity, suggesting that pulegone can open new polar pathways across the stratum corneum (Cornwell and Barry, 1993).

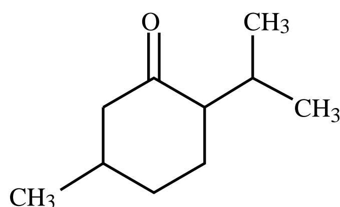
Pulegone (0.24, 0.48, or 0.72 µM) inhibited histamine-induced contractions in a dose dependent manner in isolated guinea pig ileum, demonstrating antispasmotic activity (De Urbina et al., 1990).

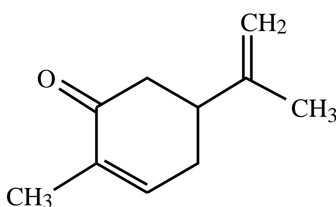
10.0 STRUCTURE-ACTIVITY RELATIONSHIPS

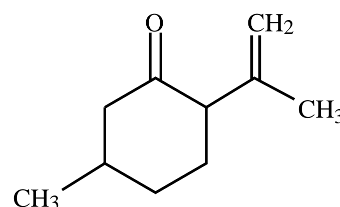
The effects on rat microsomal enzymes of the structurally similar compounds menthone (I; see the following page for structure) and carvone (II) were investigated in a study of pulegone (Madyastha et al., 1985). Both compounds were also associated with a conversion of cytochrome P-450 to cytochrome P-420, but, unlike pulegone, no loss of heme, in microsomes from rats pretreated with phenobarbital. When these two compounds were orally administered to rats at 600 mg/kg for three days, hepatic cytochrome P-450 levels were not decreased (Moorthy et al., 1989).

Another study with 16 compounds structurally similar to pulegone indicated that a particular structural feature, cyclic -isopropylidene ketone with a methyl group positioned *para* to the isopropylidene group, facilitates the *in vitro* destruction of cytochrome P-450 (Moorthy et al., 1991a).

Isopulegone (III), identified in pennyroyal oil, was also found to be a hepatotoxicant in mice after administration of single i.p. doses up to 600 mg/kg (Gordon et al., 1982). The enantiomer of pulegone, *S*-(-)-pulegone at 200 to 600 mg/kg, was less hepatotoxic than its congener, with the highest dose tested, 600 mg/kg, associated with 2641± 681 mU/mL plasma GPT at 24 hours in the 6 survivors compared to 2830 ± 1021 mU/mL in the 5 survivors from R-(+)-pulegone treatment at 400 mg/kg i.p.







11.0 ONLINE DATABASES AND SECONDARY REFERENCES

11.1 Online Databases

Chemical Information System Files

SANSS
TSCATS (Toxic Substances Control Act Test Submissions)
TSCAPP

DIALOG Files

DIOGENES
Federal Register
Foods ADLIBRA
Chem. Econ. Hdbk
FOODLINE: Current Food Legislation
FOODLINE: Food Science and Technology
FOODLINE: International Food Market Data
NIOSHTIC
KIRK-OTHMER Encyclopedia of Chemical Technology

National Library of Medicine Databases

EMIC and EMICBACK (Environmental Mutagen Information Center)

STN International Files

AGRICOLA (Agricultural Online Access)
BEILSTEIN
BIOSIS (Biological Abstracts)
CABA
CANCERLIT
CAPLUS (Chemical Abstracts)
CEN (Chemical & Engineering News)
CHEMLIST
CIN (Chemical Industry Notes)
CROPB
CROPU
CSNB (Chemical Safety News Base)
DDFB
DRUGLAUNCH
EMBASE (Excerpta Medica)
FSTA
LIFESCI
MEDLINE (Index Medicus)
PHIN
PROMT
REGISTRY
RTECS (Registry of Toxic Effects of Chemical Substances)
TOXLINE

TOXLINE includes the following subfiles:

Toxicity Bibliography	TOXBIB
International Labor Office	CIS
Hazardous Materials Technical Center	HMTC
Environmental Mutagen Information Center File	EMIC
Environmental Teratology Information Center File (continued after 1989 by DART)	ETIC
Toxicology Document and Data Depository	NTIS
Toxicology Research Projects	CRISP
NIOSHTIC7	NIOSH
Pesticides Abstracts	PESTAB
Poisonous Plants Bibliography	PPBIB
Aneuploidy	ANEUPL
Epidemiology Information System	EPIDEM
Toxic Substances Control Act Test Submissions	TSCATS
Toxicological Aspects of Environmental Health	BIOSIS
International Pharmaceutical Abstracts	IPA
Federal Research in Progress	FEDRIP
Developmental and Reproductive Toxicology	DART

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