J. Pharm. Pharmacol. 1994, 46: 618–630
Received February 23, 1994
Accepted April 22, 1994

Review

Menthol and Related Cooling Compounds

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Summary—Menthol and related cooling compounds such as 'coolant agent 10', are widely used in products ranging from common cold medications to toothpastes, confectionery, cosmetics and pesticides. The review brings together a range of information on production and chemistry of menthol, and its metabolism, mechanism of action, structure-activity relationships, pharmacology and toxicology. In particular, the coolant action and carminative actions of menthol are discussed in terms of actions on calcium conductance in sensory nerves and smooth muscle. The actions of menthol on the nose, respiratory reflexes, oral cavity, skin and gastrointestinal tract are reviewed.

Menthol and related cooling compounds are widely used in a wide range of products ranging from common cold medications to toothpastes, confectionery and pesticides. Menthol was at first considered as a flavouring agent or a fragrance which enhanced the palatability of medications and confectionery, but pharmacological studies have uncovered a range of biological activity on sensory nerves and smooth muscle. Menthol has been used for medicinal purposes for over one hundred years in the West, but our knowledge of this fascinating compound is still very limited despite its very widespread use. Research on menthol is a fertile area for those interested in developing new drugs for the treatment of respiratory, dermatological and gastrointestinal disturbances.

Production and Chemistry of Menthol

Menthol is a naturally occurring compound of plant origin which gives plants of the *Mentha* species the typical minty smell and flavour. The plant oil, often referred to as peppermint oil (from *Mentha piperita*) or cornmint oil (from *Mentha arvensis*), is readily extracted from the plant by steam distillation. Before World War II menthol was obtained primarily from plant sources, with China and Japan being the main producers. During the disruption of trade routes during World War II, Brazil took over as the main producer of raw plant oil and became the largest producer and supplier of menthol (Anon 1984).

Cornmint oil obtained by steam distillation from the flowering shrub *Mentha arvensis* contains 70–80% of (−)-menthol, which can be crystallized out from the cornmint oil mixture. Since the crystalline product contains traces of cornmint oil, menthol obtained from this plant source has a slightly herbal minty smell. *Mentha arvensis* is grown commercially in Brazil, Japan, Paraguay and China as a source of cornmint oil. Pure (−)-menthol can be obtained from cornmint oil by recrystallization from low-boiling point solvents.

Peppermint oil made from *Mentha piperita* contains up to 50% menthol, but due to its high price, peppermint oil is not used for the production of menthol. Peppermint oil is mostly produced in the USA and is used mainly as a flavouring for toothpastes, other oral hygiene products and chewing-gum. Menthol can also be extracted or synthesized from other essential oils such as cirtonella oil, eucalyptus oil and Indian turpentine oil, and synthesis of menthol from thymol competes with isolation from natural mint oils.

Menthol ($C_{10}H_{20}O$, mol. wt 156·27) is a cyclic terpene alcohol, and although this type of alcohol occurs widely in nature very few of these alcohols have the chemical properties that make them important fragrance or flavour compounds. Menthol has three asymmetric carbon atoms in its cyclohexane ring, and therefore occurs as four pairs of optical isomers; (−)- and (+)-menthol, (−)- and (+)-neomenthol, (−)- and (+)-isomenthol and (−)- and (+)-neoisomenthol, as illustrated in Fig. 1 (Bauer et al 1990). (−)-Menthol is the isomer that occurs most widely in nature

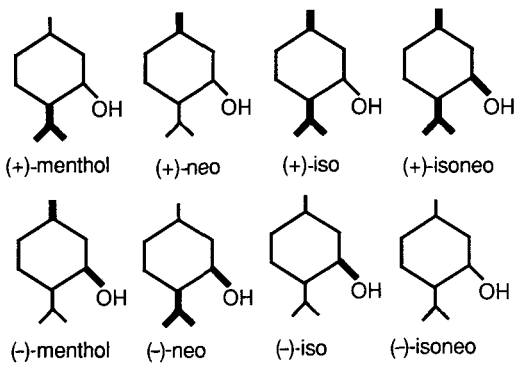

FIG. 1. The four pairs of optical isomers of menthol. (−)-Menthol is the most common naturally occurring isomer and also the isomer with the subjective cooling action.

and is the one assumed by the name menthol. It has the characteristic peppermint odour and exerts a cooling sensation when applied to skin and mucosal surfaces (Watson et al 1978). The other isomers of menthol have a similar, but not identical, odour and do not have the same cooling action as (−)-menthol (Eccles 1990). The (−)- and (+)-isomers of menthol each have identical physical properties apart from their specific optical effect on rotation of light. Neomenthol, neoisomenthol, menthol and isomenthol differ slightly in their boiling points with a range of 211·7–218·6°C. The isomers also differ in their physical characteristics, as at room temperature (+)-neomenthol is a colourless liquid and isomenthol and menthol are white crystals.

Metabolism

Menthol is a highly lipid-soluble substance and like many similar organic substances metabolism of menthol involves the formation of glucuronide compounds, which are much more water soluble and more readily excreted in the urine. Menthol administered orally or absorbed through the skin or respiratory epithelium is transported to the liver by the circulation. There may be some phase 1 metabolism of menthol in the skin and gut on absorption but most occurs in the liver. In the liver, menthol is hydroxylated by microsomal enzymes to form mainly *p*-menthane-3,8 diol, which is metabolite II as illustrated in Fig. 2. The hydroxylated menthol compound is then conjugated with glucuronide and then circulated to the kidneys for excretion in the urine. Madyastha & Srivatsan (1988) in studies on the metabolism of (−)-menthol in rats demonstrated that there were four hydroxylated menthol compounds in rat urine following oral administration of 800 mg menthol kg^{-1} per day for 20 days. The major metabolites were *p*-menthane-3,8 diol (II) and 3,8-dihydroxy-*p*-menthane-7-carboxylic acid (V) as shown in Fig. 2. Minor metabolites 3,8-oxy-*p*-menthane-7-carboxylic acid (IV) and *p*-menthane-3-9-diol (III) were also found. Oral administration of 800 mg menthol kg^{-1} per day, for up to seven days was also shown to induce the liver microsomal enzymes cytochrome P450 and NADPH-cytochrome c (P450) reductase.

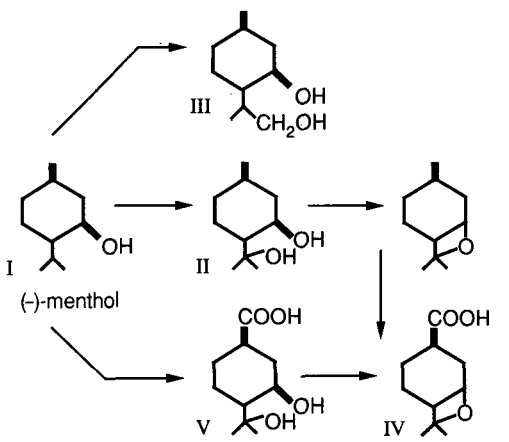

FIG. 2. Metabolism of menthol. The major metabolites are *p*-menthane-3,8 diol (II) and 3,8-dihydroxy-*p*-menthane-7-carboxylic acid (V). See text for further details. After Madyastha & Srivatsan (1988).

The glucuronidation of menthol involves UDP-glucuronyltransferase which is responsible for the glucuronidation of monoterpenoid alcohols in general (Nakaoka 1990). Menthol glucuronide compounds in urine can be readily deconjugated by treating raw urine with β-glucuronidase and the menthol content of urine can be specifically assayed by gas chromatography (Bell et al 1981; Kaffenberger & Doyle 1990).

Structure-activity Relationships

In discussing structure-activity relationships for menthol it is the cooling action of menthol which has been most studied. The cooling action can be investigated either by using panels of volunteers with subjective scores for cooling effects (Watson et al 1978), or by electrophysiological investigations on nerve cell activity (Swandulla et al 1987).

Studies on the electrophysiological activity of cultured vertebrate neurons and snail neurons have shown that (−)-menthol has a very specific dose-dependent effect on calcium flux across the cell membrane, whereas the closely related compound, cyclohexanol, was without activity and (+)-

menthol was only half as active as its stereoisomer (Swandulla et al 1986, 1987).

A systematic investigation of structure-activity relationships was undertaken by Watson et al (1978), who investigated the cooling activity of over 1200 synthetic compounds in order to develop a non-volatile coolant compound for use in shaving foams. The subjective tests used by Watson et al (1978) to determine the coolant activity of compounds were based on the application of coolant compounds onto the surface of the tongue to determine oral threshold for cooling, or directly onto the skin of the inside of the forearm to determine the degree of cooling on an eight point subjective scale. The authors established that four important criteria needed to be satisfied for a compound to possess effective cooling activity: a hydrogen bonding group, a compact hydrocarbon skeleton, a correct hydrophilic/hydrophobic balance, and, a mol. wt in the range 150–350.

The hydrogen-bonding group of menthol is the hydroxyl group, but this can be substituted with a variety of hydrogen-bonding groups and still retain coolant activity. Watson et al (1978) studied a series of *N*-alkyl-carboxamide groups which had the advantage over menthol of low volatility. Two examples of these compounds are illustrated in Fig. 3, with the average oral cooling threshold recorded (μg) given in brackets.

Studies by Amano (1986) on cooling and pungent agents for use as flavouring compounds led to the discovery of a very active cooling compound, 3-1 menthoxy propane-1,2-diol, which is often referred to as 'coolant agent 10' or MPD, and whose structure is illustrated in Fig. 3. The compound is widely used in cosmetics, soaps, dentifrices, mouthwashes, chewing-gums, tobaccos and medical plasters. Coolant agent 10 has an oral cooling threshold in man as low as 1 ppm in aqueous solution, which is only one-fifth that of (−)-menthol, and its duration of cooling action is 20–25 min at 10 ppm, which is twice as long as (−)-menthol (Amano 1986). Coolant agent 10 resembles menthol in its ability to cause a subjective sensation of coolness, but it differs in other respects being a colourless liquid with only a faint minty odour.

Cooling agent AG-3-5 (1-(2-hydroxyphenyl)-4-(3 nitrophenyl)-1,2,3,6-tetrahydropyrimidine-2-one), as shown in Fig. 3, was discovered by a chance finding when Wei (1983) noted that accidental self-contamination of the nostrils eyelids and lips with the compound caused a very strong sensation of coolness. Cooling agent AG-3-5 was found to be a potent cooling agent and Wei (1983) proposed that the compound could be very useful as it lacked the flavour and odour of menthol and was not readily absorbed through the skin.

Apart from the cooling effect, and a degree of flavour potentiation and odour modification, there is no common property of cooling compounds. For instance, there is no association between minty smell and cooling. (−)-Menthol has the greatest cooling activity of the eight stereoisomers of menthol, with (+)-menthol 45 times less active than (−)-menthol, as determined by oral threshold for cooling activity (Watson et al 1978). (+)-Menthol is also relatively inactive compared with (−)-menthol when nasal cooling sensation is used as a measure of activity (Eccles et al 1988a).

Menthol is an alcohol derivative and could act by inducing general cell membrane labilization or modifying membrane fluidity due to its lipophilic properties. However, similar lipophilic compounds such as cyclohexanol are without cooling activity. Studies on the effects of menthol on rat lingual nerve response and comparisons with a range of related alcohols demonstrated that menthol behaved quite differently from the other substances tested, and the results were consistent with menthol interacting with a specific receptor (Simon & Sostman 1991). This information, together with the observations that the effects of menthol are fully reversible, stereochemically selective and cannot be evoked by intracellular application, indicate that menthol exerts its cooling effects by interaction with a specific pharmacological receptor (Watson et al 1978; Swandulla et al 1986; Eccles et al 1988a).

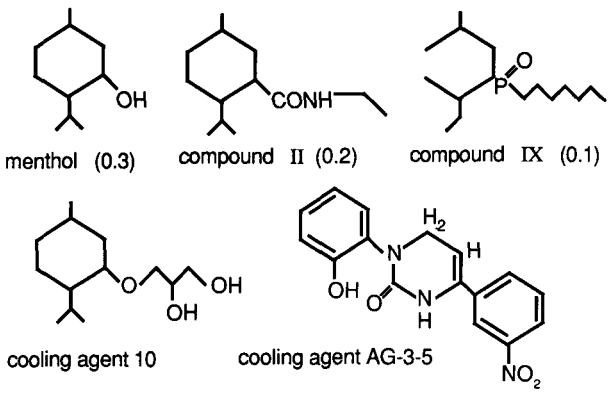

FIG. 3. Synthetic coolant compounds. The structure of menthol is shown for comparison. The figures in parentheses are the oral thresholds (μg) for cooling sensation for man. Compounds II and IX were synthesized by Watson et al (1978). Coolant agent 10 was synthesized by Amano (1986), and cooling agent AG-3-5 was discovered by Wei (1983).

Mechanism of Action

One of the major effects of menthol when applied to the skin or a mucosal surface is to cause a sensation of coolness or warmth and this was attributed to stimulation of thermoreceptors by Goldsheider as early as 1886. The perceived temperature effect is not caused by evaporation of menthol or due to vasodilation but is due to a specific action of menthol on sensory nerve endings. The sensation of cold or warmth is determined by the activity of thermoreceptors in the skin and mucosal surfaces. These cold and warm receptors are considered to be free nerve endings without any specialized end organ (Hensel 1982).

It has been known for some time that changes in the calcium concentration around thermoreceptors cause changes in the sensation of temperature. In man, intravenous injection of calcium solutions has been reported to cause a diffuse sensation of warmth (Hirschsohn & Maendl 1922).

The effects of calcium solutions on human sensations of warmth are supported by electrophysiological studies on warm and cold receptors in the nasal area of the cat. Intravenous administration of calcium solution in the anaesthetized cat caused a marked increase in the frequency of warm-receptor discharge and a depression in the discharge of cold receptors (Hensel & Schafer 1974). Subsequent studies on the cat have demonstrated that a decrease

in external calcium concentration caused by intravenous administration of the calcium-chelating agent EDTA, caused an increase in cold-receptor discharge from nasal skin (Schafer et al 1982). Similarly, a decrease in the calcium concentration in the perfusion fluid of the isolated tongue of the cat was shown to enhance cold-fibre activity in the same way as menthol (10^{-5} M) (Schafer & Braun 1992).

The above studies indicate that calcium ions play a major role in determining the activity of cold receptors and that movement of calcium across the sensory nerve-cell membrane controls the membrane potential and the electrical activity of the cold receptor. Our understanding of the transducer activity of cold receptors is still developing, but it is generally accepted that the cold receptor exhibits oscillations in the membrane potential and that whenever the membrane potential depolarizes above threshold a bursting discharge of action potentials is fired (Braun et al 1980). The oscillations in membrane potential are caused by a regenerative influx and efflux of calcium ions. The efflux of calcium from the cold receptor causes hyperpolarization of the receptor and inhibits the discharge of action potentials. It is this efflux of calcium which is believed to be sensitive to changes in external calcium concentration as the influx of calcium stimulates the efflux mechanism (Eckert & Lux 1976). Therefore, a decrease in external calcium concentrations as caused by EDTA slows the efflux of calcium from the cold receptor and the resultant depolarization of the cell membrane causes an increased electrical discharge from the cold receptor.

Menthol exerts its effects on cold receptors by interfering with the movement of calcium across the cell membrane and there is evidence that menthol acts in the same way as EDTA. Studies on the discharge activity of cat nasal and lingual cold receptors have demonstrated that intravenous infusion of an aqueous solution of menthol in micromolar concentrations caused an increase in the electrical discharge from cold receptors (Schafer et al 1986). The effects of menthol on cold-receptor discharge were abolished by intravenous infusion of a calcium solution. The authors concluded that these findings indicated that menthol caused receptor depolarization and increased nervous discharge by inhibiting the efflux of calcium from the cold receptor (Schafer et al 1986).

The inhibitory action of menthol on calcium efflux from cold receptors is not due to blocking of open calcium channels. Studies on snail neurons and vertebrate neurons in culture have shown that menthol acts on a cell membrane mechanism which regulates calcium efflux (Swandulla et al 1986, 1987; Dorshenko et al 1989). This mechanism does not require menthol to penetrate the nerve cell, as intracellular injection of saturated menthol solutions did not affect calcium efflux (Swandulla et al 1987).

The actions of peppermint oil and menthol on calcium channel-dependent processes in intestinal, neuronal and cardiac preparations were studied in detail by Hawthorn et al (1988). These investigators demonstrated that menthol was almost twice as potent as peppermint oil as an inhibitor of potassium depolarization-induced and electrically induced contractions of guinea-pig ileum and cardiac muscle. Both menthol and peppermint oil inhibited radiolabelled nitrendipine binding to smooth and cardiac muscle and the authors concluded that menthol exhibited calcium-channel blocking properties.

The early observations that administration of calcium solutions affected the sensations of warmth and cold (Hirchsohn & Maendl 1922) clearly linked temperature sensation with calcium. Later studies on menthol have now demonstrated that menthol exerts its actions on the perception of cold and warmth by influencing the movement of calcium in thermoreceptors (Schafer et al 1991). Further clarification of the mechanism of action of menthol is dependent on a better understanding of the role of calcium in modulating nerve-cell activity. Pharmacological studies comparing the effects of menthol with calcium antagonists such as verapamil and dihydropyridine (Sidell et al 1990) are starting to untangle this complex interaction of menthol on calcium flux in the nerve cell, and we can expect rapid developments in this field of research.

The cool sensation caused by application of menthol to the skin or mucosal surfaces is apparent when low or threshold concentrations of menthol are used and is related to stimulation of cold receptors, but menthol has both irritant and local anaesthetic actions when used in higher concentrations. Menthol in 5–10% concentration in mineral oil causes a burning sensation when applied to the skin and this sensation has been attributed to stimulation of nociceptors in the skin (Green 1986). The local anaesthetic action of menthol was studied by Macht (1939) who used a 1:500 solution of menthol in 9·5% alcohol. Macht (1939) demonstrated local anaesthetic activity on frog skin, rabbit eye and guinea-pig skin and he also attributed the central nervous system effects of menthol to its local anaesthetic activity. A local anaesthetic action of menthol on sensory receptors of the human tongue was reported by Hensel & Zotterman (1951). Similarly, Hellekant (1969), reported that high concentrations of menthol ($0{\cdot}2\,g\,L^{-1}$ in water) or prolonged exposure to menthol caused anaesthesia of sensory receptors in the cat tongue. Menthol in aqueous solution is used as an anaesthetic for experiments on snails where it causes a fully reversible depression of activity in central neurons (Haydon et al 1982).

The local anaesthetic activity and irritant activity of menthol may be caused by effects on calcium conductance in sensory nerves and have similarities with menthol's effects on thermoreceptors. This is quite likely for the local anaesthetic properties, but the irritant activity may be a nonspecific action which is also found in other compounds such as camphor, which have quite a different pharmacological profile from menthol.

Pharmacology

Respiratory system

Menthol is widely used in medications for the relief of common cold symptoms such as nasal congestion and cough, but there is little hard scientific evidence to support any nasal decongestant or antitussive activity.

Menthol was introduced into Europe and the United States at the end of the nineteenth century and in 1890, Potter, in a review of 'The use of menthol in diseases of the upper air passages' stated that "while menthol seems to be a drug with a future of great usefulness, it needs to be carefully

studied and investigated, so that we may become familiar with its action and know its limitations". It is now over one hundred years since Potter wrote on menthol, yet our understanding of the pharmacology of this compound is still very limited and often confused.

Nasal decongestant activity. Menthol-containing inhalations, rubs, and lozenges are often described as nasal decongestants yet there are several studies which have clearly shown the opposite effect, i.e. a tendency to increased nasal congestion. As early as 1927, Fox, in a series of experiments on dogs, demonstrated that topical application of 1 and 5% solutions of menthol to the nasal mucosa caused nasal congestion whereas topical adrenaline solution (1:1000) had a marked nasal decongestant action.

Administration of menthol via a nasal inhaler has also been shown to cause nasal congestion (Butler & Ivy 1943). In these experiments on volunteers, inhalation of menthol produced a marked increase in nasal resistance within 1 min after administration and this congestion was sustained over 210 min. Despite the nasal congestion, the subjects did not feel congested but actually believed "that their nasal passages felt clear" (Butler & Ivy 1943). The nasal congestion caused by menthol may be due to an irritant action as menthol, when applied topically to both the skin and nasal mucosa, has been shown to cause hyperaemia and a burning or smarting sensation (Glass & Bliss 1939). The irritant action of menthol is dependent on the concentration of menthol, as a 0·5% solution of menthol in liquid petrolatum was irritating to the nasal mucosa in all subjects tested, whereas a 0·1% solution in physiological saline was non-irritant in most subjects (Glass & Bliss 1939).

Despite the results of the studies by Fox (1927) and Butler & Ivy (1943), menthol and similar aromatics were still believed to have a nasal decongestant action and they were recommended as nasal decongestants in professional literature (British National Formulary 1974). Studies on healthy volunteers by Burrow et al (1983) clearly demonstrated that inhalation of camphor, eucalyptus and menthol vapour had no effect on nasal resistance to airflow as measured by rhinomanometry, but exercise caused a marked decrease in nasal resistance in the same group of subjects. This was the first investigation to use rhinomanometry as a means of investigating the effects of menthol on the nose. In the study by Burrow et al (1983), the volunteers were asked if their sensation of nasal airflow was improved after breathing the aromatics and after exercise. One hundred percent of subjects who breathed menthol reported an increased sensation of nasal airflow whereas only 20% of subjects reported improved airflow after exercise, despite a 70% decrease in nasal resistance to airflow associated with exercise and no change in nasal resistance after menthol. This study clearly demonstrated that menthol inhalation caused a subjective nasal decongestant effect without any objective decongestant action.

The method of measuring the subjective decongestant action of menthol was refined in subsequent studies which used scoring from −6 (completely obstructed nose) to +6 (completely clear nose) and a 100-mm visual analogue scale with the extremes labelled 'nose feels extremely blocked' and 'nose feels extremely clear' (Eccles et al 1987a,b, 1988a,b). These studies confirmed that menthol had no effect on nasal airway resistance but caused a marked increase in nasal sensation of airflow, which could now be quantified for the first time as a subjective score.

Menthol lozenges are widely marketed for the relief of common cold symptoms and although they may have a soothing effect on sore throat, their main action appears to be a subjective sensation of improved nasal airflow. In a study on subjects suffering from nasal congestion associated with the common cold, Eccles et al (1990), demonstrated that oral administration of an 11-mg menthol lozenge caused a subjective sensation of improved airflow without any change in nasal airway resistance. The effects of ingestion of a menthol lozenge on the subjective sensation of nasal airflow and an objective measure of nasal airway resistance are illustrated in Fig. 4.

The cool sensation of increased nasal airflow caused by inhalation of menthol is believed to be due to stimulation of cold receptors served by the trigeminal nerve supply to the nose (Jones et al 1987; Eccles 1990). Jones et al (1989) studied the distribution of thermoreceptors within the nasal vestibule and compared the sensitivity of this area with the nasal mucosa inside the nasal cavity and the malar skin lining the outside of the nose. They found evidence for thermoreceptors within the nasal vestibule and on the surface of the nose, but thermoreceptors were not found on the nasal mucosa.

The location of the cold receptors in the nose is believed to be mainly in the nasal vestibule, which is lined by a squamous epithelium similar to facial skin (Jones et al 1987; Aldren & Tolley 1991; Clarke et al 1992), but there

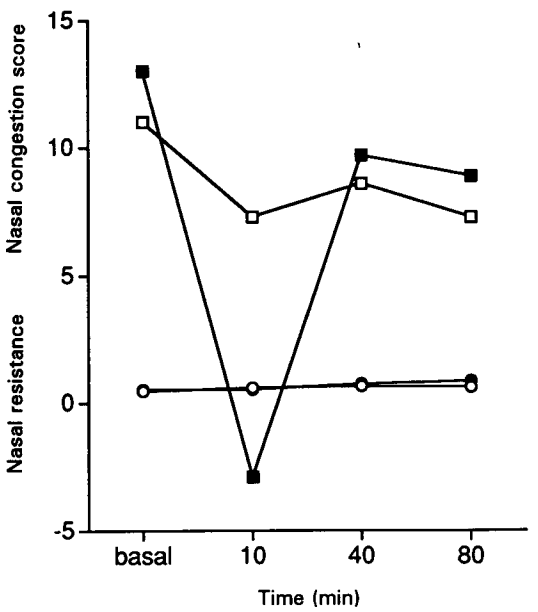

FIG. 4. The effects of ingestion of an 11-mg (−)-menthol lozenge on subjective sensation of nasal congestion (□, ■) and nasal resistance to airflow (○, ●) in volunteers with common cold. The subjective sensation of nasal congestion was significantly reduced after 10 min after ingestion of the lozenge but nasal airway resistance as measured by rhinomanometry was unaffected. Shaded symbols (■, ●) represent the values for the menthol-treated group (n = 30) and the open symbols (□, ○) represent the mean values for the placebo-treated group (n = 32). Results taken from Eccles et al (1990).

is also evidence supporting the involvement of the nasal mucosa in the sensation of nasal airflow based on a finding that local anaesthesia of the nasal mucosa reduces the sensation of nasal airflow (Eccles et al 1988c).

When menthol is taken orally in lozenge form, the increased sensation of nasal airflow is believed to be due to menthol vapour reaching the nasal cavity via the nostrils when the mouth is opened and via the nasopharynx during swallowing. However, there is evidence that menthol may influence the sensation of nasal airflow by a purely oral form of stimulation as it has been proposed that the major palatine nerve serving the hard palate has a role in nasal sensation of airflow (Naito et al 1991).

Respiratory reflexes. Inspiratory airflow cools the nose and larynx and stimulates cold receptors to cause a sensation of airway cooling which is associated with airflow. As well as providing a sensation of cool airflow, the stimulation of upper airway cold receptors causes a reflex inhibition of respiration and inhibition of upper airway accessory respiratory muscle activity in conscious man (McBride & Whitelaw 1981; Eccles & Tolley 1987) and in the anaesthetized dog (Mathew et al 1990). Inhalation of menthol causes the same reflex inhibition of respiration as a cold air stimulus but without any change in airway temperature in the anaesthetized guinea-pig (Orani et al 1991), the anaesthetized dog (Sant' Ambrogio et al 1992), and in conscious man (Eccles et al 1989; De Cort et al 1993; Sloan et al 1993). The effects of nasal airflow and menthol are not always inhibitory to respiratory activity as in the anaesthetized cat, nasal airflow enhances the activity of nasal dilator muscles and menthol potentiates this response (Davies & Eccles 1985, 1987).

The inhibition of respiration caused by cold-air stimulation of the nostrils in man may cause a brief period of apnoea in newborn infants. Similarly, administration of menthol vapour directly to the nose of infants has been shown to cause a brief period of apnoea (Javorka et al 1980). Those authors demonstrated that menthol vapour administered from an open container of menthol crystals placed 1 cm from the nostrils caused a brief period of apnoea (mean 4·7 s in 43% of cases) in a study on 44 premature infants (gestation age 19–37 weeks). It is not clear from this study if the reflex apnoea was induced solely by cold receptor stimulation; menthol crystals placed so close to the nose would deliver a high concentration of menthol vapour which could be irritant. Javorka et al (1980) proposed that the apnoea was the result of a protective reflex to prevent inhalation of irritant substances, and this is supported by the increase in heart rate observed after menthol exposure, which may represent a general arousal reaction.

Since menthol-containing medications are widely used in the treatment of common cold in infants, any tendency of menthol to cause apnoea is viewed with great concern. Following several reports in the German medical literature of adverse events in infants after treatment with menthol-containing ointments (Bettecken 1964; Moll 1964), the German medical authorities issued a statement warning against the use of menthol medications in infants (Arzneimittelkommission der Deutschen Arzteschaft 1964). The public warning by the German medical authorities stimulated interest in this area and an international symposium was held in Paris in April 1966 to debate the safety of menthol medications. The symposium proceedings bring together a large amount of clinical data on the use of menthol medication in thousands of cases in infants suffering from acute respiratory tract infection. The general conclusions of the symposium were that commonly used vaporub remedies were safe to use in infants but that they should not be applied directly to the nostrils (Dost & Leiber 1967).

Antitussive properties. The use of menthol as an antitussive goes back over one hundred years, as it was in 1890 that Lunsford Richardson developed a topical rub containing menthol for the treatment of whooping cough (Poetsch 1967). The 'vaporub' developed by Richardson is still marketed for the treatment of cough together with numerous other menthol-containing syrups, lozenges and topical rubs. Despite the very widespread use of menthol products as antitussives, there is very little literature available in the public domain to support antitussive efficacy.

Inhalation of menthol has been shown to inhibit respiration via stimulation of upper airway cold receptors (Eccles et al 1989; Orani et al 1991; Sant' Ambrogio et al 1992), and menthol may act to inhibit cough via this mechanism. Respiratory reflexes such as cough are closely linked to the brainstem centres regulating respiration and the general inhibition of respiratory activity caused by menthol could also influence the frequency and intensity of cough. Menthol may also act as an antitussive by influencing the activity of sensory nerves in the upper airway which initiate cough. It is not clear at present which sensory nerves are involved in producing cough, but it is generally agreed that the larynx is the most sensitive site for the initiation of cough (Karlsson et al 1988; Fuller & Jackson 1990). Menthol has been shown to influence the activity of cold receptors in the larynx (Sant' Ambrogio et al 1991) and may also influence the activity of laryngeal sensory receptors involved in the cough reflex. The mechanisms by which menthol may act as an antitussive are at present speculative, but they are based on established knowledge that menthol has already been shown to influence the activity of upper-airway sensory receptors and to modulate respiratory reflexes.

Mucus production and mucociliary clearance. The secretion of mucus and the continuous mucociliary clearance from the upper airway are important defence mechanisms against infection. There are conflicting reports in the literature that menthol and other aromatics may either enhance or depress these defence mechanisms. Menthol is a popular ingredient for steam inhalations taken for the treatment of cough and as an expectorant to promote clearance of mucus from the respiratory tract. Oswald (1959) concluded that the most desirable property of menthol and other aromatics taken in steam inhalation was their pleasant smell because the main virtue of steam inhalation "is the expectorant effect of hot moist air". However, studies by Boyd & Sheppard (1969) on anaesthetized rabbits demonstrated that administration of menthol by steam inhalation caused an increase in mucus production and a decrease in the specific gravity of respiratory tract fluid. This effect of menthol was produced by amounts of menthol which added no detectable odour of

menthol to the inspired air and which corresponded to a systemic absorption of less than 20 $\mu g\,kg^{-1}$ body weight. The authors concluded that the bronchomucotropic effects of menthol were due to direct local stimulation of mucus-secreting cells in the respiratory tract. The effect of menthol on mucus production was dose dependent, as inhalation of high concentrations of menthol depressed both the volume and mucus content of respiratory-tract fluid.

The stimulant action of menthol on mucus production may be beneficial, as bacteria adhere avidly to respiratory-tract mucus (Plotkowski et al 1993). Ciliary clearance of mucus is essential in order to prevent infection and therefore any effect of menthol on ciliary activity is of interest. Using an in-vitro model of the frog oesophagus, Das et al (1970) demonstrated that 0·1% menthol in Ringer solution stimulated mucociliary clearance, whereas Su et al (1993) using a rat tracheal preparation demonstrated that menthol at 0·01% concentration decreased ciliary beat frequency. Although Su et al (1993) claim that menthol is ciliotoxic in a dose-dependent manner, they also suggest that in-vivo studies need to be made on human subjects to determine any effects on mucociliary clearance and respiratory defence.

There does not appear to be any report of in-vivo studies in man on the effects of menthol on respiratory defence. However, Huber et al (1973) reported in abstract form that exposure to a vapour rub containing menthol and other aromatics resulted in a depression of respiratory defence, as measured by bacterial aerosol challenge in mice. This publication aroused great clinical interest as the vaporub preparation was widely used in children for the symptomatic treatment of common cold. In a fuller paper, Jakab & Green (1975) contradicted the earlier report by Huber et al (1973) and demonstrated that exposure to vaporub vapours did not cause any depression of pulmonary antibacterial activity. The findings of Jakab & Green (1975) were later confirmed by Goldstein et al (1976), who demonstrated that rates of pulmonary bacterial transport and inactivation in the rat and mouse were unaffected by exposure to vapours of camphor, menthol, eucalyptol and turpentine. Jakab & Green (1975) took the trouble to measure the concentrations of the vapours and they carefully controlled the conditions of exposure and assessments of respiratory defence, and their work throws considerable doubt on the early report by Huber et al (1973) that inhalation of menthol and other aromatics may compromise respiratory defence.

The studies on respiratory defence mechanisms investigated the effects of a mixture of aromatic vapours and, therefore, it is difficult to separate out any specific effect of menthol. However, the substantial studies by Jakab & Green (1975) and Goldstein et al (1976) showed that the mixture of aromatics was without effect on respiratory defence and one can conclude that menthol vapour alone would also be innocuous unless there was some complex interaction between the components of the vapour mixture.

Pulmonary function. Because of the commercial pressure to show efficacy of action for a registered product with a mixture of aromatics (including menthol) there are relatively few studies on the single ingredients. Menthol may have effects on the airways in subjects with common cold, as Cohen & Dressler (1982) reported that a mixture of aromatics (including menthol) improved a number of pulmonary function indices. However, that report which indicates a beneficial effect of aromatics inhalation has not been confirmed and it is difficult to attribute any effect of the mixture of vapours to an action of menthol.

Menthol vapour may exert some effect on lung function by influencing lung surface tension, as in-vitro studies on synthetic surfactant films have shown that menthol does lower surface tension and in-vivo studies on anaesthetized rabbits have shown that eucalyptol exhibits surfactant properties and increases lung compliance (Zänker et al 1980).

Skin

The main effect of menthol when applied to hairy skin is to create a cooling sensation similar to the nasal cooling sensation described above. The coolant action of menthol on skin made it a popular ingredient in shaving creams (Watson et al 1978) and in order to avoid the eye irritation caused by menthol vapour, relatively non-volatile menthol-like coolant compounds were developed. However, the sensory effects of menthol on skin are more complex than a straightforward stimulation of cold receptors, as menthol also modulates the sensations of warmth and skin irritation. Menthol (0·2 and 2% in mineral oil) when applied to the vermillion border of the human lip causes an attenuation of the sensation of warmth, which appears to be due to effects on warm receptors rather than a masking of the sensation of warmth due to stimulation of cold receptors (Green 1986). Higher concentrations of menthol (5 and 10% in mineral oil) caused a strong burning sensation which may be due to stimulation of nociceptors rather than warm receptors (Green 1986). Green (1992) also made psychophysical measurements on the sensory effects of menthol applied topically to the human forearm under controlled thermal conditions. Menthol as a 5% solution in ethanol heightened the perception of skin cooling and attenuated the perception of moderate skin warming. Menthol was also shown to have pungent or irritant properties which were enhanced by cooling of the skin, and Green (1992) suggested that menthol may stimulate cold-sensitive nociceptors as well as cold receptors.

Menthol's property to stimulate skin nociceptors may be responsible for the counter-irritant action of menthol, as the nociceptors initiate an axon reflex with subsequent release of vasodilator peptides. An increase in skin temperature and underlying muscle temperature has been shown after topical application of a mixture of eucalyptus oil and menthol, and this may be beneficial for pain relief and useful to athletes as a passive form of warm-up (Hong & Shellock 1991). The effects of menthol on cold receptors and nociceptors may also be beneficial in the treatment of pruritus, as itching is mediated via unmyelinated 'c' fibres which also transmit the sensation of pain. Topical menthol (0·25%) has been widely used for many years to treat pruritus but there are no controlled studies to verify the efficacy of menthol as a topical treatment for this condition (Greco & Ende 1992).

The irritant effect of menthol causes local vasodilation and this together with the lipophilic nature of menthol may aid topical drug penetration. Studies on hairless mouse skin have shown that menthol (1–5% w/v) can aid the penetra-

tion of compounds such as indomethacin and cortisone (Katayama et al 1992), morphine hydrochloride (Morimoto et al 1993) and methyl salicylate (Yano et al 1991).

Irritancy at high concentrations is not a general property of coolant compounds as coolant agent 10 has no irritant activity on human skin and rabbit eye at concentrations up to 10%, despite being a more potent coolant compound than menthol (Amano 1986; Takasago Co. 1993).

Oral cavity

Cornmint oil and peppermint oil are widely used for flavouring toothpastes, chewing-gum and confectionery. These oils contain around 70% (−)-menthol which is responsible for the pleasant cooling and refreshing taste of the oils.

Menthol has a complex sensory effect in the oral cavity as it influences the activity of both gustatory and temperature receptors. In studies on the electrical activity of the chorda tympani nerve of the anaesthetized cat, Hellekant (1969) demonstrated that rinsing the tongue with an aqueous menthol solution (0·1 g L^{-1}) caused a slowly increasing activity in all gustatory fibres. Menthol also altered the response of the taste receptors to other sapid solutions, and prolonged exposure to menthol or higher concentrations of menthol (0·2 g L^{-1} water) anaesthetized the taste receptors. Hensel & Zotterman (1951) studied the effects of an aqueous solution of menthol on temperature-induced responses from the tongue of the cat and reported that menthol caused 'chemical sensitization of the thermoreceptors to thermal stimuli'. Menthol in an aqueous solution (1:10 000) was shown to enhance cold-receptor activity without any action on warm receptors. Hensel & Zotterman (1951) also commented that the local anaesthetic action of high concentrations on menthol in oil was easily demonstrated in man, as 1 or 2 min after application of menthol to the tip of the tongue there was an obvious decrease in both cold and tactile sensibility. Dodt et al (1953), in a similar study on the thermoreceptors of the tongue of the cat, found that acetylcholine and menthol behaved in a similar way as they both sensitized cold receptors. The authors also reported that menthol influenced the activity of warm receptors and caused a shift of the range of steady discharge of the warm receptors towards the cold side, i.e. sensitized warm receptors. Dodt et al (1953) claimed that the earlier study by Hensel & Zotterman (1951) did not find any effect of menthol on warm-receptor discharge because the study was limited to very few warm-fibre preparations and to onset of heat paralysis in the warm-fibre response above 40°C.

The complex oral sensations induced by menthol were commented on by Green (1985), who found that menthol enhanced cold sensations in the mouth but could enhance or attenuate warm sensations depending on the time period of pretreatment with menthol.

The complexity of oral sensations caused by menthol may be explained by a sensitization of both warm and cold receptors together with modulation of taste-receptor activity. In high concentrations menthol has both an irritant action and a local anaesthetic effect and gradations of these effects complicate the sensory actions of menthol. Studies on the chorda tympani nerve response to menthol in the anaesthetized rat have shown that the nerve response elicited by menthol (0·64 mM in aqueous solution) only lasts around 2·5 s, but that the tongue receptors remain insensitive to menthol for up to 10 min after application of the solution (Lundy & Contreras 1993). Although stimulation with menthol prevented taste receptors from responding to subsequent presentations of menthol, the preparation responded normally to sodium and potassium solutions and solutions of quinine and citric acid, and prior stimulation with one of these solutions resulted in the recovery of the menthol response. Lundy & Contreras (1993) explained this effect of menthol as being due to cell depolarization caused by menthol binding with receptor proteins on the plasma membrane of the taste receptor.

The oral sensations of warmth and cold are accompanied by a sensation of nasal cooling and a peppermint smell, as menthol vapour from the oral cavity reaches the nose. Oral administration of menthol may also directly influence nasal sensation of airflow by effects on the major palative nerve which innervates the palatal mucosa (Naito et al 1991).

Gastrointestinal tract

Peppermint oil has been used for many years in herbal remedies for the treatment of digestive disorders and it has been shown to be effective in the symptomatic treatment of irritable bowel syndrome (Rees et al 1979), and to reduce colonic spasm when injected directly into the large intestine during colonoscopy (Leicester & Hunt 1982). When used for the treatment of irritable bowel syndrome, peppermint oil is recommended to be taken in enteric-coated capsules as release of the oil in the stomach causes relaxation of the lower oesophageal sphincter and heart burn (Somerville et al 1984). Peppermint oil is a carminative with potent antispasmodic properties and it is likely that these actions are mainly due to the high (−)-menthol content of peppermint oil, although Evans et al (1975) suggest that the carminative action of menthol and similar compounds such as camphor, thymol, eugenol, and carvone, is non-specific and related to the aqueous solubility.

Menthol has been shown to inhibit the histamine- and acetylcholine-induced contractions of the guinea-pig isolated taenia coli (Reis & Bertini 1984) and to have smooth muscle relaxant activity on the isolated vas deferens, uterus and intestine (Macht 1939). The fact that menthol has been shown to be a smooth-muscle relaxant supports the idea that the activity of peppermint oil is mainly related to its high menthol content. However, since peppermint oil is a mixture of biologically-active substances, some caution needs to be taken in attributing all the properties of peppermint oil to menthol.

Menthol has been shown to inhibit the contractions of the guinea-pig ileum by an effect on calcium conductance in smooth muscle (Hawthorn et al 1988). This inhibitory action of menthol on gut smooth-muscle calcium conductance may be the basis for the efficacy of peppermint oil in treating irritable bowel syndrome, as peppermint oil contains a high percentage of menthol. In-vitro studies on guinea-pig and human gut smooth muscle by Taylor et al (1984, 1985) indicate that menthol exerts an inhibitory effect on gut smooth muscle by decreasing the influx of extracellular calcium through potential-dependent channels, whilst

having no effect on the intracellular mobilization of calcium. Thus, the actions of menthol on both nervous tissue and smooth muscle are mediated by effects on calcium conductance across the cell membrane, and this basic action links menthol's cooling and carminative properties.

Effects on olfaction

The cooling sensation caused by inhalation of menthol is believed to be primarily due to stimulation of trigeminal nerve fibres supplying the nasal vestibule and nasal mucosa, but menthol also has a distinctive minty smell which is presumably due to stimulation of olfactory nerves. The integrity of the olfactory system is not essential for the detection of menthol as anosmics can detect menthol via pungency (Commetto-Muniz & Cain 1990). Chronic exposure to menthol has been shown to cause symptoms of occupational hyposmia (Naus 1983), whereas an acute exposure to an aqueous solution of menthol applied to the human olfactory area by means of a drop pipette has been shown to decrease the smell threshold to coffee, which is believed to be a pure olfactory stimulus (Skouby & Zilstorff-Pedersen 1954). The ability to detect menthol declines with age (Murphy 1983), and this may represent a general decline in nasal trigeminal/olfactory sensitivity due to accumulated damage of nasal infection and it indicates that caution must be taken when applying results of research on healthy young adults to the general population.

The trigeminal nerve fibres supplying the nasal mucosa have been shown to respond to a wide variety of chemical stimuli and are believed to provide a sense of irritation often referred to as a 'common chemical sense', whose prime function is to protect the animal from potentially harmful chemicals (Silver 1990). The nerve fibres involved in the sense of nasal irritation appear to be free trigeminal nerve fibres which penetrate between the epithelial cells of the nasal mucosa (Silver 1992).

Sensory impact of menthol

The sensory impact of menthol when applied to skin or a mucosal surface depends on the concentration of menthol. Low concentrations give a cool sensation whereas high concentrations of 2–5% menthol cause irritation and local anaesthesia. There is little information on the dose-response characteristics of menthol and it is often difficult to compare studies when different aqueous and organic vehicles are used for application of menthol, as the solvent influences the biological activity of the menthol. There is also evidence that there is a marked difference in sensitivity between different body surfaces for the cooling action of menthol. Watson et al (1978) stated that the eye was the most sensitive region of the body, with thresholds measured in nanograms and a simple test for cooling activity in volatile compounds being to hold an open bottle near to the eye. The sensitivity of body surfaces was ordered as eye > tongue > buccal region > ano-genital area > axilla > inside forearm-breast > other arm areas, thigh, back > hands, feet ≫ palms, soles (Watson et al 1978). This gradation of sensitivity is probably related to the density of thermoreceptors in these surfaces. The nasal-cooling response was not listed by Watson et al (1978) but from our own experience it probably comes a close second to the eye as a very sensitive area.

The sensory impact of menthol in the nose when inspired as a vapour or administered intranasally in solution is a complex mix of sensations. The cool sensation dominates at low concentrations of menthol with stimulation of trigeminal cold receptors and this is supplemented by the minty smell caused by stimulation of olfactory nerves. In higher concentrations menthol stimulates trigeminal nerves to give a 'chemical' sense related to the detection of irritant compounds, and this may be related to stimulation of nociceptors. Depending on the concentration of menthol, this irritant sensation may be just a pungent sensation, or in extreme, a sensation of burning and pain. The cool sensation is generally perceived as a pleasant sensation, especially when providing relief from nasal congestion, but high concentrations of menthol are irritant and may elicit protective respiratory reflexes and cause nasal congestion.

The sensory impact in the mouth is complex, as menthol vapour will also reach the nasal cavity and stimulate olfactory and trigeminal sensory receptors. Similarly in the oral cavity, menthol will stimulate taste receptors and thermoreceptors and, as discussed above, the effects of menthol on these sensory receptors are dependent on both the concentration of menthol and the duration of exposure. The well-known pleasant minty taste of menthol and peppermint oil is very familiar, but when it comes to dissecting out the various sensations and ascribing them to nasal and oral receptors the picture is extremely complex.

Miscellaneous

Menthol is commonly used in oral and nasal medications and comes into contact with mucosal epithelia which are frequently subject to bacterial infection and allergic reactions. Therefore, it is not surprising that investigators have studied the antibacterial and anti-allergic properties of menthol and other essential oil components.

Moleyar & Narasimham (1992) studied the antibacterial activity of fifteen essential oil components towards food-borne bacteria and demonstrated that cinnamic aldehyde was the most active compound but that menthol also had significant antibacterial activity.

Studies on the anti-allergic effects of constituents of chewing-gum by Arakawa et al (1992) have shown that intraperitoneal administration of menthol in the guinea-pig inhibits homologous passive cutaneous anaphylaxis mediated by IgE antibody. Similarly, menthol was shown to suppress antigen-induced histamine release from rat peritoneal mast cells and the authors concluded that menthol in chewing-gum would have an anti-allergic effect.

Cooling of the urinary bladder in man and other animals has been shown to cause a reflex contraction of the detrusor muscle and micturition. This reflex is believed to be mediated by cold receptors in the bladder wall. Lindstrom & Mazieres (1991) studied the effect of menthol on the urinary bladder cooling reflex in anaesthetized cats and demonstrated that the bladder cooling reflex was greatly exaggerated by intraluminal exposure of the bladder or urethra to a 0·6 mM solution of menthol. This action of menthol appeared to be a specific sensitizing action on bladder cold receptors as the activity of bladder mechanoreceptors was unaffected. Thus, menthol seems to have similar actions on respiratory cutaneous and bladder cold receptors.

Menthol causes a cool sensation when applied to the skin and this may be the cause of a behavioural response known as 'the wet shakes', which is observed following administration of menthol in rats. Wei (1983) described the wet shakes in rats treated with menthol and another coolant compound AG-3-5 as rotational body movements similar to those made by a dog when wet. The ED50 for menthol for wet shakes was found to be 35 mg kg^{-1} compared with an ED50 of only 0·18 mg kg^{-1} for compound AG-3-5 when administered by intraperitoneal injection. The wet-shakes behaviour was attributed to a cutaneous action of the drugs which probably caused a sensation of skin cooling.

An unusual use for menthol is as a pesticide in controlling tracheal mite infestations in honey bee colonies. A 50-g porous sachet of menthol is placed within the hive and the menthol vapour kills mites within the bee tracheae (Duff & Furgala 1991; Delaplane 1992). Menthol is the only pesticide approved by the US Environmental Protection Agency for bee mite control.

Toxicology

Menthol, peppermint oil and synthetic coolant compounds are widely used in the food and pharmaceutical industries; peppermint oil is the world's third most important flavouring exceeded in popularity only by vanilla and citrus flavours (Thorup et al 1983a). World production of menthol was estimated as 3500 tonnes in 1984 and is probably considerably more today (Anon 1984). Peppermint oil contains 30–50% menthol and the world production of peppermint oil is about 8000 tonnes per year. For such a commonly ingested substance it is surprising that there are relatively few studies on the toxicology of menthol and this supports the generally held opinion that menthol is a relatively nontoxic and safe substance.

The acceptable daily intake for menthol is quoted as 0–0·2 mg kg^{-1} (FAO/WHO 1976), but this figure is not supported by any toxicological data. The authors have quoted a very low value for chronic menthol intake to err on the side of safety in the absence of any hard data and they acknowledge that much larger doses have been taken by man without any ill effect.

Martindale, The Extra Pharmacopoeia (1989) states that the fatal dose in man has been estimated to be about 2 g. This is probably a gross underestimate as it represents a dose of only around 28 mg kg^{-1} and in metabolic studies on rats a daily dose of 800 mg kg^{-1} was given for 20 days without any report of serious adverse effects, apart from induction of liver enzymes (Madyastha & Srivatsan 1988). Macht (1939) reported the lethal dose in rats to be 1·5 g kg^{-1}. In a study which primarily investigated the cooling action of menthol, the LD50 in rats was found to be 700 mg kg^{-1}, with death attributed to the "well known anaesthetic properties of this compound" with loss of the righting reflex and depression of respiration (Wei 1983). Macht (1939) commented that the most striking pharmacological property of menthol was its effect on the central nervous system, as with high doses animals soon became depressed and gradually lost consciousness, an action which he attributed to the local anaesthetic activity of menthol. Menthol has been shown to reversibly depress the activity of snail neurons, where it is often used as an anaesthetic agent and there is also some evidence that menthol inhibits synaptic transmission in the snail (Haydon et al 1982). In comparison with menthol, camphor exhibited marked convulsant activity in the rat (Macht 1939).

The synthetic coolant compounds have been found to be even less toxic than menthol when judged by the rat LD50. Coolant agent 10 has an acute oral LD50 of 5·7 g kg^{-1} (Amano 1986), and coolant agent AG-3-5 has an acute LD50 of 1·5 g kg^{-1} (Wei 1983).

Neuropathy has been induced in rats given peppermint oil (40–100 mg kg^{-1} per day), with histological changes in the white matter of the cerebellum (Thorup et al 1983a). Since peppermint oil contains 1–3% pulegone, which is known to be toxic, a comparison of the toxicology of menthol with pulegone was undertaken in order to determine if menthol could also be implicated as a cause of neuropathy. Rats were given menthol by gavage for 28 days at 0, 200, 400 and 800 mg kg^{-1} and showed few toxic effects (Thorup et al 1983b). For menthol, the only effects were an increase in liver weight and increased vacuolization of hepatocytes which was evident at all doses, and probably represents an induction of liver enzymes. In contrast, pulegone, at doses of 80 and 60 mg kg^{-1}, caused atonia and histopathological changes in both liver and brain tissues. No sign of encephalopathy was observed in rats given menthol. Thus, the neurotoxicity of peppermint oil is probably related to the pulegone content rather than menthol.

Chronic exposure to high concentrations of menthol vapour has been shown to have no gross toxic effects in rats (Rakieten et al 1954). Those investigators exposed rats to (−)-menthol vapour at several concentrations between 0·087 and 0·166 ppm for over 6 h a day, for up to 79 days, and found only slight changes indicative of irritation of the eyes and lungs at the highest concentration. The authors also report that after the rats had been killed, tracheal ciliary activity was normal in all animals, although no objective measurement of ciliary beat frequency or mucociliary clearance was used by the authors to support this claim.

In-vitro studies on a variety of animal tissues including hamster brown adipocytes have shown that the major effect of menthol in-vitro at a concentration of 0·32–0·76 mM is to cause deterioration of biological membranes (Bernson & Pettersson 1983). The authors also demonstrated that noradrenaline-induced respiratory activity in the adipocytes was inhibited by menthol.

Menthol is used in many brands of cigarettes and the smoking of mentholated cigarettes represents a chronic form of menthol intake. The percentage of mentholated-cigarette users has been shown to be much higher in American Blacks and Asians than in Whites, especially in the younger age groups (Sidney et al 1989). Since the incidence of oesophageal cancer mortality among American Blacks is over three times the rate for Whites and the increase in oesophageal cancer roughly parallels the increase in menthol cigarette sales, it has been proposed that there is a link between smoking mentholated cigarettes and oesophageal cancer (Herbert & Kabat 1989). However, studies looking at the consumption of mentholated cigarettes and oesophageal cancer have not shown any clear link, and the

issue of menthol-cigarette smoking and oesophageal cancer is unresolved (Herbert & Kabat 1989). In-vitro studies on genotoxicity on animal and human tissues have not found any chromosomal-damaging effects of menthol and at present there is no evidence to implicate menthol as a carcinogen (Murthy et al 1991).

In general, menthol appears to be a substance of very low toxicity in acute studies, but more information is required to define a safe daily intake for chronic intake.

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