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## CHAPTER 3: THE PHYSIOLOGICAL EFFECTS OF MENTHOL CIGARETTES

### INTRODUCTION

Menthol is a flavor additive widely used in consumer and medicinal products. It can be natural or synthetic, has a minty taste and aroma, and may have cooling, analgesic or irritating properties. As noted in chapter 1, menthol is an active ingredient in certain medicinal products, such as cough drops, and when used in medicinal products, it is regulated as a drug. The use of menthol in tobacco products is not regulated. Menthol is present in varying concentrations in 90 percent of tobacco products, including cigarettes that are not marketed as menthol cigarettes.

The Family Smoking Prevention and Tobacco Control Act charges the Tobacco Products Scientific Advisory Committee (TPSAC) with developing a report and recommendations that address "the issue of the impact of the use of menthol in cigarettes on the public health including such use among children, African Americans, Hispanics, and other racial and ethnic minorities." Chapter 3 reviews the physiological effects of menthol in cigarettes. It reviews menthol's chemical structure, its mechanism of action, its interaction with key constituents of tobacco and tobacco smoke, and its affect on the sensory experience of smoking.

Specifically, chapter 3 will address the following questions:

- Does menthol have cooling and/or anesthetic properties that moderate the harshness of cigarette smoke?
- Does menthol make low-tar, low-nicotine cigarettes more acceptable to smokers?
- Does menthol have an effect on nicotine or nicotine-derived nitrosamine metabolism?
- Is it biologically plausible that menthol increases the addictiveness of cigarette smoking?

The answers will assist TPSAC in addressing the nine overarching questions listed and discussed in chapter 1 that are the subject of this report. While the information in chapter 3 is relevant to all nine questions, it is of particular importance to those examining the impact of menthol cigarettes on individual smokers.

### METHODS

Chapter 2 provided the general framework for this report and the Tobacco Products Scientific Advisory Committee's approach to gathering, reviewing and weighing the evidence. Using this framework, chapter 3 draws on sources that provide information about the physiological effects of menthol or necessary background information. The sources of information includes papers published in peer-reviewed literature, documents supplied to the committee by tobacco companies, FDA white papers and unpublished tobacco company documents. Chapter 3 relies in part on animal and human studies that biochemically and/or behaviorally assess the physiological effects of exposure to menthol.

### WHAT IS MENTHOL?

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Chemically, menthol is a monocyclic terpene alcohol. It is a naturally occurring chemical chiefly derived from the peppermint plant (*Mentha piperita*) or the corn mint (*Mentha arvensis*), but it can also be synthetically produced. The chemical structure of menthol is shown in Figure 1. Menthol can exist as one of eight stereoisomers—molecules with identical formulas but different three-dimensional shapes. These isomers include menthol, isomenthol, neomenthol and neoisomenthol, each of which can exist as l, also called (-), or d, also called (+). Each of the stereoisomers has distinct pharmacologic characteristics. The l, or (-), isomer of menthol is the natural isomer and conveys the typical taste and sensory characteristics of menthol. The d, or (+), isomer is active but less so than l-menthol (Eccles 1994).

Tobacco companies use both natural and synthetic menthol in cigarettes. The natural menthol found in cigarettes (l isomer) is typically crystallized from steam-distilled oil of the corn mint plant (R.J. Reynolds 2010, p.6). Synthetic menthol (dl - menthol) is racemic, meaning it contains both the d and l isomers and has different taste characteristics from natural menthol (Lorillard Tobacco Company 2010, p.11, Heck 2010). Some cigarette manufacturers use natural menthol only; others use a mixture of natural and synthetic menthol. Natural menthol has been reported to impart greater cooling and mintness and less sharpness, perhaps due to trace chemicals in the natural extract (Wayne and Connolly 2004). Peppermint and spearmint oils may also be added along with menthol to some cigarettes to modify the taste and other sensory characteristics of the smoke (Wayne and Connolly 2004).

Menthol is volatile and has a relatively low boiling point (212 degrees C) (Heck 2010). Consequently, menthol readily vaporizes during cigarette smoking and easily transfers from the cigarette smoke to the smoker, with little pyrolysis, or decomposition. (Jenkins et al. 1970). In mainstream smoke, the vast majority of menthol is in the particulate phase (Jenkins et al. 1970).

Menthol is added to cigarettes in numerous ways: (1) spraying the cut tobacco during blending; (2) application to the pack foil; (3) injection into the tobacco stream in the cigarette maker; (4) injection into the filter on the filter maker; (5) insertion of crushable capsule in the filter; (6) placement of a menthol thread in the filter; and (7) a combination of the above (R.J. Reynolds 2010, p.7, Altria Client Services 2010). Over time, menthol diffuses throughout the cigarette irrespective of where it was applied. Menthol cigarettes are typically blended using more flue-cured and less burley tobacco (Wayne and Connolly 2004). This is because some of the chemicals in burley tobaccos create an incompatible taste character with menthol.

Menthol in cigarettes can be measured either by weight or yield. When measured by weight, menthol content is expressed either as the ratio of the weight of menthol to the weight of the tobacco in the cigarette (mg menthol/gm tobacco), or the weight of menthol in the entire cigarette (mg menthol/cigarette). Ratios also can be expressed as parts per million (ppm), where 1000 ppm is equivalent to 0.1 percent. Yield per cigarette measures menthol in cigarette smoke and is expressed in mg. Though the menthol-in-smoke measurement is more biologically relevant, it is important to note that menthol yield is generated using standard smoking machine test methods and may not reflect how individual smokers consume menthol cigarettes. Smokers on average take in larger amount of smoke that the machine predicts, particularly when smoking lower yield cigarettes. Thus smokers of menthol cigarettes are likely to be exposed to more than the machine determined menthol yield per cigarette.

Menthol produces a minty taste and aroma and elicits cooling sensations. At low concentrations menthol has a soothing effect, but at high concentrations menthol is irritating. Menthol is reportedly added to cigarettes both as a characterizing flavor (higher levels) and for other taste reasons (lower levels). These other taste reasons include brightening the flavor of tobacco blends and/or smoothing or

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balancing the taste of the blend (R.J. Reynolds 2010, p.15). The lowest detectable concentration identified by smokers as menthol characterizing is about 0.12 percent (Lorillard Tobacco Company 2010, p.13). Most menthol cigarettes contain 0.30 percent or higher. Menthol concentrations in non-menthol cigarettes average about 0.01 to 0.03 percent (Wayne et al. 2004). (b) (4)

. In addition to taste, menthol also contributes to smoke impact and to modulation of the irritation from nicotine.

In a recent survey of 48 U.S. menthol cigarette brands and sub-brands, the average menthol content in cigarettes by weight was 2.64 mg/ cigarette, with a range from 1.61 to 4.38 mg (Celebucki et al. 2005). The average menthol content in tobacco by weight was 3.89 mg/ gm tobacco, with a range from 2.35 to 7.76. Menthol concentrations tended to be highest in cigarettes with the lowest machined-measured tar deliveries, for reasons discussed below. Thus ultralight cigarettes typically had the most menthol, followed by light cigarettes and full flavor cigarettes. Altria presented data on menthol concentration in tobacco and in smoke for U.S. menthol cigarettes marketed in 2008 and 2009 (Altria Client Services 2010, p.25). The median menthol in tobacco was about 0.6 percent (6 mg/gm tobacco) and the median menthol in smoke was about 0.6 mg/cigarette. The lowest menthol in smoke was 0.35 mg/cigarette and the highest 1.29 mg/cigarette. The latter was in Camel LT KS Men HP cigarettes in which a menthol capsule is crushed prior to machine smoking. Menthol is also present in many non-menthol cigarettes at lower concentrations.

Examples of the menthol contained in the cigarettes and delivered in the smoke (as tested by standard condition machine smoking) for common full flavor menthol cigarette sub-brands are as follows (units are mg): Marlboro FF DS Men HP – 4.1, 0.71; Camel Crush KS HP, breaking capsule – 5.3, 0.87; Camel FF KS Men HP – 3.6, 0.71; Kool FF 100 HP/SP – 4.4, 0.74; Salem FF KS HP Green Label – 3.3, 0.61; Newport FF LS Men HP – 2.3, 0.46 (Altria Client Services 2010).

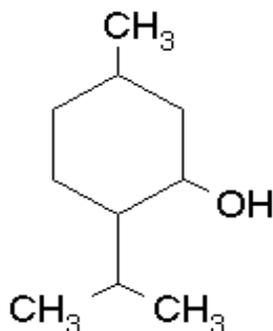
Low yield cigarettes – light and ultralight brands – are low yield primarily due to increased ventilation or air dilution. Compared to full flavor menthol cigarettes, light and ultralight menthol cigarettes have lower transfer efficiency—the percentage of menthol in the smoke compared to the menthol in the cigarette. The increased filtration and ventilation of lower tar delivery products decreases transfer efficiency. In full flavor menthol cigarettes, the transfer efficiency of menthol averages 10–20 percent, while the transfer efficiency in ultralight menthol cigarettes can be as low as 5 percent (Altria Client Services 2010, p.22–24; Cook et al. 1999). To cite a specific example, menthol transfer from the Newport cigarette is 20 percent, while transfer from Newport Light is 12 percent (Lorillard Tobacco Company 2010, p.6). The higher menthol content in light and ultralight cigarettes compensates for the lower transfer efficiency. The transfer efficiency can change with storage of cigarettes as menthol moves from the tobacco to the filter, from which it may be less available for elution (Altria Client Services 2010).

Tobacco companies have explored adding chemicals with menthol-like cooling effects to cigarettes. A number of cooling agents were developed by Wilkinson Sword Ltd in the 1970s and are identified as WS compounds (Leffingwell & Associates 2010). Several of these chemicals including WS-3, WS-5, WS-12, WS-14 and WS-23, act on the same receptors as menthol and have similar cooling effects, but lack menthol's minty taste and aroma (Ma et al. 2008). Other cooling chemicals have been developed by other companies. (b) (4)

but to TPSAC's knowledge, they were never added to mass marketed cigarettes. (b) (4)

. In any case, when considering regulation of menthol in cigarette, the presence of menthol analogs or alternative should also be considered.

Figure 1



## MENTHOL'S MECHANISMS OF ACTION

Menthol acts on receptors expressed primarily on sensory nerves, including in the trigeminal nerves that innervate the nose, mouth and airways (Abe et al. 2005). Specifically, menthol acts on Transient Receptor Potential (TRP) channels that contribute to the detection of physical stimuli, including temperature and chemical irritation (Levine et al. 2007; Macpherson et al. 2006). Menthol has been reported to act on three of these receptors: the TRPM8 (transient receptor potential melastatin 8), TRPA1 (transient receptor potential ankyrin1) and TRPV3 (transient receptor potential, vanilloid family, member 3). (b) (4)

The TRPM8 receptor, which is responsive to cold, and the TRPA1 receptor, which is a chemosensory receptor, are expressed in the sensory neurons of the trigeminal and dorsal root ganglia. The TRPV3 and TRPV1 receptors are responsive to heat and capsaicin. The TRPV3 receptors are expressed in skin cells, and TRPV1 in trigeminal nerve and dorsal root ganglia cells. All of these receptors have roles in mediating sensations of pain or irritation (Eid et al. 2009).

The TRPM8 receptor is activated by both cold and by menthol (Voets et al. 2004; Macpherson et al. 2006; Bautista et al. 2007), explaining why menthol elicits sensations of cooling. Menthol decreases cold pain thresholds and enhances pain responses to noxious cold stimuli (Hattem et al. 2006; Wasner et al. 2004). TRPM8 receptors are located on sensory, or afferent, nerves. At low doses menthol produces cooling and analgesia and at high doses menthol can cause irritation and pain via effects on these receptors. With prolonged stimulation menthol desensitizes TRPM8 receptors (Kuhn et al. 2009).

The TRPA1 receptor chiefly mediates the pain response to irritant chemicals, including the unsaturated aldehydes in cigarettes smoke (Andre et al. 2008; Bessac and Jordt 2008). This receptor also transmits responses to noxious cold (Karashima et al. 2009), and inflammatory pain (Bautista et al. 2006). Chemicals interact with TRPA1 to produce cough and airway inflammation (Geppetti et al. 2010). Menthol activates and inhibits the TRPA1 receptor, through which menthol can produce or reduce the irritation from tobacco smoke (Bressac and Jordt 2008; Talavera et al. 2009; Xiao et al. 2008; Karashima

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et al. 2007). Nicotine, a known irritant, also activates TRPA1 receptors (Karashima et al. 2007; Xiao et al. 2008). Menthol activates TRPV3 receptors to induce cooling in skin (Macpherson et al. 2006).

TRPV1 receptors, found in airway sensory fibers as well as the nasal mucosa, respond to chemical stimuli including capsaicin and many other irritant chemicals (Bessac and Jordt 2008). Nicotine induces irritation by effects both on nicotinic cholinergic receptors and on TRPA1 and TRPV1 receptors (Talavera et al. 2009; Dussor et al. 2003; Simons et al. 2003; Lee et al. 2009).

Menthol acts on olfactory nerves to produce a minty aroma and pungency, effects that decrease as people age (Murphy 1983). When applied to skin, menthol has cooling and antipruritic effects (Bromm et al. 1995). These anti-itching effects have been attributed to menthol's interaction with cold receptors and possibly with kappa opioid receptors (Galeotti et al. 2002).

In addition to its ability to relieve itching, menthol is a topical analgesic. Menthol desensitizes nociceptive C receptors, which are responsible for sending pain signals to the brain; this activity may contribute to analgesia (Cliff and Green 1994). Given in high doses orally (10 mg/kg) or in smaller doses into the brain (10 mcg intracerebroventricularly) menthol has potent analgesic effects in rodents, effects that depend on activation of the endogenous opioid system, acting on kappa opioid receptors (Galeotti et al. 2002). Thus in high concentrations, menthol acts on the brain. However, the concentration threshold for effects on the brain is not known. Menthol increases skin blood flow at the site of application, which may also contribute to local analgesia (Harris et al. 2006). Menthol's other attributes include antibacterial and antifungal properties and the ability to enhance of penetration of topical drugs and chemicals (Iskan et al. 2002).

## MENTHOL DESENSITIZATION AND INTERACTION WITH NICOTINE

With repeated or prolonged administration, menthol is known to cause desensitization to its own cooling and irritant effects. Menthol is also reported to reduce sensitivity to noxious chemicals, including nicotine. The irritating effects of nicotine on the airway are mediated by activation of nicotinic cholinergic receptors and TRPA1. In cellular electrophysiology studies and in a rodent model of nicotine-induced airway constriction reflex response, menthol inhibits effects of nicotine (Talavera et al. 2009). Other in vitro studies have reported that menthol results in desensitization of nicotine-induced neuronal activation (Hans et al. 2006; Reeh et al. 2006).

(b) (4)



In an experimental study, people whose tongues were repetitively dosed with menthol in solution became less sensitive to menthol's irritating and cooling effects (Dessirier et al. 2001). Menthol also

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reduced irritation from nicotine when applied to the tongues of people (Dessirier et al. 2001). (b)

(4)

. Menthol did however reduce burning pain both in baseline and nicotine conditions. Higher levels of nicotine reduced the subjects' ability to discriminate dose-related odor and cooling effects of menthol compared to lower nicotine levels. While both menthol and nicotine have the potential to desensitize responses with repeated exposure, a study comparing olfactory thresholds for menthol and nicotine in smokers and non-smokers found the smokers had a much higher olfactory threshold for nicotine but no difference in threshold for menthol (Rosenblatt et al. 1998). The same was seen in both menthol and non-menthol smokers. Thus the effects of menthol are persistent in smokers.

## **MENTHOL KINETICS, METABOLISM AND METABOLIC INTERACTIONS WITH NICOTINE AND TOBACCO-SPECIFIC NITROSAMINES**

Menthol moves from cigarette smoke into the lungs and then into the bloodstream. Smokers systemically absorb an average of 5–20 percent of the menthol in a menthol cigarette, depending on the extent of ventilation (Altria Client Services 2010, Benowitz et al. 2004). For a cigarette containing 3 mg of menthol (0.3 percent), a smoker of 20 cigarettes per day is exposed to an average systemic dose of 12.5 mg menthol per day.

Once it enters the general circulation, menthol is rapidly metabolized, making it difficult to measure free menthol in the blood or urine. Menthol is metabolized primarily through glucuronidation, a process that takes place in the liver to detoxify substances, and through oxidation, which also takes place in the liver. Glucuronidation primarily is driven by the liver enzyme UDP-glucuronosyl transferase 1A4 (Green and Tephly 1998). The result of this process is a compound called menthol glucuronide. Oxidation of menthol to hydroxylated metabolites has been observed in studies in rats (Yamaguchi et al. 1994; Madyastha and Srivatsan 1988). In humans, approximately 50 percent of an oral dose of menthol is excreted in the urine as menthol glucuronide (Gelal et al. 1999). The half-life of menthol glucuronide after oral menthol dosing is about 50 minutes in plasma and 74 minutes in urine, although there appears to be a longer terminal half-life, most likely due to the slow release of the highly lipid-soluble menthol from body tissues and/or due to enterohepatic recirculation (Gelal et al. 1999). It is difficult to do pharmacokinetic studies with inhaled menthol because the dose absorbed cannot be known with certainty. Urine menthol glucuronide concentrations have been measured in a cross-sectional study of smokers of menthol and non-menthol cigarettes (Benowitz et al. 2010). On average, menthol levels are higher in menthol smokers, but many non-menthol smokers also have high menthol levels due to consumption of menthol-containing foods.

While free menthol concentrations are quite low in blood, they are high in tobacco smoke. As a result, menthol concentrations will be high in the mouth, throat and lungs. Estimating concentrations in smoke is important to assess the plausibility that menthol has effects on sensory nerves and possible drug metabolism in the upper and lower airways in relation to concentrations that have effects in animals or cell preparations. Assuming that a menthol cigarette delivers 0.8 mg of menthol in smoke and that a smoker takes 8 puffs on a cigarette, the menthol per puff is 0.1 mg. Assuming that all of the menthol in a puff is absorbed and that the inhalation volume associated with one puff (puff volume plus air) is 800 ml, the concentration of menthol would be 1250 mcg/L, which would be 8.0 uM/L. There is uncertainty about the partition of menthol between smoke and lung tissue, but this gives some rough approximation about what levels might act in the lungs, where there are drug metabolizing enzymes. Concentrations could be considerably higher in the mouth and throat, before the inhaled smoke is fully diluted with the fresh air inhaled with the smoke. These high concentrations are in contrast with the

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low concentrations of free menthol in the blood stream and presumably in the liver, as discussed in more detail below.

## Interactions with nicotine

Menthol may alter the metabolism of constituents of tobacco smoke, including nicotine. Menthol inhibits the metabolism of nicotine in liver microsomal test systems (MacDougall et al. 2003). (b) (4)

[REDACTED]. The IC 50 (concentration that inhibits metabolism by 50%) was 70.5 uM for l menthol and 37.8 uM for d menthol in the MacDougall study. This concentration is higher than the concentrations typically detected in the blood of smokers, raising the question of whether circulating menthol levels in smokers would be adequate to inhibit liver metabolism of nicotine. However, nicotine is also metabolized in the lungs (Turner et al. 1975), where, as described previously, menthol levels in smoke are likely to be high enough to inhibit nicotine metabolism. In an experimental study of smokers, Benowitz et al. (2004) found that smoking menthol cigarettes inhibits nicotine metabolism in smokers. This was a two-week crossover study in which 14 smokers smoked menthol or non-menthol cigarettes on alternating weeks. After smoking a particular type of cigarette for several days, each subject was given an intravenous infusion of deuterium-labeled nicotine and cotinine to determine the effects of menthol cigarette smoking on the disposition kinetics of nicotine and cotinine. Nicotine clearance was on average 10 percent slower while smoking menthol cigarettes. Menthol inhibited both oxidative metabolism of nicotine to cotinine, and glucuronidation of nicotine. Menthol had no effect on cotinine metabolism. Potential limitations of this study include its small sample size, that its subjects were all heavy smokers and that its subjects were predominantly men.

Studies that used a different measure of nicotine oxidative metabolism found that menthol had no statistically significant effect on the breakdown of nicotine. These studies measured the ratio of the nicotine metabolites trans-3' hydroxycotinine to cotinine (Dempsey et al. 2004), which result from the activity of the enzyme CYP2A6, the major enzyme involved in the oxidation of nicotine. The ratio of trans-3' hydroxycotinine to cotinine, which can be measured in blood, saliva or urine, is highly correlated with the clearance of nicotine. Using this ratio, three studies found no difference in nicotine metabolism between menthol and non-menthol smokers. One was a cross-sectional multi-site study of 1044 menthol and 2297 non-menthol smokers conducted by Altria (Total Exposure Study, Wang et al. 2010). Another was a study of 755 African American smokers participating in a clinical trial of smoking cessation (Ho et al. 2009). The third was a study of 89 smokers with schizophrenia and 53 controls (Williams et al. 2007). The lack of a menthol effect is consistent with either no effect or a small effect of menthol on oxidative metabolism. The ratio would not be sensitive to an effect of menthol on nicotine conjugation. The Altria Total Exposure Study did look at urine ratios of nicotine glucuronide to nicotine, and found no effect of menthol cigarette smoking, arguing against an effect of menthol on nicotine conjugation (Altria Client Services 2010).

## Interaction with tobacco-specific nitrosamines

Menthol may also inhibit the detoxification of the tobacco-specific carcinogen 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanol (NNAL). NNAL is formed as a major metabolite of the potent tobacco-specific nitrosamine and carcinogen 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone NNK (Hecht). NNK is present in cigarette tobacco, and is formed primarily by nitrosation of nicotine in the curing process. A major pathway of detoxification of NNAL is by glucuronidation, considered to be

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mediated by the isoenzymes UGT2B7 (Ren et al. 2000) UGT2B10 (Chen et al. 2007) and UGT2B17 (Lazarus et al. 2005). A substance that inhibits the detoxification of NNAL could potentially increase the risk of cancer. Richie et al. (1997) found in a study of 34 African American smokers and 27 Caucasian smokers that the ratio of NNAL glucuronide / NNAL in urine was significantly lower in African Americans. This finding suggested slower glucuronidation detoxification of NNAL in African American smokers. Since African Americans predominantly smoke menthol and Caucasians predominantly non-menthol cigarettes, Ritchie et al. hypothesized that menthol inhibits NNAL glucuronidation. Muscat (2009) specifically compared 67 menthol smokers to 80 non-menthol smokers, and found that the glucuronidation ratio was significantly lower in white menthol smokers and menthol smokers overall, with a non-significant trend in the same direction for African American smokers. Muscat et al. also found that menthol inhibited NNAL glucuronidation in vitro using human liver microsomes. In the latter study, the IC 50 values for inhibition of N-glucuronidation and O-glucuronidation of NNAL were 0.26 and 0.41 mM, respectively. These levels are higher than those found in the blood and presumably liver of menthol cigarette smokers. Whether such glucuronidation can occur in the lung is not clear. The Altria-sponsored Total Exposure Study, which included 1044 menthol and 2297 non-menthol cigarette smokers, mentioned previously, found no effect of menthol cigarette smoking within racial groups on the ratio (Altria Client Services 2010).

## **MENTHOL AND SENSORY RESPONSE TO CIGARETTE SMOKING**

### **Effects on smoke smoothness and impact**

Sensory attributes of tobacco smoke can be considered as a combination of taste, smell and chemesthesis (the latter referring to the feel, such as cooling, biting and burning) (Carpenter et al. 2007). These occur in the context of stimulation of physiological responses in olfactory and trigeminal nerves. These responses have been described by Philip Morris as tobacco smoke flavor, which includes attributes derived from aromatic volatile substances, tastes and feeling qualities such as dryness and cooling (Philip Morris 1999). Sensory attributes overall include resistance to draw, throat response (such as smooth, stinging, peppery, cool), mouth response, mouth fullness, dryness and harshness, tobacco taste, aftertaste strength and cooling effect.

As noted above, menthol produces a variety of sensory effects, including a minty taste and aroma, cooling/ soothing effects, anesthetic effects and irritant effects. Menthol contributes to many of the sensory effects of cigarette smoke, including strength, taste, harshness, smoothness, mildness, coolness taste, and aftertaste. (R.J. Reynolds 1984). The effects of menthol are related to concentration. Lower menthol concentrations produce cooling and anesthetic effects, while higher menthol concentrations produce burning and irritation.

At the very low menthol concentrations used in non-menthol cigarettes, menthol is likely to make smoke smoother and less harsh even though the distinctive minty taste and aroma is not detectable (Wayne and Connolly 2004). At the concentrations found in menthol cigarettes, smokers report that menthol reduces irritation and that menthol cigarettes are less harsh and smoother than non-menthol cigarettes. Smokers of high menthol cigarettes appear to particularly like the taste and aroma of menthol.

Menthol also has irritant effects, as noted above. Throat irritation is an important contributor to smoke impact, which is a key component of the perceived strength and satisfaction of the cigarette. Both nicotine and menthol stimulate the trigeminal nerve in the mouth and throat to jointly produce the sensory effect of “bite,” or “throat grab.” Reviews of tobacco company documents and a submission

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from Altria describe the interaction between menthol, nicotine and tar in producing impact and other sensory effects (Wayne et al. 2004; Kreslake et al. 2008; Altria Client Services 2010; RJ Reynolds 1985). In cigarettes with low levels of tar and nicotine, the addition of menthol can enhance the “bite” or “throat grab” of the smoke, making such cigarettes more acceptable to consumers. Conversely, the addition of menthol to cigarettes high in tar and nicotine can reduce the irritating effect of nicotine, perhaps by cross desensitization, making these cigarettes more palatable. Among menthol cigarette smokers, perception of strength and impact correlate better with menthol delivery than with nicotine delivery (Perfetti 1982).

Thus menthol is not simply a flavoring agent but has drug-like characteristics that modulate the effects of nicotine on the smoker. The consequences of these effects for menthol cigarette smokers are twofold: the sensory stimulation from the “throat grab” of menthol could provide greater reinforcement of smoking behavior, and the reduced irritation provided by lower levels of menthol could lessen aversion to initial self-administration of nicotine among novice smokers, thereby facilitating continued smoking that leads to addiction. Additionally RJR documents (Carpenter et al. 2007) found a relationship between sensory preferences and smoking topography. Smokers who desired a strong cigarette took larger puffs compared to individuals who desired less strength. Since menthol is a determinant of perceived strength, this could be another reason for a relationship between menthol and greater intake of cigarette smoke.

(b) (4)

## Genetic interactions

Individual differences in taste perception, such as the ability to taste bitter chemicals, are well known. These differences are at least in part genetically determined. There has been much research on genetic differences in response to the bitter chemicals phenylthiocarbamate (PTC) and 6-n-propylthiouracil (PROP). Some people can taste bitter taste (“tasters”) and some cannot (“non-tasters”). Tasters are less likely to become a smoker, suggesting that bitter taste makes smoking more aversive (Enoch et al. 2001; Cannon et al. 2005; Snedecor et al. 2006). The family of bitter receptors, TAS2R (taste receptor type 2) contribute substantially to the ability to taste bitter. One of the genes, TAS2R38, accounts for 85% of individual variability in response to bitter (Wooding 2004). The two most common genetic variants (haplotypes) of TAS2R38 are PAV and AVI. PAV homozygotes are most sensitive and AVI homozygotes are least sensitive to PTC/PROP. Among people of European descent, smokers with the AVI genotype rate higher taste/sensory and cue exposure-related motivations for smoking compared to smokers with the PAV genotype (Cannon et al. 2005). Thus the ability to perceive bitter taste seems to decrease taste-related motivations for smoking. This study found however that an intermediate taste sensitivity genotype, AAV, was protective against smoking, which seems inconsistent with earlier studies based on the taste sensitivity phenotype. Among African Americans the taster PAV genotype was inversely associated with smoking quantity, whereas the non taster AVI genotype was positively associated with smoking quantity (Mangold et al. 2008). Furthermore, in women, the non-taster genotype was associated with the level of nicotine dependence. Neither the Cannon nor the Mangold study examined interactions between genotype and menthol cigarette smoking. However, since menthol reduces bitterness for some cigarettes, and since reduced bitterness is associated with smoking

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more, the genetic data support the idea that menthol may affect smoking behavior and associated dependence. These studies also raise the possibility that menthol might interact with genetically determined taste sensitivity to facilitate smoking. That is, menthol could mask bitterness to allow smokers who are genetically more sensitive to bitterness to better tolerate tobacco smoke and therefore to become a smoker.

## **Respiratory effects**

Menthol is used medicinally in decongestant products. Menthol produces a sensation of increased nasal patency, although nasal congestion is unaffected (Eccles 1990; Nishino et al. 1997; Kenia et al. 2008). Menthol inhibits ventilation (Harris 2006) and increases breath-hold time in humans (Sloan 1993). Menthol also acts as a cough suppressant (Laude et al. 1994; Morice et al. 1994). The respiratory effects of menthol—a sensation of cooling, increased breath-hold time and cough suppression—could promote deeper inhalation and/or longer retention of smoke in the lungs while smoking menthol cigarettes. In animal studies, menthol promotes bronchodilation (Wright et al. 1997) and the clearance of mucous from the lungs (Nishino 1997).

## **Other effects**

Orally dosed menthol can cause vasodilation and relaxation of intestinal smooth muscle (Hawthorne et al. 1988). These effects, which are believed to be related to inhibition of calcium currents in smooth muscle (Hawthorne et al. 1988; Taylor et al. 1984), may explain the medical utility of menthol as a treatment for gastrointestinal disturbances. The relevance to the pharmacology of inhaled menthol is unclear. Oral menthol also has been found to increase heart rate, possibly a reflex response to menthol-induced vasodilation (Gelal et al. 1999). However, studies comparing menthol and non-menthol cigarettes have not found any cardiovascular effects of menthol (Pritchard et al. 1999; Pickworth et al. 2002). Studies of electroencephalographic responses to smoking found that response correlated with perceived impact and liking, which may be determined in part by menthol (Gullotta et al. 1989a, 1990, cited in Wayne, 2004). However menthol added to cigarettes had no direct effect on the electroencephalogram (Pritchard et al. 1999).

## **EVIDENCE SYNTHESIS**

Chapter 3 set out to answer four questions relating to the physiological effects of menthol pursuant to TPSAC's charge. The responses to those questions are given below. TPSAC considered this information, along with other evidence gathered, reviewed and synthesized in this report, to assess the overall public health impact of menthol cigarettes and to make its recommendations to the FDA.

### **Does menthol have cooling or anesthetic properties that moderate the harshness of cigarette smoke?**

The evidence is sufficient to conclude that menthol has cooling and anesthetic effects that reduce the harshness of cigarette smoke. Research indicates that menthol acts on both thermal and nociceptive receptors. This dual action results in both cooling and counter-irritant effects. Menthol desensitizes receptors by which nicotine produces irritant effects, thereby, reducing the irritation from nicotine in tobacco smoke.

The implications of these findings are that by reducing the harshness of tobacco smoke menthol could facilitate initiation or early persistence of smoking by youth. Also, by reducing the harshness of smoke, menthol could facilitate deeper and more prolonged inhalation of tobacco smoke, resulting in greater smoke intake per cigarette.

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## **Does menthol make low-tar, low-nicotine cigarettes more acceptable to smokers?**

The evidence is sufficient to conclude that menthol makes low-tar, low-nicotine cigarettes more acceptable to smokers. Like nicotine, menthol has irritant effects that contribute to the impact or “throat grab,” of tobacco smoke. In light or ultralight cigarettes with lower nicotine delivery, menthol can be used to provide impact.

The implications of these findings are that menthol is likely to make low-yield cigarettes more satisfying, and smokers who switch to low-yield cigarettes for health concerns may be more likely to continue to smoke rather than quit.

## **Does menthol have an effect on the metabolism of nicotine or tobacco-specific nitrosamines?**

The evidence is sufficient to conclude that it is at least as likely as not that menthol inhibits the metabolism of nicotine in smokers. The evidence is not sufficient to conclude that it is at least as likely as not that menthol inhibits the glucuronidation of NNAL in smokers. Studies using liver microsomes demonstrate that menthol can inhibit the metabolism of nicotine. One experimental within-subject human study, using a state-of-art method of measuring the rate of nicotine metabolism, indicates that menthol cigarette smoking inhibits the metabolism of nicotine by about 10 percent. Menthol could be affecting nicotine metabolism in the lungs, where some nicotine metabolism is known to occur and where menthol concentrations are likely quite high in menthol cigarette smokers. Several cross-sectional studies show menthol has no effect on the nicotine metabolite ratio, a biomarker of the rate of nicotine oxidation. However cross-sectional studies may not have adequate power to detect a 10 percent difference in the metabolite ratio. Given the small magnitude of the menthol effect on nicotine metabolism in the positive human experimental study, it is unlikely that such a metabolic difference would have much, if any, effect on smoking behavior.

Menthol in high concentrations has been shown to inhibit the metabolism of the tobacco-specific nitrosamine, NNAL, in isolated liver preparations. One cross-sectional study found lower ratios of NNAL glucuronide to NNAL in menthol cigarette smokers, but another larger study did not find such an effect. On balance the evidence to date is not sufficient to demonstrate a significant effect. However if menthol does inhibit NNAL metabolism, this could be a basis for higher cancer risk in menthol cigarette smokers.

Menthol is known to enhance the dermal penetration of a variety of drugs, and might in theory enhance the pulmonary absorption of nicotine and/or tobacco carcinogens. The data on menthol and exposure to tobacco toxins is reviewed in chapter 6.

## **Is it biologically plausible that menthol enhances the addictiveness of cigarette smoking?**

The evidence is sufficient to conclude that it is biologically plausible that menthol makes cigarette smoking more addictive. The evidence reviewed suggests several mechanisms by which menthol could contribute to the initiation and persistence of cigarette smoking.

- Nicotine is required for the acquisition and maintenance of addiction to cigarette smoking. But as described previously, menthol can modulate nicotine effects and may act directly on nicotinic cholinergic receptors to alter nicotine response.
- While nicotine is required for nicotine addiction, the addictiveness of cigarettes is also influenced by sensory factors (Rose 2006; Henningfield et al. 2011 in press). Menthol provides an unmistakable

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- Sensory experiences can contribute to conditioned aspects of smoking behavior. Once drug self-administration has been established, taste and other sensory factors can function as stimuli that can substantially enhance the strength and persistence of drug self-administration (Carroll and Meisch 2011; Panlilio et al. 2005).
- Stimuli associated with drug intake and/or withdrawal can come to evoke craving that promotes resumption of self-administration of the drug after a period of abstinence. Thus, menthol from food or toothpaste could serve as a sensory cue to prompt relapse to smoking. These mechanisms have been demonstrated in a variety of animal and human studies with a variety of addictive drugs (Wilson et al. 2004; Sayette and Griffin 2010).
- Another potentially relevant issue is the relationship between menthol and genetic differences in perception of taste. As noted above, various studies raise the possibility that menthol might interact with genetically determined taste sensitivity to facilitate smoking. Thus, there may be a genetically susceptible population for whom menthol cigarettes facilitate smoking.