Food and Chemical Toxicology 48 (2010) S1–S38

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Review

A review and assessment of menthol employed as a cigarette flavoring ingredient

J. Daniel Heck *

Scientific Affairs, A.W. Spears Research Center, Lorillard Tobacco Company, P.O. Box 21688, Greensboro, NC 27420-1688, USA

ARTICLE INFO

Article history:
Received 26 June 2009
Accepted 1 November 2009

Keywords:
Menthol
Cigarette
Tobacco
Smoking
Epidemiology

ABSTRACT

Cigarette smoking is established as a substantial contributor to risks for cancer, cardiovascular and respiratory diseases. Less is known about the potential of cigarette composition to affect smoking risks. The use of cigarette flavoring ingredients such as menthol is currently of worldwide public health and regulatory interest. The unique conditions of menthol inhalation exposure that occur coincident with that of the complex cigarette smoke aerosol require specialized studies to support an assessment of its safety in cigarette flavoring applications. The present state of knowledge is sufficient to support an assessment of the safety of the use of menthol in cigarettes. Scientific, smoking behavioral and epidemiological data available through mid-2009 is critically reviewed and a broad convergence of findings supports a judgment that menthol employed as a cigarette tobacco flavoring ingredient does not meaningfully affect the inherent toxicity of cigarette smoke or the human risks that attend smoking. There remains a need for well-designed studies of the potential of menthol to affect smoking initiation, cessation and addiction in order to differentiate any independent effects of menthol in cigarettes from those imposed by socioeconomic, environmental and peer influences on these complex human behaviors.

Contents

* Tel.: +1 336 335 6662; fax: +1 336 335 6640.
E-mail address: dheck@lortobco.com

0278-6915/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2009.11.002

1. Introduction

A variety of flavoring substances are employed in the manufacture of contemporary American-style blended cigarettes to provide distinctive, brand-specific flavor signatures to the mainstream smoke that is inhaled by the smoker. The most familiar and most intensely-studied of these flavoring ingredients is menthol, which has been the subject of scores of chemistry, toxicology and epidemiology investigations relating to its use in cigarettes. A conference organized by the National Cancer Institute and the Centers for Disease Control in 2003 identified a number of research needs and priorities to address remaining uncertainties in regard to the questions of whether the use of menthol as a cigarette flavoring ingredient may increase the inherent disease risks of smoking or introduce novel risks to the smoker (Clark et al., 2004; Ahijevych and Garrett, 2004). A considerable number of new studies has appeared in the peer-reviewed scientific literature since the time of that conference, and the present review presents a synthesis of published and heretofore unpublished information available through mid-2009 on menthol research topics identified in the NCI/NIH conference proceedings summarized by Clark et al. (2004).

An assessment of the potential of menthol used as a cigarette flavoring to affect the risks of diseases caused by smoking is founded upon an understanding of the intrinsic toxicity of menthol itself, as well as any effects this ingredient may have on the chemistry of the complex smoke aerosol. Such an assessment must certainly include the comparative appraisal of the toxicity of menthol and non-menthol cigarette smoke in experimental toxicology tests, and beyond this an understanding of the potential of menthol to affect population risks developed from epidemiological studies of cigarette smokers. These topics are considered in the present review.

The substantially greater preference for menthol cigarettes that has been reported in recent decades among African-American smokers relative to American smokers of European ancestry has given rise to speculation that menthol may in some way account for the elevated incidence and severity of some smoking-related diseases among African-Americans (Richardson, 1997; Ahijevych and Garrett, 2004). Approximately 75% of the estimated 5.5 million African-American adult smokers and 23% of the estimated 37 million white adult smokers in the United States prefer menthol brands (Adams and Schoenborn, 2006; CDC, 2008). Since twice as many European-Americans smoke menthol cigarette brands as do African-Americans, consideration of any independent effects of menthol on the adverse health consequences of smoking is important across all demographic groups. The interplay of demographic and socioeconomic factors and smoking behaviors, including a preference for mentholated cigarette brands, constitutes a daunting challenge to field investigators seeking to study menthol and its potential effects outside of the controlled experimental laboratory environment (USDHHS, 1998).

Speculation unsupported by original data has been offered that menthol may elevate smoking-related disease risks through effects on breathing patterns (Garten and Falkner, 2004), by masking early indications of respiratory disease through its antitussive effects (Garten and Falkner, 2003) or through sensory effects mediated by the same thermal receptors that produce the cooling sensation evoked by other mentholated consumer products (Rabinoff et al., 2007). Such effects are hypothesized to result in deeper smoke inhalation and higher exposures to cigarette smoke constituents consequent to a damping of sensory cues by added menthol. Available scientific evidence relating to the notion that mentholated cigarettes may be smoked differently that non-mentholated brands is reviewed and discussed in a subsequent section reviewing smoking topography studies. An additional data-based perspective is provided by reviewed comparative studies of biomarkers of cigarette smoke exposure in smokers of menthol and non-menthol cigarettes.

Hypotheses that menthol may also impact societal disease burdens by influencing the rates of smoking initiation and cessation also require consideration. Although some of these hypotheses are not readily amenable to direct testing with controlled experimental approaches, the available observational studies in these areas are considered and discussed in this review.

The volume of scientific literature that is available for menthol and its use as a cigarette flavoring is considerable, far exceeding that of any other commonly used tobacco ingredient. Although some research needs remain, the present state of knowledge in regard to menthol in cigarettes is sufficient to support an assessment of the safety of that usage based upon the information summarized in this review.

This report was developed under the aegis of a Tobacco Ingredients Expert Panel (Table 1) which has reviewed the underlying science and accepted its conclusions. This Expert Panel was originally convened in 1986 to provide expert review, critique and opinion on biomedical and toxicology topics related to the use of ingredients, other than tobacco, that are employed in the manufacture of

Table 1
Expert review panel.

Richard A. Davis, Ph.D.	ToxSolutions
John Doull, Ph.D., M.D.	University of Kansas Medical Center
Donald Gardner, Ph.D.	Inhalation Toxicology Associates
William T. George, Ph.D.	Tulane University School of Medicine
Roger A. Renne, D.V.M.	Toxpath Consulting
Steven L. Taylor, Ph.D.	University of Nebraska Department of Food Science
Gary M. Williams, M.D.	New York Medical College

cigarettes in the USA. The typical "American blended" style cigarette contains added flavorings, humectants, and other added functional ingredients that serve to facilitate manufacturing and maintain the stability of the finished product and its components. Importers or producers of cigarettes marketed in the USA have been required since 1985 to report these ingredients annually to the US Centers for Disease Control's Office on Smoking and Health under section 7(a) of the Federal Cigarette Labeling and Advertising Act. Additional requirements for cigarette ingredients reporting are under development at this writing as one element of a new regulatory authority over tobacco products that was granted to the US Food and Drug Administration in mid-2009.

The Expert Panel has provided an independent review of available published and unpublished scientific information that relates to the safety of added cigarette ingredients, including a review of tobacco and smoke chemical analyses as well as *in vitro* and *in vivo* toxicology assessments that have been conducted by the major tobacco companies that participate in the US market. The Panel comprises experts in the fields of general toxicology, respiratory toxicology and pathology, carcinogenesis, and immunology.

The composition of the Expert Panel has changed over the years since its inception. Emeritus members include T.A. Loomis, Ph.D., M.D., J.P. Frawley, Ph.D., R.A. Squire, D.V.M., Ph.D., and R.A. Ford, Ph.D. The Panel has provided ongoing review, detailed comments and critique to the author throughout the development of the present assessment of menthol employed as a cigarette flavoring ingredient.

1.1. General information

Menthol is a monocyclic terpene alcohol having three asymmetric carbon atoms in the cyclohexane ring, yielding a variety of isomers (Fig. 1). The *l*-menthol isomer exhibits the characteristic balanced peppermint odor and flavor and exerts a cooling effect when applied to the skin (Eccles, 1994). The other menthol isomers exhibit significantly different taste characteristics and are lacking in the familiar cooling sensation imparted by *l*-menthol (Clark, 2007). While *l*-menthol constitutes the predominant isomer in natural botanical sources, the racemic mixture *dl*-menthol is produced synthetically and is similarly employed to impart the characteristic cooling menthol note to various consumer product formulations. The *dl* racemate exhibits about half of the cooling properties of *l*-menthol, and finds use mainly in topical skin care products (Derfer and Derfer, 1983). Both *l*-menthol and *dl*-menthol are used in tobacco products.

1.2. Characteristics

Natural plant sources of commercial menthol include several members of the mint family *Labiatae* (*Lamiaceae*), most prominently members of the *Mentha* genus such as peppermint (*Mentha piperita*), cornmint (*Mentha arvensis*) and spearmint (*Mentha spicata* L. or *Mentha viridis* L.). Major producers have historically included the United States, Japan, Taiwan, and Brazil (Leung and Foster, 1996). China became a major menthol producer in more recent years. However, India has very recently emerged as the predominant world source of menthol following the abandonment of the cultivation and processing of menthol-producing mint plants in other growing regions (Clark, 2007).

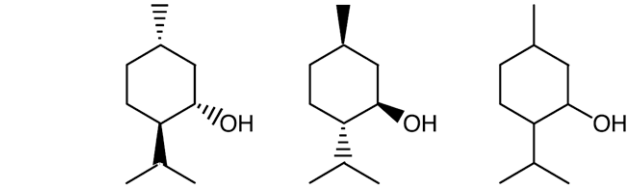

Fig. 1. Structure of menthol.

Menthol is widely used in foods, topical therapeutic preparations, oral hygiene and dentifrice formulations, and tobacco products by virtue of the pleasant minty flavor and a cooling sensation it imparts upon contact with the skin or oral membranes. Recent work has established that menthol's cooling properties are manifested through the same cell membrane receptors (TRPM8) that serve as sensors for thermal coldness through activation of a cation channel function (reviewed by Patel et al. (2007)). Since this characteristic cooling sensation is produced by interaction with thermal receptors rather than the taste buds, it is prominently expressed in tissues other than those of the oral cavity. This sensation has long been known to be manifested upon skin application, and as a result menthol is a familiar component of shaving creams, antipuritic salves and other topically-applied consumer product preparations. Menthol produces the sensation of coolness in the oral and olfactory regions only at low concentrations, as higher concentrations induce a burning sensation coincident with some modest degree of desensitization (Eccles, 1994; Green and McAuliffe, 2000; Patel et al., 2007).

There is evidence that stimulation of respiratory tract cold receptors is accompanied by a slight, transient decrease in respiration (Eccles, 1994; Nishino et al., 1997). While the breathing of menthol vapor results in a marked increase in the sensation of increased airflow due to its agonist action at respiratory tract cold receptors, several human clinical studies have shown no actual increase in respiratory flow or a measurable decrease in respiratory airflow due to stimulated mucus production by menthol (Eccles, 1994). Menthol provides some degree of symptomatic relief of upper respiratory congestion by stimulation of cold receptors, achieving a modest therapeutic effect analogous to that by which cold air reduces the sensation of breathlessness associated with loaded breathing in normal subjects (Schwartzstein et al., 1987; Nishino et al., 1997).

Selected chemical information and physical properties

CAS#	Common name
89-78-1	Menthol
2216-51-5	*l*-Menthol
15356-60-2	*d*-Menthol
15356-70-4	*dl*-Menthol
FEMA # 2665	

$C_{10}H_{20}O$.
FW = 156.
Vapor pressure, *l*-menthol: 0.8 mm Hg @ 20 °C.
Boiling point, *l*-menthol: 212 °C (FCC, 1996).
Melting point, *l*-menthol: 43 °C (FCC, 1996).
Solubility: Soluble in ethyl alcohol, several nonpolar solvents, glacial acetic acid, essential oils, esters. Slightly soluble in water [0.04% @ 20 °C] (Clark, 2007).
Reactivity: Menthol is subject to all of the chemical reactions typical of a cyclic secondary alcohol, including dehydrogenation or oxidation to menthone [10458-14-7] and isomenthone [491-07-6] and esterification to menthyl acetate [16409-45-3] and other esters (Derfer and Derfer, 1983).

1.3. Regulatory status

Menthol is approved by the US FDA for use in familiar over-the-counter lozenges, topical preparations and vapor inhalation

products by virtue of its antipuritic and antitussive properties (21CFR 341(2)(b); 21CFR 310.545). Menthol is employed as a food flavoring in the USA and elsewhere and has been declared to be Generally Recognized as Safe (GRAS) for food usage by the Flavoring and Extract Manufacturers Association (FEMA) (Hall and Oser, 1965; Adams et al., 1996). Menthol is similarly listed among essential oils, oleoresins, and natural extractives regarded as GRAS by FDA (21 CFR 182.20) and is approved for use as a synthetic flavoring substance and adjuvant (21CFR 172.515) in foods with no limitation on usage except good manufacturing practices and no food category restrictions other than those specified in food standards of identity. It is acknowledged that such safety assessments and regulatory approvals of the use of menthol in foods and other consumer products were not intended to address its use in tobacco products and cannot be used solely as a basis for judgments of menthol's safety when used as a flavoring in smoking tobacco products.

Similar approvals for food uses by other authoritative bodies have been made (Council of Europe: CE No. 63, Category A – Approved; IOFI: Nature Identical). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has specified an acceptable daily dietary intake (ADI) of 0–4 mg/kg bw/day for *dl*-menthol (JECFA, 1998) on the basis of an available chronic feeding study that demonstrated a no observed effect level (NOEL) of >375 mg/kg bw/day (NCI, 1979). A comparison of this experimental NOEL to an estimated maximum US/European per capita daily intake of 3.05×10^{-1} mg/kg/day indicates a 1229-fold margin of safety for oral intake of menthol (Munro and Kennepohl, 2001). While the scientific basis for the wide approval of menthol for oral intake cannot be unconditionally extended to tobacco usage, consideration of any additional intake of menthol from smoking sources suggests that a generous margin of safety exists for potential systemic toxicity consequent to all-source menthol exposure.

Menthol is at the time of this writing explicitly or categorically approved for use, or is listed as used as a flavoring ingredient in tobacco products around the world. Countries having regulatory approvals or oversight of cigarette ingredients have approved, permitted, or acknowledged menthol's use as a cigarette flavoring ingredient. Legislation granting the US Food and Drug Administration new regulatory authority over tobacco products in the USA was signed into law in June of 2009. The text of the legislation specifies menthol as the sole characterizing cigarette flavoring ingredient (other than tobacco) that is permitted under the US FDA regulations, and that use will be subject to further review and rulemaking as the implementation of the new FDA authority proceeds.

2. Tobacco uses, chemistry, pyrolysis and smoke transfer

2.1. Tobacco uses

Menthol was first used as a cigarette flavoring ingredient in the late 1920s (Reid, 1993). The application of menthol to cigarette and pipe tobaccos constitutes a major end use of the natural and synthetic articles of North American commerce, accounting for approximately 25% of annual usage volume. About 28% of the annual menthol production is employed in oral hygiene products, and about 27% of the total volume finds use in pharmaceuticals (Clark, 2007). The physical characteristics of menthol enable its addition to cigarette packaging materials and filters in addition to direct application to tobacco as a means to impart the distinctive flavor note to the mainstream smoke of commercial cigarettes (Borschke, 1993).

The pleasant taste and cooling sensation imparted by menthol is manifested in cigarette smoke at concentrations lower than those employed in some other types of mentholated consumer products. A slight menthol effect is apparent at tobacco addition rates of 0.1–0.2%, and a stronger flavor note is achieved at 0.25–0.45% (2500–4500 ppm) (Technical Resources Inc., 1993; Hopp, 1993). While earlier reviews of menthol usage in cigarettes stated that addition rates did not typically exceed 0.3%, several major US cigarette manufacturers have recently released information indicating that some cigarette tobaccos may contain on the order of 2% w/w menthol. It might be noted that reported levels of menthol application in cigarette manufacturing may be in excess of the target levels found in the finished products' tobacco filler due to unavoidable losses by vaporization during manufacturing, packing and storage; as well as equilibration of menthol among tobacco, filter, paper and packaging materials.

Celebucki et al. (2005) performed determinations of menthol on a per cigarette and per milligram tobacco basis for 48 brands of mentholated cigarettes sampled from the US market. The authors' analytical measurements properly included extraction and analysis of the entire cigarette in order to account for any menthol that may have migrated from the tobacco filler to the filter or paper following the products' manufacture. A mean value of 2.64 (±0.71, S.D.) mg menthol/cigarette and 3.89 (±1.89, S.D.) mg menthol/g tobacco filler was reported for all 48 mentholated brands surveyed. Tobacco blend filler weights ranged between 0.58 and 0.80 g tobacco/cigarette among the brands tested.

The authors' abstract reported menthol content (both menthol content per cigarette and per gram of tobacco filler) to be significantly greater in "ultralight" and "light" cigarettes than in "regular/full-flavor" brands, "*...belying the common consumer perception that "light" means less*". No substantiation for the assertion that consumers interpret product descriptors as being relevant to menthol content is provided in the report. Celebucki et al. (2005) further speculate that their reported general trend toward higher menthol in brands having lower FTC smoke yields is "*...consistent with prior research that suggests menthol may be used to offset reductions in smoke delivery or impact and to facilitate compensatory smoke inhalation behaviors in smokers of cigarettes with reduced machine-measured smoke delivery*". These conclusions appear contradictory, as the offsetting of reductions in smoke delivery or impact and the facilitation of compensatory smoke inhalation by menthol would suggest the simultaneous increasing and decreasing of local sensory effects in the upper-middle respiratory tract.

An alternative rationale for the relatively higher application rates of menthol in lower-yielding commercial cigarette brands was provided in the report of Best (1993), which documented a measurably lower transfer efficiency of menthol in cigarettes having higher filter efficiencies and substantial levels of filter tip ventilation. Such low-yield cigarette designs were reported to deliver menthol into the mainstream smoke much less efficiently – on the order of 10% – than did earlier cigarettes made without filters or tip ventilation (Best, 1993). Continuing work in the field has further documented the reality that menthol and other flavoring constituents of cigarette tobacco blends are reduced in the delivered smoke by cigarette filtration and ventilation to varying degrees relative to common reference analytes for mainstream smoke yield such as "tar", nicotine and carbon monoxide (Jing et al., 2005). Specific flavoring constituents may be more or less reduced by ventilation than is "tar", for example, as a function of their boiling point, vapor pressure, molecular weight, water solubility or other incompletely characterized factors. It is therefore not unexpected that certain flavoring ingredients may be incorporated at different levels in cigarettes of varying design to achieve a desired sensory note in a smoke aerosol produced by different combinations of filter efficiency, air dilution, paper porosity and other parameters. Although further research is indicated to fully characterize the effects of contemporary low-yield cigarette construction and design variables on the delivery of menthol relative to that of other smoke

constituents, the relatively higher levels of menthol in unburned "light" cigarettes reported by Celebucki et al. (2005) does not necessarily reflect a concomitant increased delivery of menthol into the smoke or to the smoker.

Kreslake et al. (2008) reported an analysis of internal tobacco industry documents that had been produced in litigation, supplemented by limited laboratory analyses of the menthol content of the tobacco blend and the mainstream smoke of several major cigarette brands sampled from store shelves in the Boston area. The authors interpreted their findings as evidence that major mentholated cigarette manufacturers had strategically lowered the menthol levels in popular brands as a strategy to encourage smoking initiation among adolescent smokers. However, neither the internal documents cited nor the single-point menthol analyses reported provided a compelling scientific basis for the authors' assertion that cigarette menthol levels had been strategically lowered in diverse brands of cigarettes produced by several US manufacturers.

At least one of the major US cigarette manufacturers, the Lorillard Tobacco Company, issued a public denial of the assertion by Kreslake et al. (2008) that it had lowered the menthol level in the current leading US mentholated cigarette brand, Newport, by 16% during the time interval discussed in the paper (2000–2007). This manufacturer cited tens of thousands of data points from validated production quality assurance analyses performed over this same time interval, and hundreds of additional menthol analyses from Newport samples collected from retail store shelves in the Boston area, showing that no change in the menthol level for this cigarette brand had been made over the previous decade. The manufacturer argued that this overwhelming body of data from fully validated sampling, analytical and statistical protocols provided clear evidence that the published speculation that the menthol level in Newport had been strategically lowered to attract young smokers, or indeed for any reason whatsoever, was without merit.

2.2. Chemistry, pyrolysis and smoke transfer

Several major reviews of published work and reports of major research programs conducted by tobacco companies have collated and summarized available information on the effects of added cigarette ingredients on the chemistry and biological effects of cigarette smoke (Paschke et al., 2002; Rodgman, 2002; Baker et al., 2004). Menthol has been the subject of a number of these research efforts, and it is perhaps the most thoroughly-studied flavoring ingredient among those in use at the present time.

Menthol's volatility and boiling point of 212 °C support an expectation that ready vaporization of intact menthol into the smoke stream will predominate over pyrolytic destruction of the molecule. That this is the case was seen in early analytical smoking studies in which radiolabeled menthol was found to transfer intact with high efficiency into the mainstream smoke particulate matter by distillation (Bass et al., 1975). The majority of the remainder of applied menthol was found in the side stream smoke or in the cigarette butt. However, the mainstream smoke transfer efficiency of contemporary cigarettes is generally on the order of about 10% of added menthol due to the implementation of changes in filters, ventilation, and construction to reduce "tar" deliveries (Best, 1993; Cook et al., 1999). Higher or lower delivery efficiencies have been reported in other studies, as a function both of differences in laboratory smoking and analytical chemistry methods as well as the composition and construction variables characteristic of different experimental and commercial cigarettes (Gaworski et al., 1997; Jing et al., 2005).

Benowitz et al. (2004) reported an estimate of daily menthol exposure from the smoking of mentholated cigarettes based upon renal clearance of urinary menthol, and collection of excreted metabolites. The authors estimated an average daily systemic exposure to 12.5 mg (80 μmol) menthol per day for a smoker of 20 cigarettes/day. These authors' analysis of commercial mentholated cigarettes found them to contain approximately 3 mg of menthol, a value in good agreement with that reported by Celebucki et al. (2005) and other authors. The daily total exposure was based upon an assumption that the efficiency of menthol absorption upon inhalation is similar to that following oral consumption. This exposure estimate amounts to 0.625 mg menthol (4 μmol) per cigarette, or about 20% of that initially present in the cigarette (Benowitz et al., 2004).

2.2.1. Evaluation of the effect of menthol addition on smoke chemistry

It is noted that late in 2008 the US Federal Trade Commission (FTC) rescinded its prior guidance in regard to the term of reference employed to describe a standard analytical smoking method that has previously been employed in the USA for many years to generate comparative mainstream smoke yield information for different cigarettes. This method, formerly referred to as the "FTC method", is now more properly referred to simply as the "Cambridge filter method". The use of the term "FTC method" herein reflects the historical common usage that term of reference in the discussed scientific literature. This terminology should not be taken as an endorsement of the Cambridge filter method or any other machine smoking method by the FTC.

An exceptionally comprehensive evaluation of the effects of the addition of various flavoring materials on the chemical composition of the particulate and vapor phases of cigarette smoke has been reported (Carmines, 2002; Rustenmeier et al., 2002). Experimental cigarettes containing flavoring ingredients at high levels of addition were prepared from a typical "American-style" blend and smoked under conditions specified by the International Organization for Standardization (ISO) Standard 3308. Reference cigarettes of an identical ventilated filter construction were prepared without any added ingredients and were smoked under identical conditions. Subsequent chemical analyses of 59 smoke constituents that are generally regarded to include those of greatest toxicological significance were performed to assess the potential of added flavoring ingredients to affect their mainstream smoke deliveries. These analytes included those compounds that have been classified as animal or human carcinogens by the International Agency for Research on Cancer (IARC).

One of the experimental cigarettes, identified as "Ingredient Group 3", contained 18,000 ppm (1.8%) *l*-menthol in addition to a simple flavoring/casing mixture comprising corn syrup, licorice extract, and cocoa shells. The authors reported that it was physically impossible to reliably add menthol to the experimental cigarettes at levels higher than 18,000 ppm tobacco due to crystallization and consequent unacceptable variability in achieving target application levels at higher rates of addition. The low and high level ingredient applications for the Ingredient Group 3 cigarettes therefore contained low and high level applications of the other tested ingredients and a fixed level of 18,000 ppm menthol.

Significant increases of 23% and 16% were reported for the smoke yields of total particulate material (TPM; "tar", water and nicotine) at the low and high levels of ingredient application, respectively. Such increases are familiar to workers in this field and are attributed to the relatively efficient smoke transfer of many commonly-used, semi-volatile cigarette flavoring ingredients into the mainstream smoke, as opposed to their pyrolytic destruction into gaseous products that do not measurably contribute to the measurable mass of the particulate phase of the smoke aerosol. The smoke yields of individual analytes are therefore most meaningfully considered relative to TPM for each experimental cigarette. Increases in TPM-relative (mg/mg TPM) formaldehyde (low level: 23%; high level: 45%), resorcinol (low level: 23%; high level: 45%) and lead (high level: 13%) were reported for the two levels of Group

3 ingredients inclusion. Most of the other smoke constituents, including benzo[*a*]pyrene, 1,3-butadiene, benzene, and numerous phenols, were reduced by 10–20% in the ingredient-containing test cigarette compared to the reference cigarette prepared without added flavoring ingredients. The test cigarette containing ingredients exhibited substantial reductions in naphthalene (low level: 31%; high level 36%) and *N*-nitrosamines (26 and 37%, respectively). In summary, the extensive smoke chemistry analyses of Rustenmeier et al. (2002) provided evidence that experimental cigarettes prepared with 18,000 ppm added *l*-menthol and two levels of commonly-added casing ingredients did not exhibit substantive changes in the TPM-relative quantities of many of the most biologically significant constituents of cigarette smoke.

Baker et al. (2004) reported findings from an extensive evaluation of the effects of added flavoring and functional ingredients on the smoke chemistry of experimental cigarettes designed and made to be representative of typical American-style blended tobacco cigarettes. The smoke constituents analyzed included the "Hoffmann list", an inventory of compounds that are believed to be the most toxicologically significant among the tens of thousands of cigarette smoke constituents that have been identified to date (Hoffmann and Hoffmann, 1998; Rodgman and Perfetti, 2009). The experimental menthol cigarette contained 2.34% added *l*-menthol in addition to a representative product formulation that included humectants, casings and processing aids. The tested ingredient levels were stated to be at or above the levels actually used in commercial cigarette production. Notable findings for the experimental menthol cigarette, identified as cigarette B4 in the authors' extensive reporting of smoke chemistry, included a consistent and significant reduction in ammonia, NO_3^-, nicotine, minor alkaloids, total tobacco-specific nitrosamines, phenol, cresols, and benzo[*a*]pyrene in mainstream smoke generated under standard ISO smoking conditions. The experimental menthol cigarette also exhibited a modest but statistically significant increase in formaldehyde yield, which the authors attributed to the sugar components of the added casing mixture. Subsequent work confirmed that this was the case (Baker, 2006). Changes to other smoke constituents were described as modest and within the ranges of measurement variability (Baker et al., 2004).

Another investigation of the effect of added menthol on mainstream smoke chemistry has been performed by investigators at the R. J. Reynolds Tobacco Company. These data, heretofore unpublished, are presented in Appendix A (RJRT, 2000). Filtered cigarettes of matching construction and blend, differing only in the addition of 1.03% menthol to the test cigarette, were smoked under the standard conditions of the Cambridge filter method and chemical analyses were performed on collected smoke particulate material and gas/vapor phases. Mainstream smoke chemistry profiles were broadly similar ($p > 0.05$) between the test and reference cigarettes for most analytes. Confirming the findings of Rustenmeier et al. (2002), a moderate but statistically significant elevation in mainstream smoke formaldehyde was reported for the menthol test cigarette (4.2 ± 0.59 μg/cigarette *vs.* 3.4 ± 0.27 μg/cigarette for the non-menthol reference cigarette, $p < 0.05$). A statistically significant elevation in smoke vapor phase 2-furfural was also reported for the menthol test cigarette. The values for these and all other analytes were well within the ranges of published values for cigarettes on the US market at the time of the study (Chepiga et al., 2000), and concurrent toxicology evaluations showed no indication that these differences in smoke chemistry had any toxicological significance (Appendix B; RJRT, 2000).

2.2.2. Laboratory bench pyrolysis studies of the fate of menthol in a burning cigarette

Laboratory bench furnace pyrolysis studies intended to model the fate of added tobacco ingredients in a burning cigarette are best pursued with caution and reservation, since the complexities of the thermal, chemical and physical interactions that characterize the conditions within a burning cigarette are now known to have been only poorly approximated in some early work in this field. Modifications to older laboratory pyrolysis methods have recently been instituted in an attempt to more meaningfully approximate the events that may occur in a burning cigarette (Baker and Bishop, 2004).

Although a crude pyrolysis study of neat *dl*-menthol reported by Schmeltz and Schlotzhauer (1968) has since been followed by more meaningful studies of the fate of menthol in actual burning cigarettes, this early work is worthy of mention since it is still frequently cited in support of statements that menthol may produce benzo[*a*]pyrene (B[*a*]P) when used as a cigarette flavoring ingredient. These investigators pyrolyzed a sample of menthol in a quartz tube under an inert stream of dry nitrogen at fixed temperatures of 600 and 860 °C, and collected evolved pyrolysis products for identification by methods of the day, including paper and thin layer chromatography. At 860 °C, only 16% of the menthol was recovered intact; and among its pyrolysis products was B[*a*]P, formed at a rate of about 400 μg/g menthol. Other pyrolysis products included phenol, benzene, toluene, and vinyl methylcyclohexane. At the lower, fixed pyrolysis temperature of 600 °C, 78% of the menthol was recovered intact and no B[*a*]P was formed. Extrapolation of this trend downward to the range of the boiling point of menthol (212 °C) indicates that essentially all menthol applied to cigarette tobacco might be expected to volatilize intact in the temperature gradient of the heated zone ahead of the cigarette's advancing burning cone. That this is indeed the case was shown clearly in subsequent studies of the behavior of menthol under conditions of actual cigarette combustion.

An investigation of the fate of menthol in burning cigarettes was reported by Newell et al. (1968), who added 0.38 mg randomly labeled ^{14}C-menthol to the first 45 mm of five filtered cigarettes in each of two reported experiments. Fully 96.4% of the radioactivity recovered from mainstream smoke particulate material was found to represent intact menthol, as did 91.7% of recovered sidestream smoke activity. Approximately 70% of added menthol was recovered from these smoke solids, with a substantial portion of remaining menthol trapped in the butts and filters. This study demonstrated clearly that, in contrast to the extensive pyrolytic degradation of menthol seen under the extreme conditions of Schmeltz and Schlotzhauer (1968) work, very little pyrolytic destruction of menthol occurs in the environment of a burning cigarette.

The subsequent work of Jenkins et al. (1970) confirmed Newell's observation that under conditions of cigarette smoking the vast majority of menthol is in fact vaporized intact rather than pyrolyzed. A 3 mg quantity of menthol and uniformly labeled ^{14}C-menthol was applied throughout unfiltered 70 mm cigarettes, which were smoked to a 20 mm butt length in a material balance study. An efficient volatilization of intact menthol in the heated zone just proximal to the burning zone of the cigarette was evident. The mainstream smoke was found to contain 28.9% of the total recovered activity, while 44.3% and 26.9% were recovered in the sidestream smoke and butt, respectively. Unchanged menthol accounted for fully 98.9% of the mainstream smoke activity, with apparent menthol pyrolysis products, including CO_2 (0.1% total), accounting for only 0.4% of the total menthol radioactivity recovered.

A comprehensive chemical analyses of the particulate and vapor phase constituents of collected cigarette smoke generated from reference cigarettes without any added ingredients and test cigarettes containing a variety of added flavoring materials has been reported (Carmines, 2002; Rustenmeier et al., 2002). Menthol was added at a rate of 18,000 ppm (1.8%) to the tobacco of a test cigarette containing a simple four-component mixture (corn syrup,

licorice, cocoa shells, and menthol) representative of several major ingredient categories. Analyses of smoke particulate material (TPM) generated under conditions approximating ISO standard 3308 (1991) indicated yields of 0.53 and 0.55 ng B[*a*]P/mg TPM, compared to 0.59 ng B[*a*]P/mg TPM reported for the reference cigarette containing no added ingredients. Other compounds reported by Schmeltz and Schlotzhauer (1968) to be pyrolysis products of menthol in the laboratory furnace (*i.e.* benzene, toluene, and phenol) were also found at lower or significantly lower concentrations in the particulate material from the menthol-containing test cigarette relative to that of the control cigarette.

A heretofore unpublished analysis of the smoke particulate material generated under analytical smoking conditions from a non-mentholated reference cigarette and a matched test cigarette containing 1.03% added menthol reported B[*a*]P yields of 2.87 and 2.65 ng/cigarette, respectively [Appendix A; (RJRT, 2000)]. Other laboratory furnace pyrolysis products of menthol reported in the early work of Schmeltz and Schlotzhauer (1968), including benzene, toluene and phenol, were not significantly different between the control and mentholated cigarettes. Such reports of very different results from laboratory and actual cigarette pyrolysis techniques are not uncommon. It is presently well-appreciated that cigarette ingredient fate studies conducted with laboratory furnace pyrolysis/combustion methods should be applied and interpreted with caution due to the substantially different thermal and chemical conditions present in the laboratory furnace and a burning cigarette (Baker and Bishop, 2004).

The reports of Newell et al. (1968) and Jenkins et al. (1970) provide convincing evidence that menthol applied to cigarette tobaccos is transferred into the smoke particulate phase almost entirely as the intact parent molecule, with pyrolytic degradation accounting for only a small fraction of menthol present in the unburned cigarette. The exhaustive smoke chemistry analyses reported by Carmines (2002) and Rustenmeier et al. (2002) and the heretofore unpublished work by investigators at the R.J. Reynolds Tobacco Company (Appendix A; RJRT, 2000) further demonstrate that B[*a*]P and other products reportedly generated from menthol under conditions of laboratory pyrolysis in an inert atmosphere are not produced in measurable excess under actual conditions of mentholated cigarette combustion in air. It therefore seems reasonable to expect that the predominant biological effects of menthol employed as a cigarette flavoring ingredient would be those of the parent compound rather than those of menthol degradation products produced under the artificial conditions of laboratory pyrolysis.

2.2.3. Smoke transfer studies

Wilson (1993) and Best (1993) have reviewed the early studies of the transfer efficiency of menthol on cigarette tobacco into the mainstream smoke. These early investigations frequently employed unfiltered cigarettes or cigarettes having low-efficiency, unventilated filters that yielded mainstream smoke menthol transfer efficiencies as high as 30–50%. A more recent report has described menthol smoke transfer efficiencies for commercial cigarettes sampled from the marketplace in the early 1990s (Cook et al., 1999). These cigarettes represented a spectrum of contemporary filtered designs, with measured filter ventilation rates ranging from zero to over 70%. Menthol transfer efficiencies into the mainstream smoke of conditioned, filtered king-size products varied from a low of about 3% for highly ventilated designs to about 18% for non-ventilated products. Menthol transfer was found to be somewhat less efficient for longer 100-mm commercial cigarettes (Cook et al., 1999).

Analytical smoke studies performed by the R.J. Reynolds Tobacco Company, presented here in Appendix A (RJRT, 2000), found that experimental filter cigarettes containing 6.68 mg menthol (1.03% w/w tobacco) delivered 0.41 mg menthol into the mainstream smoke under the standard Cambridge Filter analytical smoking conditions. This 6.14% mainstream smoke transfer efficiency yielded mainstream smoke "tar" with menthol content of around 10%. This figure is in reasonable agreement with independently-performed analyses which indicated that a smoke condensate preparation from experimental cigarettes containing 5000 ppm added menthol comprised about 6% menthol (Cochran, 1995). These values are also in reasonable agreement with the estimated 20% delivery of cigarette menthol content to the smoker reported by Benowitz et al. (2004) in a clinical biomarkers study discussed below.

2.2.4. Conclusions regarding tobacco uses, smoke chemistry, pyrolysis and smoke transfer

Menthol has a long history of use as a tobacco flavoring ingredient and such use is reported to or acknowledged by tobacco regulatory authorities around the world. A considerable body of scientific data indicates that menthol is transferred largely as the intact parent molecule into cigarette mainstream smoke and that it has no substantive effects on the delivery of other smoke constituents.

3. Toxicology information

3.1. Metabolism

Humans rapidly metabolize menthol at doses resulting from its use in consumer products by direct glucuronidation or to oxidized metabolites such as polyols and hydroxy acids that are subsequently excreted as such or, most predominantly, as glucuronide conjugates (Atzl et al., 1972; Kaffenberger and Doyle, 1990; Adams et al., 1996). Menthol clearance has in fact been employed as a clinical test for glucuronidation capacity in humans, typically by oral administration of menthol at a dose (~1 g) that is greatly in excess of the exposure that has been estimated to occur from smoking a menthol cigarette (<1 mg; Benowitz et al., 2004). Yamaguchi et al. (1994) characterized the metabolism of menthol in the rat with identification of both direct glucuronide conjugation of the parent molecule and hydroxylation of the methyl and isopropyl moieties followed by excretion of carboxylic acid metabolites or their glucuronide conjugates. Enterohepatic recirculation appeared to account for increased quantities of hydroxylated metabolites, both free and conjugated, in rats relative to humans (Yamaguchi et al., 1994). Metabolism and clearance of menthol is similarly efficient in rodents, rabbits and humans, with about 30–60% of an administered dose accounted for, primarily in the urine, as glucuronide conjugation products of the parent menthol structure or its oxidized metabolites (OECD, 2003; Belsito et al., 2008).

A recent investigation of the disposition kinetics and physiological effects of menthol employed a 100 mg dose of *l*-menthol administered to male and female non-smoking human subjects in an oral capsule (Gelal et al., 1999). Plasma and urine levels of menthol and menthol glucuronide were evaluated over an interval of frequent sampling, as objective and subjective data were recorded for a number of physiological variables. Menthol was rapidly metabolized and glucuronidated; only the conjugate was measurable in either body fluid. The plasma half-life for menthol glucuronide was determined to be 56.2 min, and the urinary recovery amounted to 45.6 % of the administered dose, appearing with a urinary half-life for excretion of 75 min. The liver enzyme accounting for menthol glucuronidation in humans is UDP-glucuronosyltransferase 1A4 (Green et al., 1998).

Azzi et al. (2006), citing prior reports of elevated risks for esophageal cancer among both drinkers and smokers, reported an exper-

imental investigation of the potential of menthol and/or ethyl alcohol to affect the tissue penetration and retention of two cigarette smoke constituents that have been identified by IARC to be carcinogenic to humans. Benzo[*a*]pyrene and 4-(N-nitrosomethylamino)-1-(3-pyridinyl)-1-butanone (NNK) were suspended with an added surfactant and dissolved, respectively, in an aqueous medium retained as a donor solution on the luminal side of a freshly-isolated segment of porcine esophagus mounted in a Franz cell. Sampling of an aqueous solution bathing the other side of the tissue was conducted to determine the rate of transfer of B[*a*]P or NNK through the tissue. Ethyl alcohol (5%) or 0.08% menthol was added to the luminal solution to determine any effects on B[*a*]P and NNK permeation kinetics. The menthol concentration employed was stated by the authors to be "*typical. . .of the amount of menthol delivered from a typical mentholated cigarette*", but the method used to translate cigarette menthol delivery into a concentration in the test system's model esophageal lining fluid was not detailed.

The permeation of NNK through the experimental esophageal tissue was reported to be markedly lower in the presence of menthol. The statistically-significant retardation of NNK tissue penetration was largely negated by the presence of 5% ethyl alcohol in the donor solution. Tissue penetration by B[*a*]P was not detectable under any of the experimental treatment conditions.

Tissue reservoir formation, a measure of NNK retention by the experimental tissue apparatus, was increased from 3.666 mg under the saline treatment condition to 4.405 mg ($p = 0.02$) in the presence of 0.08% menthol, but decreased to 3.004 mg in the presence of both menthol and ethyl alcohol. Tissue reservoir accumulation of B[*a*]P was not meaningfully affected by menthol.

The authors concluded that their findings "*. . .support the theory that the use of mentholated cigarettes, or the concomitant consumption of alcohol while smoking them, may have marked effects on the absorption and squamous membrane fate of tobacco chemicals*". However, the decreased NNK permeation and increased membrane retention reported for the static, *in vitro* menthol test condition by Azzi et al. (2006) may not be predictive of phenomena occurring in functioning tissue *in vivo*. The constant perfusion of respiratory tissues by circulating blood and draining lymph fluid, as well as the removal of smoke constituents by local tissue metabolism, are known to exert significant effects on tissue diffusion gradients for such compounds (Ewing et al., 2006).

The brief report of Alakayak and Knall (2008) described an investigation exploring potential differences between the smoke of mentholated (Newport) and non-mentholated (Marlboro) cigarettes in altering the transepithelial electrical resistance of cultured Calu-3 human bronchial epithelial cells. Transepithelial electrical resistance, a measure of the integrity of epithelial tight junctions between adjacent cells, may provide information on the potential of cigarette smoke exposure to facilitate the penetration of inhaled substances through the respiratory epithelium. The author's experiment employed the Vitrocell exposure apparatus that permits exposures of cells to a freshly-generated smoke aerosol at an air–liquid interface that in some respects models the conditions in the lung. The changes in transepithelial electrical resistance elicited by the menthol and the non-menthol cigarette were not statistically different in the authors' analysis.

Richie et al. (1997) described a laboratory investigation of the potential of menthol to affect the capacity of Fischer 344 rats to form glucuronide conjugates of 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanol (NNAL), the primary urinary metabolite of the tobacco-specific nitrosamine NNK. It had been hypothesized that menthol may compete for available glucuronidation capacity and thereby compromise the primary metabolic detoxification and excretion pathway for NNK, with a potential to exacerbate NNK carcinogenicity. A group of control rats received a standard diet, while the experimental group received a diet supplemented with 5000 ppm menthol for 15 days. All rats received 2 ppm NNK in the drinking water throughout the study. On the 16th day of the study, the rats were placed in metabolism cages and 24-h urine was collected for NNAL and NNAL-glucuronide analyses. A blood sample was collected for quantification of HPB-releasing hemoglobin adducts, a surrogate marker for DNA adducts that are believed to be central to NNK's carcinogenic mechanism.

Notably, menthol appeared to enhance the detoxification of co-administered NNK rather than compromise the glucuronide conjugation pathway. The urinary clearance of glucuronide-conjugated NNAL was increased to a statistically significant degree, from 1098 to 1360 pmol/24 h ($p = 0.011$); and the ratio of NNAL-Gluc:-free NNAL was also increased from 0.91 to 1.20 ($p = 0.0006$). HPB-releasing adducts were also moderately reduced in the menthol-treated animals, from 76.4 to 71.6 pmol/g hemoglobin, consistent with a more efficient elimination of NNK from systemic circulation in the presence of menthol, possibly through induction of enzymes active in detoxification pathways.

3.2. General toxicology

The topical, inhalation and systemic toxicity of menthol is generally unremarkable and has been extensively reviewed and periodically updated (Technical Resources Inc., 1993; Adams et al., 1996; OECD, 2003; Bhatia et al., 2008a,b; Belsito et al., 2008). A very low potential for acute toxicity is indicated by a number of published LD50 values for menthol in the grams/kilogram range. Given the availability of a number of comprehensive reviews of the toxicology literature in support of menthol's food usage, the present review includes only selected citations from this general toxicology literature that in the author's view may have some relevance to cigarette-associated menthol usage.

3.3. Allergenicity and sensitization

Although a number of instances of dermal and mucous membrane irritation and sensitization by menthol have been reported and reviewed (Anderson and Maibach, 1980; FEMA, 1992; Ale et al., 2002; OECD, 2003), these studies are generally isolated case reports of an anecdotal nature rather than structured toxicological investigations. Synthetic *l*-menthol was reported not to invoke skin sensitization in a guinea pig model (Hopp, 1993). The OECD summary of toxicology information on all menthol isomers (OECD, 2003) provides a concise summary of available published and unpublished investigations of menthol's sensitizing potential in both animal and human tests. Neither animal test systems (Buehler Test, Local Lymph Node Assay) nor a human evaluation (Maximization Test) were reported to evoke sensitization reactions (OECD, 2003). An 8-h closed-patch test of 8% *l*-menthol or *dl*-menthol in petrolatum did not irritate the skin, and these experimental menthol preparations did not evoke sensitization reactions in a human maximization test (FEMA, 1992). However, all menthol isomers are reported to possess some potential to cause mild skin irritation; as well as ocular, nasal and oral irritation at relatively high exposure levels.

A case report of mild, erythematous dermatitis was reported in a smoker of mentholated cigarettes (Camarasa and Alomar, 1978). The patient demonstrated sensitization to menthol in a skin patch test and symptoms resolved after cessation of menthol cigarette smoking. A similar case report of a 25-year old woman described chronic dermatitis of the upper lip in association with the smoking of menthol cigarettes. Her symptoms resolved upon discontinuing menthol cigarette smoking and reappeared when she resumed the practice (Chrisman, 1978). A generalized uticaria was reported upon oral challenge with 10 mg menthol in a 31-year old patient

who habitually consumed peppermint candy, mint-flavored toothpaste and mentholated cigarettes (FEMA, 1992). A German study of sensitivity to topical drug ingredients among 1440 patients reported the occurrence of menthol sensitivity, more frequently among the longer-term users of menthol-containing preparations. A published letter by Luke (1962) provided an anecdotal case report of a 58-year-old female patient complaining of insomnia, unsteady gait, mental confusion, depression, and a bizarrely overactive and irritable state after reporting consumption of 80 mentholated cigarettes daily for the previous 3 months. She recovered following a hospital stay in which she was supplied with a smaller daily quantity of non-menthol cigarettes. Her physician subsequently administered daily doses of 3 g of menthol for 1 week "to test her reaction", and some of the prior symptoms reappeared. The physician attributed the patient's physical and mental symptoms to an "excessive consumption and craving for menthol cigarettes".

Although available experimental studies predict that menthol has a low potential to evoke sensitization, its wide occurrence in foods and consumer products and periodic anecdotal reports of symptoms consistent with some manifestations of sensitization reactions suggest that certain individuals may manifest menthol sensitization.

An investigation of occupational exposures to menthol vapors occurring in the manufacture of mentholated Sucrets® throat lozenges was conducted in response to employee complaints of respiratory and ocular irritation (NIOSH, 1979). Air sampling indicated that menthol was present in the air of production and packaging areas at varying levels up to 39.4 mg/m^3. Inflammation of upper respiratory tissues, runny noses, watery eyes and ocular redness comprised the primary symptoms among the evaluated employees. Pulmonary function testing indicated that non-smokers and former smokers among the affected individuals exhibited significant reductions in forced vital capacity and 1-second forced expiratory volume (FEV_1) at the end of a day's workplace exposure, while currently smoking workers showed no significant changes in any of the evaluated parameters of respiratory function. While the design of this study was inadequate to support many specific conclusions regarding menthol's inhalation toxicity in humans, it did clearly demonstrate that the inhalation of high concentrations of menthol vapor over the course of a workday can induce signs of irritation in some persons. This report is similar to a number of other investigations that document the fact that exposure to high concentrations of menthol may in certain circumstances induce a transient irritation to the skin and mucous membranes.

3.4. Reproductive toxicology

No studies are available. However, histopathological examinations of animals dosed repeatedly or chronically with menthol have shown no evidence that reproductive organs are targets for menthol toxicity and available developmental toxicology studies provide no evidence of adverse effects on reproductive performance or embryotoxicity at doses below maternally toxic levels (185–425 mg/kg bw/day) in several species (OECD, 2003).

3.5. Developmental toxicology

A series of developmental toxicity (teratology) evaluations of natural Brazilian menthol were conducted by the oral administration of corn oil vehicle or menthol solutions at four dose levels to pregnant females of each of four species during the critical period of organogenesis. Menthol produced no indications of any potential to adversely affect development in these studies performed in CD-1 mice, Wistar-derived rats, Golden hamsters, or rabbits (FDA, 1973; OECD, 2003; Belsito et al., 2008). The developmental toxicity study in rabbits is representative of this work. Pregnant test animals were administered menthol in doses of 4.25–425 mg/kg bw/day on gestational days 6–18, with no adverse effects on fetal survival and no elevated incidence of skeletal or soft tissue anomalies. No maternal or fetal toxicity was manifested by the menthol dosing regime, and a NOEL of 425 mg/kg bw/day was derived (OECD, 2003).

3.6. Genetic toxicology

Numerous investigations of menthol's potential to induce genetic toxicity have been reported; these data support a conclusion that menthol does not pose a genotoxic or mutagenic hazard under conditions of its use as an ingredient in consumer products (FEMA, 1992; Technical Resources Inc., 1993; OECD, 2003; Belsito et al., 2008).

Representative Ames *Salmonella* mutagenicity tests of *l*-menthol performed in batteries of tester strains both in the presence and absence of an S-9 metabolic activation system were negative (Andersen and Jensen, 1984; Ishidate et al., 1984). Testing performed under the National Toxicology Program's screening program found *dl*-menthol to be non-mutagenic in the sensitive L5178Y mouse lymphoma cell TK +/− forward mutation assay (Myhr and Caspary, 1991), and inactive in both an *in vitro* sister chromatid exchange assay and chromosome aberrations assay in CHO cells (Ivett et al., 1989). These findings were in agreement with earlier testing of *dl*-menthol which generated negative responses in a host-mediated Ames *Salmonella* assay, cytogenetics studies, and a dominant lethal assay (Litton Bionetics Inc., 1975). The report of Hilliard et al. (1998) is of interest as it described an investigation of the phenomenon of "false positive" results for model non-genotoxic test articles, including *d,l*-menthol, in an *in vitro* CHO cell chromosome aberrations assay and a human lymphoblastoid TK6 cell mutagenesis assay. The positive findings for menthol were evident only at relatively high tested levels that induced substantial cytotoxicity (reductions in cell viability of about 50% or more) in the test cells, and were characterized by the authors as a "false positive" result that is not predictive of a genetic toxicity hazard for menthol in intact mammalian systems.

3.7. Tumorigenesis

Available chronic and subchronic test data for menthol provides no indication of any carcinogenic potential, while a number of studies suggest a modest anticarcinogenic efficacy for this material.

A traditional, two-species chronic rodent bioassay performed by the National Cancer Institute found *dl*-menthol to be without carcinogenic activity when administered to F344 rats of both sexes at 3750 or 7500 ppm in the diet (approximately 187 or 375 mg/kg/day) for 103 weeks. Similarly, administration of menthol to B6C3F1 mice of both sexes at dietary concentrations of 2000 or 4000 ppm (approximately 300 or 600 mg/kg/day) for 103 weeks did not produce any indication of a carcinogenic effect. Mean group body weights of the rats and mice receiving menthol were only slightly depressed relative to those of the control groups, and no other clinical signs of toxicity were noted in the menthol test animals. Female rats dosed with menthol exhibited lower incidences of mammary gland fibroadenomas and lung bronchial/alveolar adenomas and carcinomas than did the control animals (NCI, 1979).

An investigation of menthol's potential to enhance spontaneous lung tumor development in the strain A/He mouse model was conducted by the administration of 20 *i.p.* injections totaling 0.5 or 2 g/kg over a period of 24 weeks. While a dose-related decrease in

animal survival was observed, tumor incidence and tumor multiplicity were somewhat decreased among surviving animals. No indication of any enhancement of spontaneous lung adenoma incidence was reported (Stoner et al., 1973).

An investigation of a series of monoterpenoids for anticarcinogenic activity found *l*-menthol to be a potent inhibitor of tumor initiation in the rat mammary carcinogenesis model (Russin et al., 1989). Menthol was administered in this study at 0.5% in the diet for 2 weeks before and 1 week after the oral administration of a single 65 mg/kg injection of the experimental carcinogen 7,12-dimethylbenz(*a*)anthracene (DMBA). While DMBA is not found in cigarette smoke, this potently tumorigenic polycyclic aromatic hydrocarbon is often employed as a model compound in tobacco-related biological studies. Menthol significantly reduced tumor incidence ($p < 0.001$), extended tumor latency ($p < 0.01$), and reduced tumor multiplicity ($p < 0.003$) when administered during the initiation stage of DMBA mammary carcinogenesis.

Another investigation of the anticarcinogenic potential of *l*-menthol showed neither inhibition nor enhancement of azoxymethane-induced intestinal tumor development by menthol addition at 5 mg/g in the diet beginning 3 days after carcinogen administration to male F344 rats (Wattenberg, 1991).

3.8. Inhalation toxicology

The sensory irritation potential of menthol was evaluated in 30-min exposures of Swiss-Webster mice to seven menthol concentrations ranging from 18 to 31 ppm (115–198 mg/m^3) (Burleigh-Flayer, 1988). Periocular wetness was observed in several animals 24 h following exposure to concentrations of 22 ppm (140 mg/m^3) and above, and mortalities were recorded among the 20 and 30 ppm (140 and 191 mg/m^3) exposure groups. An inhalation RD50, defined as that concentration inducing a 50% reduction in mean respiratory frequency (a measure of sensory irritation), was determined to be 45 ppm (287 mg/m^3).

An investigation of the potential of a menthol-containing topical preparation (Vicks Vaporub®) to affect mucociliary and phagocytic clearance following a challenge with *Staphylococcus aureus* bacteria was conducted in rats and mice (Goldstein et al., 1976). Animals were exposed for 4 or 8 h to "normal" and "4 times normal" concentrations of the medicated vapors prior to an aerosol challenge with radiolabeled bacteria. These exposure conditions resulted in peak menthol vapor concentrations of about 0.5 and 1.5 μg/l, along with similar to substantially higher concentrations of camphor, eucalyptol and turpentine. No adverse effects on mucociliary or phagocytic clearance were observed at any exposure concentration in either rodent species. These authors mentioned a previous 1975 study of Jakab and Green in which continuous exposure to a 30-fold higher vapor concentration of the medicinal preparation had similarly been found to have no adverse effect on pulmonary bacterial clearance.

An investigation of the ciliastatic potential of a model inhalation cold remedy comprising roughly equal quantities of menthol, eucalyptus oil and pine needle oil was reported by Riechelmann et al. (1997). Freshly-collected human nasal cells were exposed *in vitro* to very substantial vapor concentrations of the mixture, and a maximum inhibition of ciliary beat frequency of −22.6% was observed at a concentration of 10 g/m^3. However, chemical analysis indicated that menthol constituted perhaps 5% or less of the test vapor and collateral experiments demonstrated that the eucalyptus oil and pine needle oil components likely accounted for the majority of the ciliastatic potency of the mixture. The study findings do not indicate any substantive concern in regard to menthol as a ciliastatic agent.

3.9. Menthol cigarette pyrolysis toxicology studies

The representative toxicology studies discussed above provide a strong assurance that menthol does not pose a safety hazard when employed as an ingredient in food or consumer product applications. Oral and dermal toxicology studies provide some assurance that systemic exposures to menthol from tobacco use is unlikely to induce systemic manifestations of toxicity. However, special toxicology assessments are appropriate for an evaluation of menthol employed in tobacco products that are burned and inhaled. A number of these special assessments are discussed below.

3.9.1. In vitro cytotoxicity and genetic toxicity

Roemer et al. (2000, 2002) performed an *in vitro* comparison of the mutagenic and cytotoxic activity of smoke condensates from a reference cigarette having no flavoring ingredients to that of a test cigarette containing 18,000 ppm (1.8%) *l*-menthol added with a 3-component casing mixture. No differences in mutagenic activity were observed in an Ames *Salmonella* assay employing strains TA102, TA1535, TA1537 and TA98 in the presence or absence of an S9 metabolic activating system in a comparison of smoke particulate material from the control and menthol-containing cigarettes.

These authors also reported a comparison of the cytotoxicity of the smoke particulate material as well as of the water-soluble constituents of the smoke gas phase from the test cigarettes containing 18,000 ppm (1.8%) added menthol to that of the matched reference cigarette without added ingredients. A neutral red dye uptake assay was performed in four replicate 96-well microtiter plates seeded with BALB/c 3T3 cells per test concentration. Each of eight concentrations of the smoke particulate and gas phase preparations was replicated six times on each plate. The aqueous extracts of all of the test cigarettes' smoke gas phase were somewhat more cytotoxic than were the particulate material samples collected from the same volumes of smoke. The cytotoxicity of the smoke particulate material and gas phase extracts from cigarettes containing menthol or mixtures of other commonly-employed cigarette flavoring materials were moderately less cytotoxic (on the order of 15%) than were the smoke preparations from cigarettes without added flavorings.

A series of heretofore unpublished assessments of the potential of added menthol to affect the *in vitro* genotoxic and cytotoxic activity of cigarette smoke condensates has been conducted (RJRT, 2000). These studies are outlined below, and additional experimental detail is presented in Appendix B. Experimental cigarettes were prepared from a typical American tobacco blend to which menthol had been applied at 1.03% w/w tobacco (6.68 mg/cigarette); matched reference cigarettes of identical blend and construction were prepared without any added menthol. Smoke particulate material collected under standard FTC smoking conditions was subsequently evaluated in a series of bacterial and mammalian cell test systems.

A comparison of the bacterial mutagenic activity of these menthol and non-menthol cigarette smoke particulate material samples was performed in the Ames *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 both in the presence and absence of an S9 metabolic activation mixture. Menthol addition was found to have no effect on the mutagenic activity of the cigarette smoke particulate material (RJRT, 2000).

The potential of menthol addition to affect the inherent mammalian cell cytotoxicity of cigarette smoke condensates was evaluated in a neutral red assay employing Chinese Hamster Ovary (CHO) cell cultures treated with smoke condensates prepared as described above. The CHO cell cultures were treated with smoke particulate material from reference cigarettes containing no menthol and from experimental cigarettes containing 1.03% (6.68 mg/cigarette) added menthol. A range of smoke particulate material

concentrations ranging from 10 to 150 μg/ml culture medium was evaluated; no indication of an effect of menthol addition to the test cigarettes on the inherent cytotoxicity of the smoke particulate material preparations was observed (RJRT, 2000).

The potential of menthol addition to cigarettes to affect the *in vitro* genetic toxicologic activity of the smoke particulate material was further evaluated in a sister chromatid exchange (SCE) assay in CHO cell cultures in the presence and absence of S9 metabolic activation. The experimental cigarettes containing 1.03% added menthol were made and smoke particulate preparations were collected as described above for comparison to reference cigarettes containing no added menthol. Concentrations of reference and menthol cigarette smoke particulates ranging from 10 to 75 μg/ml in the absence of S9 and from 150 to 300 μg/ml in the presence of S9 induced a wide range of toxicity in the assay and yielded linear dose–responses in the SCE assay. The SCE activity of cigarette smoke particulate material from menthol cigarettes was not significantly different than that of the non-menthol reference cigarette under the conditions of the study (RJRT, 2000).

3.9.2. Tumorigenesis studies

The reviewed abstract of an early [German language] paper reported no differences in mouse skin tumorigenic activity between condensates prepared from mentholated and non-mentholated cigarettes (Schievelbein, 1969). While reported experimental details were incomplete, the test cigarettes probably contained a moderate amount of menthol (1–4 mg per cigarette) representative of the quantities found in German cigarettes of the day.

A more recent study (Gaworski et al., 1999) compared the mouse skin tumor promoting potential of smoke condensates from a menthol-containing test cigarette to that of a similarly-constructed reference cigarette containing no added flavoring materials. The test cigarettes contained 5000 ppm *l*-menthol as a major constituent of an added flavoring mixture comprising a combination of ingredients representative of those employed in contemporary US cigarette manufacturing. Cold trap-collected smoke condensates were applied thrice weekly at rates of 10 and 20 mg per application, for 27 weeks, to the 7,12-DMBA initiated shaved dorsal skin of SENCAR mice, a strain selectively bred for high susceptibility to skin tumorigenesis. Gas chromatographic analyses indicated that menthol comprised 2.41% (w/w) of the experimental menthol cigarette smoke condensate, while the reference cigarette condensate contained only trace quantities of menthol (0.04%) (Cochran, 1995). The condensate of the mentholated test cigarette smoke was found to exhibit no significant differences from that of the reference cigarette smoke in any parameter of tumorigenic response (% tumor-bearing animals, tumor latency, and tumor multiplicity).

3.9.3. Cigarette smoke inhalation studies

A 13-week, nose-only cigarette smoke inhalation study was conducted in F344 rats to determine whether the addition of flavoring ingredients to cigarettes could affect the site or severity of respiratory tract changes normally observed in this animal model consequent to subchronic cigarette smoke exposure (Gaworski et al., 1997). The rats were exposed to 200, 600 and 1200 mg smoke total particulate material/m^3 for 1 h daily, 5 days/week throughout the course of the 13-week study. These exposure levels resulted in blood carboxyhemoglobin, nicotine, and cotinine levels far in excess of those reported to occur in human smokers. The clinical chemistry and histopathologic responses elicited by the smoke of reference cigarettes without flavoring ingredients were compared to those of test cigarettes of matched construction containing 5000 ppm *l*-menthol as the predominant constituent of a model flavoring ingredient mixture believed to be representative of those employed in contemporary US cigarette manufacturing. Analysis of Cambridge filter-collected smoke particulate material generated under conditions approximating those employed in the animal exposures revealed menthol deliveries of 41.4 μg per 35 ml puff (1.2 mg/l). This puff volume contained an average of 2.094 mg total particulate material, indicating that menthol comprised 1.97% of mainstream particulate material delivered into the animal inhalation chamber (Cochran, 1995).

No significant differences in the onset, site, or severity of smoke-associated respiratory tract changes were observed between the two cigarette types. Nor were any dose-related differences in blood nicotine or cotinine noted. Small but statistically significant reductions in CO levels of the diluted smoke inhalation atmosphere were noted for the menthol cigarette relative to those for the matched reference cigarette. The reason for the lower CO yield of the menthol test cigarette was not determined, but it may be attributable to minor differences in the combustion process accompanying the experimental addition of exaggerated levels of the tested flavoring ingredients to the model cigarettes.

Another 90-day subchronic rat smoke inhalation study compared the responses elicited by the smoke of cigarettes containing 18,000 ppm added *l*-menthol to those of the smoke of a matched reference cigarette without any added ingredients (Vanscheeuwijck et al., 2002). The menthol test cigarette also contained a simple casing component comprising corn syrup, licorice extract and cocoa shells. Groups of 10 Sprague–Dawley rats of both sexes were exposed for 6 h per day, 7 days a week to 150 μg total smoke particulate matter/liter air; and a battery of physiological, clinical chemistry, hematologic, and histopathologic parameters were evaluated immediately after the 90-day smoke exposure period. Additional groups of 10 animals per sex were maintained for a 42-day recovery period following the smoke exposure to evaluate the reversibility of any noted effects. No significant differences in body weight effects, respiratory rate and volume, blood carboxyhemoglobin, blood nicotine or the relative distributions of nicotine metabolites were observed between the menthol-containing and reference cigarette group in either sex. No significant differences were observed between the reference and menthol-containing cigarette test groups in any organ weight changes with the exception of the thymus; this organ was less affected by exposure to the menthol cigarette smoke than by the reference cigarette smoke in both sexes. A comprehensive histopathologic evaluation of the respiratory tract found no meaningful differences in the character or severity of cigarette smoke-related changes attributable to the inclusion of menthol in the test cigarette. Two incidental statistical differences in histopathologic severity grade between the reference and menthol cigarette were noted in the larynx, but these differences (one increase and one decrease) were not regarded as biologically significant. It was concluded that the toxicity of the smoke of the menthol-containing test cigarette did not appear to differ in any substantive way from that of the non-menthol reference cigarette.

3.9.4. Conclusions regarding menthol cigarette toxicology

An extensive and reassuring weight of *in vitro* and experimental animal investigation indicates that menthol does not pose a toxic or carcinogenic hazard, consistent with its long history of safe use in consumer products, its benign chemical structure, and its ready metabolism by mammalian systems. The available experimental cigarette smoke toxicology data is consistent with a further conclusion that menthol cigarettes and non-menthol cigarettes produce similar results in the available testing systems that have traditionally been employed in tobacco smoke toxicology testing. The presence of menthol at realistic or exaggerated levels in experimental test cigarettes has not been found to introduce novel manifestations of toxicity to the smoke or smoke condensate, nor does it increase the inherent toxicity of the smoke in these tests.

Table 2
Overview of menthol epidemiology studies.

Reference	Study type	Outcome	Comparison	Adjusted[a] Menthol risk estimate (95% CI)	
				Male	Female
Hebert and Kabat (1988)	Case control	Esophageal cancer	Non-menthol *vs.* Menthol (10+ years of smoking)	0.7 (0.29–1.73)[b]	1.53 (0.61–3.86)[b]
Hebert and Kabat (1989)	Case control	Esophageal cancer	Non-menthol *vs.* Menthol	1.00 (0.95–1.05)[c]	1.05 (0.75–4.17)[c]
Kabat and Hebert (1991)	Case control	Lung cancer	Non-menthol *vs.* Menthol in current smokers (15+ years of smoking)	0.98 (0.70–1.38)[c]	0.76 (0.53–1.16)[c]
Kabat and Hebert (1994)	Case control	Oropharyngeal cancer	Non-menthol *vs.* Menthol (15+ years of smoking)	0.9 (0.5–1.6)[c]	0.7 (0.5–1.7)[c]
Sidney et al. (1995)	Cohort	Lung cancer	Non-menthol *vs.* Menthol (20 years of smoking)	1.59 (0.96–2.63)[b]	0.70 (0.40–1.23)[b]
Friedman et al. (1998)	Cohort	All smoking-related cancers	Non-menthol *vs.* Menthol in current smokers	0.76 (0.52–1.11)[d]	0.79 (0.53–1.18)[d]
Carpenter et al. (1999)	Case control	Lung cancer	Non-menthol *vs.* Menthol (32+ years smoking)	1.48 (0.71–3.05)[c]	0.41(0.15–1.11)[c]
Brooks et al. (2003)	Case control	Lung cancer	Non-menthol *vs.* Menthol (15+ years smoking)	0.91 (0.57–1.46)[c]	1.00 (0.63–1.60)[c]
Stellman et al. (2003)	Case control	Lung cancer	Non-menthol *vs.* Menthol in current smokers	W-0.83 (0.63–1.09)[c] B-1.34 (0.79–2.29)[c]	W-0.61 (0.44–1.06)[c] B-0.79 (0.41–1.54)[c]
Jöckel et al. (2004)	Case control	Lung Cancer	Ever smoking menthol	All smokers 1.12 (0.68–1.83)[c]	
Pletcher et al. (2006)	Nested case control	Coronary calcification	Non-menthol and Menthol per 10-pack year increase	Menthol 1.16 (0.91–1.47)[c]	Non-menthol 1.23 (0.98–1.55)[c]
Murray et al. (2007)	Prospective study	All cause mortality	Non-menthol *vs.* Menthol	0.99 (0.83–1.20)[d]	
		CHD mortality		1.31 (0.77–2.22)[d]	
		CVD Mortality		1.03 (0.70–1.52)[d]	
		Lung cancer		0.96 (0.70–1.32)[d]	
Etzel et al. (2008)	Case control	Lung cancer	Non-menthol *vs.* Menthol in current and former African-American smokers	Current-0.69 (0.26–1.03)[c]	Former-0.99 (0.62–1.56)[c]

W = White and B = Black.
[a] Adjusted for age, race, sex (where relevant), smoking habits and in some studies for other variables.
[b] Relative risk (95% CI).
[c] Odds ratio (95% CI).
[d] Hazard ratio (95% CI).

4. Epidemiology of menthol cigarette smoking

Menthol is unique among cigarette flavoring ingredients in that a number of epidemiological investigations have been conducted in an attempt to determine whether menthol may contribute as an independent risk factor to the development of various smoking-associated cancers (Alberg et al., 2007). The available studies are considered individually below in chronological order, with findings summarized in Table 2.

Hebert and Kabat (1988, 1989)

Hebert and Kabat (1988, 1989) employed an existing inter-institutional database developed by the American Health Foundation from 1969 to 1984 to perform a hospital-based case–control study of the relationship between mentholated cigarette smoking and esophageal cancer. No statistically significant differences in esophageal cancer incidence were found for either male smokers, (relative risks ranging from 0.50 to 1.03), or female smokers (relative risks ranging from 1.05 to 1.53) reporting the smoking of menthol cigarettes compared to those smoking regular cigarettes.

Kabat and Hebert (1991)

These same authors subsequently performed an analysis of lung cancer incidence among self-reported current smokers of menthol or regular cigarettes in a hospital-based case–control study (Kabat and Hebert, 1991). Neither the short-term (1–14 years; odds ratios (OR) of 1.14 in males and 0.82 in females), nor long-term (15+ years; OR of 0.98 in males and 0.76 in females) use of mentholated cigarettes was associated with any significantly increased risk for the development of any of the major histological subtypes of lung cancer.

Kabat and Hebert (1994)

A third case–control study by this group investigated the rate of menthol cigarette smoking among oropharyngeal cancer patients in the American Health Foundation cohort (Kabat and Hebert, 1994). Crude and adjusted OR for oropharyngeal cancers associated with both short-term menthol cigarette smoking (1–14 years; risks ranging from 0.5 to 0.6 in males and 0.8 to 1.0 in females) and long-term menthol smoking (15+ years; risks ranging from 0.7 to 0.9 in males and 0.6 to 0.7 in females), were at or below unity for both sexes relative to those for regular cigarette smoking. The authors concluded that the use of mentholated cigarettes is unlikely to be an important independent factor in oropharyngeal cancer development (Kabat and Hebert, 1994).

Sidney et al. (1995)

A prospective investigation of the possible association between menthol cigarette usage and lung cancer risk was conducted among participants in the Kaiser Permanente Medical Care Program in northern California (Sidney et al., 1995). Substantial cohorts of men and women identifying themselves as 20-year continuous and current smokers of either regular or mentholated cigarettes were established from questionnaires administered from 1979 to 1985, with follow-up of 318 new lung cancer diagnoses carried out through 1991.

The authors reported a statistically significant elevation in the relative risk of lung cancer associated with menthol cigarette use

by men of 1.45 (1.03–2.02, 95% CI) after adjustment for age, race, education, smoking rate, and smoking duration. The lower relative lung cancer risk for female menthol smokers, 0.75 (0.51–1.11, 95% CI), did not differ statistically from that of matched female smokers of non-menthol cigarettes. Further examining the risks for lung cancer in current smokers by duration of mentholated cigarette use, the authors reported statistically indistinguishable relative risks for menthol cigarettes of 1.59 (0.96–2.63, 95% CI in males) and 0.70 (0.40–1.23, 95% CI) in females.

The reported association of menthol smoking with a marginally elevated risk for lung cancer reported by Sidney et al. (1995) among males of all races combined is puzzling in light of the fact that the females in the study exhibited a higher prevalence of menthol usage than males (34.6% *vs.* 27.4%, respectively), and a somewhat longer duration of menthol smoking to total duration of smoking (55% *vs.* 47% for males) accompanied by a lower lung cancer risk that fell short of statistical significance. Furthermore, despite the fact that both black and Asian subjects reported higher rates of menthol preference (41.5% and 36.6%, respectively) than did white participants (24.4%), no statistically significant elevations in lung cancer risk relative to whites was apparent in the race-specific analyses. Asian men, in fact, exhibited a significantly lower relative risk of only 0.13 (0.02–0.91) relative to whites, despite their substantially higher preference for menthol cigarettes. The authors acknowledged that their finding in regard to menthol and lung cancer in males was in conflict with the only other such lung cancer epidemiology study then extant (Kabat and Hebert, 1991), and pursued a follow-up investigation of cancers at other sites.

Friedman et al. (1998)

Friedman et al. (1998) sought to determine whether their previous finding of an association between menthol cigarette smoking and elevated lung cancer incidence in males (Sidney et al., 1995) was apparent for a variety of other smoking-associated tumor sites, including the upper aerodigestive tract. No evidence of an increased tumor rate associated with menthol cigarette preference was found at any site in either sex. While the 95% CI for all menthol rate ratios included 1.0, it is interesting to note that for 9 of the 11 tumor rate comparisons between menthol smokers and regular smokers, the point estimates for menthol/regular cigarette rate ratio was actually less than 1.0. For all smoking-related cancers surveyed, the menthol/regular rate ratio was 0.76 (0.52–1.11, 95% CI) for males and 0.79 (0.53–1.18, 95% CI) for females. These findings prompted the authors to comment in regard to their previous report (Sidney et al., 1995) that "*. . .the association of mentholation with lung cancer in this study population may be merely a chance finding, particularly as it was absent in women and has not been replicated elsewhere*" (Friedman et al., 1998).

Carpenter et al. (1999)

Carpenter et al. (1999) employed data extracted from a large, population based, case–control study of genetic markers for lung cancer risk in an investigation of the association between mentholated cigarette smoking and lung cancer incidence. Three hundred and thirty-seven incident lung cancer cases, comprising both present and former smokers, were compared to age-, sex-, and race-matched controls. The adjusted odds ratio (OR) for exclusive menthol smokers was indistinguishable from that of regular cigarette smokers (menthol OR = 1.04; 95% CI: 0.62–1.75). Similarly, comparisons of OR by sex, race, and duration of mentholated cigarette smoking revealed no significant differences between menthol and non-menthol cigarettes. The investigators concluded in summary that "*. . .the results from this study suggest little or no increase in lung cancer risk associated with mentholated cigarette smoking compared to non-mentholated smoking*".

Scanlon et al. (2000)

Scanlon et al. (2000) reported a prospective analysis of participants in the multicenter Lung Health Study cohort to compare the rates of lung function decline among non-smokers, ex-smokers and continuing smokers in the cohort of 3818 participants. Declines in lung function correlated with smoking histories and intensities. The authors briefly observed in regard to menthol cigarettes that "*. . .Smoking mentholated cigarettes did not affect the rate of decline in lung function in Year 1 or between Year 1 and Year 5 (p = 0.229 and 0.64, respectively, data not shown)*". This observation of a null effect of menthol on the decline in smokers' lung function was not further detailed in this paper, but was later confirmed in the study of Pletcher et al., 2006 discussed below.

Brooks et al. (2003)

Brooks and colleagues (2003) examined data from a multi-hospital case–control study (n = 643 cases and 4110 controls) to examine whether smoking menthol cigarettes is associated with higher lung cancer risk than is characteristic of non-menthol cigarette smoking. The authors found that subjects who smoked menthol cigarettes did not have an elevated risk of lung cancer (OR = 0.89, 95% CI: 0.69–1.14) relative to those reporting smoking of regular cigarettes. Further analysis revealed that risk estimates for smoking menthol cigarettes for more that 15 years (OR = 0.97, 95% CI: 0.70, 1.34), for 50% or more of reported smoking history (OR = 0.89, 95% CI: 0.65, 1.22) or exclusively (OR = 0.91, 95% CI: 0.59, 1.43) were also not elevated compared to those of the reference groups who had smoked non-menthol cigarettes. The authors concluded that "*. . .the results of this study do not support the hypothesis that smoking menthol cigarettes increases the risk of lung cancer relative to smoking non-menthol cigarettes*".

An invited commentary (Hebert, 2003) accompanying the Brooks paper stated that "*. . .it is becoming clear that if there is an elevation in risk of lung cancer from smoking mentholated cigarettes beyond that from smoking regular, filter-tipped brands, it is either subtle or refractory to the methods we have used thus far*".

Stellman et al. (2003)

Stellman et al. (2003) conducted a large, case–control, hospital-based study (n = 1710 cases) to investigate the potential basis for the elevated risk of smoking-related lung cancer among black males relative to whites. While a greater proportion of blacks in the sample reported themselves to be smokers, they smoked fewer cigarettes per day than did the white subjects. Among current smokers, the OR for lung cancer did not differ significantly between black and white males (21.0 *vs.* 18.2, respectively) or between black and white females (19.3 *vs.* 17.2, respectively). Furthermore, the authors found that the "*. . .*ORs *among smokers of menthol cigarettes were practically the same as among smokers of non-menthol cigarettes*".

Relative to the respective reference group ORs determined for non-menthol smokers, the ORs and 95% CI for menthol cigarette-smoking black and white males were 0.83 (0.63–1.09) and 1.34 (0.79–2.29), and for black and white females, 0.61 (0.44–1.06) and 0.79 (0.41–1.54), respectively. The authors concluded that "*. . .lung cancer risks were similar for whites and blacks with similar smoking habits, except possibly for blacks who were very heavy smokers*", and further that "*. . .[s]mokers of menthol flavored cigarettes were at no greater risk for lung cancer than were smokers of unflavored brands*". The authors opined in regard to black/white

differences in lung cancer risk that "*. . .[w]hile black smokers in our study were more likely to choose menthol than non-menthol brands. . ., our data provide no evidence that menthol cigarettes per se produce greater lung cancer risk than do non-menthol brands*". Stellman et al. (2003) also cited laboratory toxicology data consistent with their epidemiological findings, stating that "*. . .[e]xperimental data show no increase in NNK-induced adduct formation in NNK-treated rats that were administered menthol in their drinking water (NNK is a tobacco-specific nitrosamine, which experimentally produces lung adenocarcinoma in rodents), further supporting our conclusion that menthol does not play a role in risk for lung cancer*".

Jöckel et al. (2004)

The published abstract of a meeting presentation (Jöckel et al., 2004) reported a hospital-based case–control study of 1004 German lung cancer patients (839 males and 165 females) and a like number of controls (matched for age, sex and region of residence) that explored the possibility that the smoking of mentholated cigarettes may be associated with a disease risk differing from that of regular cigarette smokers.

The authors reported a lung cancer OR of 1.12 (95 % CI: 0.68–1.83) for having ever been a smoker of mentholated cigarettes, adjusted for the total intensity and duration of smoking history. They concluded that their study gave no indication that the smoking of menthol cigarettes entails a risk for lung cancer different from that of regular cigarettes. Once published in the peer-reviewed scientific literature, the findings of Jöckel et al. (2004) will constitute the first such epidemiological data on menthol cigarette risks from a non-US population sample. It appears from the presented abstract that these data are consistent with prior findings from the US indicating that there are no differences in lung cancer risks between menthol and non-menthol cigarettes (Werley et al., 2007).

Pletcher et al. (2006)

Pletcher et al. (2006) reported an investigation of the potential effects of cigarette mentholation on the development of atherosclerotic disease, decline in lung function and success in smoking cessation among smokers of regular and mentholated cigarettes. Discussion of the smoking cessation findings is presented below in a subsequent section. Study subjects comprised 1535 African-American and European-American smokers from a cohort of 5115 males and females in four major US cities participating in the Coronary Artery Risk Development in Young Adults (CARDIA) study. Study subjects provided questionnaire and clinical data at enrollment and 2, 5, 7, 10 and 15 years thereafter (calendar years 1985–2000).

Atherosclerotic disease progression was assessed by estimates of coronary calcification by two different imaging techniques at the end of the 15-year follow-up. Data were adjusted to achieve comparability between the two techniques. Decline in lung function over the first 10 years of the study was assessed and reported as decline in FEV_1 adjusted for smoking rate and duration. The potential of cigarette mentholation to affect success in smoking cessation was expressed as OR for comparisons of menthol and regular cigarette smokers for smoking cessation, recent successful cessation attempts and reports of smoking relapse following previously-reported cessation.

The 1535 smoking study subjects included 563 non-menthol cigarette smokers and 972 menthol cigarette smokers. The 63% preference for mentholated brands recorded for smokers participating in the CARDIA study is considerably higher than that previously reported for the US population as a whole. The African-American study subjects stated a significantly higher preference for menthol brands than did European-American subjects (89% *vs.* 29%, $p < 0.001$). The stated preference for mentholated cigarettes among African-American smokers in the present study is somewhat higher than that previously reported for this demographic group (approximately 70%) (Giovino et al., 2004). The authors stated that adjustment for ethnic, demographic and social factors; as well as for smoking rate (cigarettes per day) had been performed in their data analyses.

The authors reported no increase in coronary calcification in association with menthol cigarette smoking relative to that observed for non-mentholated cigarette smokers. OR of 1.27 (1.01–1.60, 95% CI) for menthol cigarettes and 1.33 (1.06–1.68, 95% CI) for regular cigarettes were reported for this measure of atherosclerosis progression ($p = 0.75$). Additionally, no significant differences in decline of lung function, expressed as adjusted excess FEV_1 decline per 10-pack years, were observed for menthol cigarettes [84 (32–137, 95% CI)] relative to non-mentholated cigarettes [80 (30–129, 95%CI)] ($p = 0.88$).

The negative findings for menthol and decline in respiratory function in smokers reported by Pletcher et al. (2006) confirmed a similar observation that had been briefly noted previously by Scanlon et al. (2000). Pletcher and coworkers concluded from their 2006 analyses that "*. . .[m]enthol and non-menthol cigarettes seem to be equally harmful per cigarette smoked in terms of atherosclerosis and pulmonary function decline. . .*".

Werley et al. (2007)

Werley et al. (2007) reviewed the literature regarding the potential of cigarette mentholation to affect various aspects of smoking-related health consequences and performed a formal meta analysis of five available studies of long-term menthol cigarette smoking and lung cancer risk. A stated preference for mentholated cigarettes by both sexes combined was associated with a risk of 0.93 (0.84–1.03, 95% CI) relative to that of smokers of non-mentholated brands. Similarly, the combined studies/sexes relative risk for long-term mentholated cigarette use was marginally lower than was that for non-menthol cigarettes, 0.95 (0.80–1.13, 95% CI). The authors' meta analyses of menthol smoking risks for males and females separately were likewise statistically indistinguishable from those of the respective non-mentholated cigarette smokers. This meta analysis reported no evidence that cigarette mentholation increases lung cancer risk relative to non-mentholated cigarettes, and further that "*. . .mentholation cannot explain the higher risk for lung cancer in African American male smokers*". The review further concluded that "*. . .[l]imited data on other cancers also suggest no risk from mentholation. . .*". The weight of epidemiological data suggesting that the risks of mentholated and non-mentholated cigarettes are similar that was available to the authors at that the time of their writing has since been further strengthened by additional studies that are included in the present review.

Other studies discussed in the following sections are consistent with the additional 2007 conclusions of Werley and colleagues that "[*t*]*he scientific literature suggests that cigarette mentholation does not increase puff number or puff volume of smoked cigarettes, and has little or no effect on heart rate, blood pressure, uptake of carbon monoxide, tar intake or retention, or blood cotinine concentration*". Additional recent studies discussed herein also provide further evidence consistent with their conclusion that "*. . .[m]entholation has little effect on other smoke constituents and no apparent effect on nicotine absorption, airway patency and smoking initiation, dependency or cessation. . .*" (Werley et al., 2007).

Murray et al. (2007)

Murray et al. (2007) reported an investigation of mortalities from a variety of causes among a cohort of 5887 adult smokers

followed for 14 years as participants in the Lung Health Study (LHS) of smoking cessation and COPD prevention.

The authors sought to determine whether self-reported smokers of mentholated cigarettes exhibited any differences in hazard ratios for several smoking-associated diseases compared to smokers of non-mentholated brands. Several other questions relating to potential relationships between menthol, successful smoking cessation and nicotine dependence were also explored.

The authors acknowledged that the study cohort was developed from participants in a clinical smoking cessation study rather than from a representative community sample, and that the 114 black subjects (4% of total) underrepresented the proportion of blacks in the general population. Nevertheless, the total number of menthol smokers in the LHS smoker cohort (1216 of 5887 participants) was adequate to support the authors' evaluation of menthol as a potential independent contributor to the investigated disease endpoints.

Analyses employing proportional hazards regression methods revealed no differences between menthol and regular cigarettes in risks for coronary heart or cardiovascular disease, lung cancer, or death from other causes. Nor were menthol-associated differences in smoking cessation success observed between smokers of regular and mentholated cigarettes. Menthol cigarette smokers had in fact smoked fewer pack-years at baseline than had regular cigarette smokers.

The authors concluded that their study provided no evidence that the use of menthol in cigarettes contributes to the hazards of smoking. Notably, these authors made passing mention in their introductory remarks of a manuscript in preparation that may report findings which suggest that "*...menthol cigarettes are indeed protective against cancer...*" relative to regular cigarettes (Murray et al., 2007). The report under development that is referred to has not yet appeared in published form at the time of this writing (June, 2009).

Etzel et al. (2008)

Etzel et al. (2008) recruited 491 African-American lung cancer patients and 497 race-matched control subjects from the M.D. Anderson Cancer Center and the M.E. DeBakey VA Medical Center in Houston to develop and validate a lung cancer risk prediction model applicable to African-Americans. Subject characteristics were extensively documented in terms of occupational exposures to wood dusts and asbestos, as well as for diagnosis of non-cancer respiratory conditions. The duration and intensity (pack-years) of subjects' menthol or non-menthol cigarette smoking habits as well as any smoking cessation histories were also considered in the model.

Consistent with numerous prior studies, both current and former smokers exhibited significantly increased risks for lung cancer, with ORs of 6.20 and 3.38, respectively. However, current smokers of menthol cigarettes were found to have a lung cancer odds ratio of 0.69 (0.4–1.03, 95% CI) relative to smokers of non-mentholated brands. The authors stated that "...[*t*]*he use of mentholated cigarettes seemed to be protective in current smokers, although the* OR *did not reach statistical significance ($p > 0.05$) even after stratification by pack-years (⩽40 vs. >40 pack-years; data not shown)*". The authors discussed this finding with references to other epidemiology studies (Kabat and Hebert, 1991; Stellman et al., 2003; Brooks et al., 2003) that had previously reported lower cancer risks for mentholated cigarettes relative to non-menthol cigarettes.

The menthol cigarette ORs for former smokers was 0.99 (0.62–1.56, 95% CI). Etzel and co-authors stated in summarization of their analysis of the menthol cigarette findings for African-American smokers that "*...we observed no significant* [excess] *risks of lung cancer among former or current smokers who reported smoking mentholated cigarettes (OR range, 0.69–0.99) and our data suggested a possible protective effect of mentholated cigarettes for current smokers*".

4.1. Conclusion regarding menthol cigarette epidemiology

Menthol is unique among commonly-added cigarette ingredients in that there is extant a considerable epidemiological literature providing a compelling weight of evidence that its use does not meaningfully increase smoking-related disease risks (Table 2). The body of available epidemiological evidence to date provides a substantial basis for a conclusion that the risks for the development of cancers and other diseases associated with the smoking of menthol cigarettes are no different, qualitatively or quantitatively, than those associated with non-mentholated cigarette smoking. Other authors and commentators (Alberg et al., 2006; Hebert, 2003; Werley et al., 2007) have previously come to similar conclusions, and all of the most recent studies summarized in the present review (Pletcher et al., 2006; Murray et al., 2007; Etzel et al., 2008) strengthen those prior judgments. Two of these recent reports extend the body of evidence that the risks accompanying the smoking of menthol cigarettes are similar in magnitude to those of non-mentholated cigarettes to include data on overall mortality as well as cardiovascular and respiratory disease.

5. Menthol cigarette smoking topography and smoke exposure biomarkers studies

There is an emerging overlap in the contemporary scientific literature among menthol cigarette epidemiology studies, experimental menthol smoking topography studies and menthol cigarette smoke biomarkers reports. One result of this promising trend accompanying the pursuit of increasingly sophisticated and informative study designs is the inclusion of one or more of these complimentary approaches in a single report.

5.1. Menthol and smoking topography

It is widely recognized that no two individuals smoke a cigarette in precisely the same way. Inter-individual differences in smoking behavior are manifested by differences in puff volume, number and frequency; depth of inhalation; duration of smoke retention in the lungs; percentage of cigarette smoked and other variables that are referred to collectively as elements of "smoking topography". Certain of these elements of smoking behavior may be quantified directly, while others are developed or calculated from primary physiological or observational data. Many of the available studies of the potential of menthol to affect human smoking topography have employed measurement of elevations in exhaled breath carbon monoxide (CO) to assess smoking intensity. While convenient, the utility of exhaled CO as a biomarker of smoke intake is compromised somewhat by its lack of specificity, its protracted and variable elimination half-life, and its variable quantitative relationship to other smoke constituents across cigarette designs.

Wagenknecht et al. (1990) and a number of other authors (Garten and Falkner, 2004; Ahijevych and Garrett, 2004) have speculated that the smoking of mentholated cigarettes may result in an increased nicotine intake relative to that experienced by smokers of non-mentholated cigarettes due to an "anesthetic" effect of menthol that increases the depth of inhalation. Although menthol is among many substances that have been shown experimentally to exhibit some capacity to evoke both sensitization (irritation) and desensitization at different levels of exposure, its modest and transient action as a desensitizer would appear unlikely to

be manifested at realistic levels of menthol exposure that accompany cigarette smoking (Green and McAuliffe, 2000).

There is considerable evidence that, aside from its familiar minty taste, menthol's predominant sensory effects at levels employed in cigarette flavoring applications is manifested through the compound's stimulation of cold receptors (Patel et al., 2007). Experimental menthol stimulation of respiratory tract cold receptors is accompanied by a slight, transient decrease in respiration (Eccles, 1994; Nishino et al., 1997). While the breathing of menthol vapor results in a marked increase in the sensation of increased airflow due to its stimulation of respiratory tract cold receptors, several human clinical studies have shown either no actual increase or a measurable decrease in respiratory airflow (Eccles, 1994). A similar reduction in ventilation by menthol's interaction with respiratory tract cold receptors was observed in a guinea pig model, and this effect was attenuated by application of a topical anesthetic (Orani et al., 1991).

Nil and Battig (1989)

Nil and Battig (1989) investigated the influence of different commercial cigarette taste categories and of different machine-measured cigarette smoke yields on various measured parameters of smoking behavior. An ascending trend in tidal CO boost after smoking was observed across the menthol, dark tobacco, blond tobacco, and preferred brand taste categories; reflecting a number of statistically significant reductions in puffing parameters (reduced volume and frequency) for the menthol cigarettes. Overall, it was apparent that the mentholated cigarettes were smoked less intensely than were the cigarettes of any of the other taste categories.

Caskey et al. (1993)

Caskey et al. (1993) tested the hypothesis that the "cooling and topical anesthetic effects" of menthol would result in smokers taking more puffs from a mentholated cigarette than from a regular cigarette with a rapid-smoking procedure performed with cardiovascular monitoring. No difference in the number of puffs taken was evident between the menthol and regular cigarette, nor did the subjects' stated preference for menthol or regular cigarettes affect the puffing stop point. None of the study's findings provided any support whatsoever for the authors' *a priori* hypothesis that cigarette smoke mentholation would increase smokers' cumulative smoke intake.

Ahijevych and Wewers (1994)

Ahijevych and Wewers (1994) investigated the relationship between self-reported cigarette smoking rate and salivary cotinine concentration in a study of 142 black women. The mean salivary cotinine concentration for these menthol smokers (394 ng/ml; $n = 130$ smokers) was not significantly different from that recorded for non-menthol smokers (369 ng/ml; $n = 12$ smokers). Expressed on a per cigarette basis, the salivary cotinine value for menthol smokers was reported to be 37.9 ng/ml/cigarette, while that for non-menthol smokers was 33.6 ng/ml/cigarette. However, since the nicotine yields of the cigarettes smoked by participants were not reported, and the menthol-preferring subjects reported a somewhat higher smoking rate than did the regular cigarette smokers (14.8 ± 9.7 *vs.* 11.4 ± 5.7 cigarettes/day; difference not statistically significant), it is difficult to draw any firm conclusions from this study beyond the fact that it provides no evidence for any meaningful effect of menthol on salivary cotinine levels in the black female subjects.

Ahijevych et al. (1996)

A follow-up study by Ahijevych et al. (1996) extended their initial investigation into the effects of cigarette mentholation on the levels of smoke biomarkers in women. Menthol and regular cigarette smokers consumed one of their usual brand cigarettes during the experiment, and pre- and post-smoking samples of end-expired CO and blood nicotine and cotinine were collected as smoke intake biomarkers. Smoking topographic variables were also recorded. The regular cigarette smokers exhibited significantly higher mean end-expired CO "boost" (elevation from smoking; 10.6 ppm) than did menthol smokers (6.5 ppm). Neither FTC CO ratings of the cigarettes nor differences in puffing topography appeared to account for these differences. No differences in blood nicotine boost were associated with menthol, nor were any significant differences in puff topography noted in any of the comparisons. The authors' initial hypothesis that cigarette mentholation would increase levels of smoke biomarkers in female smokers was not borne out. If anything, the findings of this study suggest that menthol cigarettes may be inhaled to a modestly lesser degree than are regular cigarettes.

Ahijevych and Parsley (1999)

Ahijevych and Parsley (1999) reported a third investigation into smoke constituent exposure and smoking topography among black and white women smokers of mentholated and regular cigarettes. A statistically significant greater puff volume was reported for menthol smokers relative to regular cigarette smokers (45.8 ml *vs.* 37.8 ml, respectively, $p = 0.03$), an observation that is in marked contrast to previous reports of significantly (Nil and Battig, 1989; Jarvik et al., 1994; McCarthy et al., 1995) or marginally (Ahijevych et al., 1996) reduced puff volumes for mentholated smoke relative to regular cigarette smoke. Menthol smokers were also reported to exhibit higher baseline cotinine levels than those of regular cigarette smokers (239 *vs.* 189 ng/ml, respectively; $p = 0.02$).

Unfortunately, the study participants were instructed *not* to abstain from smoking before the laboratory smoking session and reported uncontrolled smoking of their own cigarettes at a mean of 95 min (median 35 min) before the controlled laboratory smoking. Self-reported daily smoking rates were not confirmed by any independent means, and the nicotine and CO yields of the subjects' cigarettes were not reported. In light of these methodological shortcomings, the authors' attribution of the differences seen to menthol is difficult to accept. Cotinine is but one of a number of major known nicotine metabolites, and since it exhibits a plasma half-life of about 17 h it is reasonable to conclude that the reported cotinine values included a substantial contribution from uncontrolled smoke constituent intake prior to the laboratory smoking session.

Since the manner of presentation of the CO boost and nicotine boost data from the laboratory smoking session does not permit an assessment of the potential effect of cigarette mentholation as an independent variable, the paper's statement that "[*t*]*here were several significant differences on smoke constituent exposure by menthol preference*" is not supported by any controlled smoking data whatsoever in the published paper. The author's previous work employing a similar experimental protocol had reported a significantly *lower* CO boost and marginally *lower* nicotine boost for menthol smokers than for regular cigarette smokers (Ahijevych et al., 1996).

Miller et al. (1994)

Miller et al. (1994) attempted to evaluate the effect of the addition of menthol to cigarettes on inhaled puff volume and CO

exposure (as assessed by CO exhalation). Following overnight smoking abstinence, 12 subjects smoked two commercial cigarettes that had been injected with 40 μl of an alcoholic solution containing 0, 4, or 8 mg menthol. The subjects smoked each of these three experimental cigarettes in two separate sessions using a mechanical device that delivered puffs at 30-s intervals until the subjects had inhaled a volume of 600 cc smoke per cigarette. Breath samples were collected prior to smoking for CO analysis, and then again after the first 600 cc smoke inhalation session, and a third time after completion of the second cigarette 600 cc smoke inhalation. Blood pressure and heart rate data were collected in parallel with the CO exhalation measurements.

Neither menthol addition *per se*, nor the quantity of added menthol had any significant effect on puff volume or number, nor were any effects of menthol on heart rate or blood pressure evident. However, the authors reported that at the highest, 8 mg addition level of menthol, the elevation in CO exhalation of 8.1 ppm was significantly greater than both the 6.1 ppm CO exhaled after the 4 mg menthol cigarettes and the 5.6 ppm CO exhaled following the non-menthol reference cigarettes.

The authors concluded that the results "*demonstrated that menthol influences the absorption of one constituent of cigarette smoke: exhaled carbon monoxide*", and further speculated that it may similarly increase the absorption of other smoke constituents. However, the six study participants who were self-reported menthol smokers exhibited a statistically significant ($p < 0.05$) greater CO increase (7.6 ppm) than did the participants who normally smoked regular cigarettes (5.6 ppm) after the entire 1200 cc smoke inhalation session, suggesting that a taste preference for mentholated smoke among the menthol-preferring subjects, manifested as increased puff retention times (a smoking parameter not recorded) may have contributed to the modest menthol-associated increases in CO exhalation observed in the experimental smoking session. The findings of this small study are contrary to those of several others, and the authors' suggestion that menthol may somehow increase CO transfer across respiratory membranes is unsupported by a biologically plausible mechanism. It would seem reasonable to view this speculation with caution pending independent confirmation under a protocol employing a naturalistic smoking regime.

Jarvik et al. (1994)

Jarvik et al. (1994) investigated the potential of menthol to affect the depth and retention of inhaled smoke due to a postulated "local anesthetic" effect. Ten regular and ten menthol cigarette smokers consumed a single commercial cigarette of each type having identical FTC smoke yields (1.2 mg nicotine, 16 mg "tar", and 15 mg CO) in two laboratory sessions. Smoking parameters were recorded with a pressure transducer placed in the airstream entering a glass chamber containing the lit cigarette. Mainstream smoke exiting the cigarette filter was split into two pathways; one passing through a Cambridge filter to obtain an estimate of inhaled "tar" mass, and another leading directly to the mouthpiece. Smoke puffs were exhaled into a collecting device having a Cambridge filter to capture exhaled particulates; end-expired air samples and blood obtained by an indwelling venous catheter were analyzed for CO and carboxyhemoglobin (COHb), respectively.

Mentholated cigarettes were found to produce significantly smaller mean puff volumes and significantly smaller numbers of total puffs, for a smaller cumulative puff volume than regular cigarettes ($p < 0.001$). Mean puff flow rates were significantly lower for menthol cigarettes; while other smoking parameters such as puff duration, interpuff interval, and lung retention times were similar for both menthol and regular cigarettes.

Clearly, and in contrast to the authors' hypotheses, no indication of any increased intensity of puffing was found to be associated with cigarette mentholation. No significant effects of cigarette mentholation or subjects' stated cigarette mentholation preference on the quantities of particulate material inhaled were apparent. However, black smokers were found to retain a significantly smaller ($p < 0.01$) percentage of inhaled smoke particulates than did whites under the study conditions.

Although no significant difference in end-expired CO boost was evident between regular and menthol cigarettes, both the end-expired CO boost and the elevation in blood COHb by menthol cigarettes were statistically significantly greater than those values seen after regular cigarettes when expressed relative to the cumulative puff volumes inhaled. This finding led the authors to speculate about a "*. . .menthol-related increase in either the diffusivity of the alveolar capillary membrane for CO transfer or in the affinity of hemoglobin for carbon monoxide. . .*", these explanations seem highly improbable as CO is well known to diffuse quite readily through the alveolar membrane and to exhibit an extremely high affinity for hemoglobin in the absence of menthol.

The authors' conclusions regarding menthol and CO absorption were further compromised by the fact that the subjects were asked to smoke one of their usual brand cigarettes a mere 30 min before the experimental smoking session "*. . .to ensure that they would not be nicotine-deprived at the beginning of the experiment*". Carbon monoxide is eliminated from carboxyhemoglobin as exhaled CO, with a half-life of 4–6 h under conditions of breathing room air (Ernst and Zibrak, 1998). Thus the end-expired CO collections that constitute the basis for the study's significant findings unquestionably included a substantial and uncontrolled contribution of CO from pre-study smoking, rendering conclusions founded on those baseline values tenuous.

McCarthy et al. (1995)

McCarthy et al. (1995) reported experiments in which 29 male subjects smoked either a regular or a menthol cigarette in two sessions of rapid and intensive smoking conducted 1 week apart. Subjects took fewer puffs and a smaller puff volume when rapidly smoking menthol cigarettes than when smoking regular cigarettes. Cumulative menthol smoke intake volume was a significant 38.8% less than was the intake of regular smoke. There were no significant menthol-associated effects on heart rate, blood pressure, or expired CO under the rapid smoking conditions of the experiment.

The investigators concluded that their finding that CO exhalation and cardiovascular measurements were not reduced proportionately by the reduced puffing intensity observed with mentholated smoke is consistent with an increased efficiency of CO uptake in the presence of menthol. However, the menthol cigarette employed in the study delivered a nominal 13% more CO than did the regular cigarette, and there was apparently no pre-study smoking abstinence period. Nor was any validation of the sensitivity of the cardiovascular functional assessment protocols to relatively minor differences in the intake of the purported active smoke constituents reported. Considered together, these shortcomings in the study design render the authors' conclusions purely speculative.

Clark et al. (1996)

An attempt to evaluate the effect of menthol on biochemical markers of smoke exposure among black and white smokers was reported by Clark et al. (1996). One hundred and sixty-one subjects of both sexes provided cigarette brand preference and other questionnaire data and collected their daily cigarette butts for 1 week as measures of smoke intake. The participants were held in the laboratory for 1 h of smoking abstention before a blood sample was collected for baseline serum cotinine analysis, and an end-expired

breath sample was obtained to establish a baseline CO exhalation value. The subjects then smoked one of their own self-provided cigarettes in a normal manner, whereupon post-smoking blood and breath samples were collected.

Post-smoking serum cotinine levels were reported to be significantly higher for menthol smokers (478.2 ng/ml) than for regular cigarette smokers (349.1 ng/ml). The menthol smokers' mean cotinine level remained 84.5 ng/ml higher than that of regular cigarette smokers after adjusting for race, cigarettes per day, and mean millimeters of each cigarette smoked ($p = 0.03$). The mean unadjusted expired CO level of menthol smokers was not significantly different from the level reported for the regular cigarette smokers (40.3 and 35.8 ppm, respectively). However, menthol was described as a significant contributor to expired CO levels after adjustment for cigarettes per day and the amount of each cigarette smoked ($p = 0.02$).

The authors concluded that "*. . .menthol was associated with higher cotinine levels. . . and carbon monoxide concentrations*" and, further, that "*[t]he use of menthol may be associated with increased health risks of smoking*". However, a number of shortcomings in the design of the study call into question the adequacy of experimental support for these conclusions. The nicotine yields of the subjects' preferred brand cigarettes employed in the study were not considered, nor were CO yields reported or included in the model. These omissions compromise the CO and cotinine boost findings attributed to the laboratory smoking session that constitute the essential findings of the study. Furthermore, it is quite clear that the 1 h pre-study smoking abstention interval employed in the Clark study was entirely inadequate to permit clearance of CO (elimination half-time 4–6 h) (Ernst and Zibrak, 1998) and cotinine (plasma half-life approximately 17 h) (Benowitz, 1996), from uncontrolled pre-study smoking. An indication of the substantial contribution from this irrelevant source to the measured post-smoking cotinine and CO is seen in the authors' model, in which "*. . .the most important predictors of serum cotinine levels were cigarettes per day and the mean amount of each cigarette smoked. . .*". The values of these parameters were determined entirely by uncontrolled smoking prior to the laboratory session, consistent with a likelihood that a substantial portion of the detected post-smoking cotinine and CO was actually derived from smoking outside of the study.

Pritchard et al. (1999)

Pritchard et al. (1999) performed tidal breath CO measurements in subjects smoking mentholated and non-mentholated "denicotinized" cigarettes in the course of their investigations of possible pharmacological and physiological effects of menthol. The experimental 85-mm filter cigarettes were closely matched in terms of nominal FTC smoke yield and appearance [mentholated: 0.06 mg nicotine, 7.8 mg "tar", 8.4 mg CO; non-menthol: 0.06 mg nicotine, 8.2 mg "tar", 8.6 mg CO]. The experimental subjects were instructed to smoke as little as possible on the morning before the laboratory smoking sessions, but no formal smoking abstention interval was enforced. Pre-study breath CO measurements were not significantly different between the 10 participants stating a preference for regular cigarettes and the 12 who self-reported to be menthol cigarette smokers. Each participant smoked one regular and one menthol cigarette in a balanced order with an intervening session of data collection and a rest period. No significant difference in tidal breath CO boost was evident between the menthol and non-menthol cigarettes.

Pickworth et al. (2002)

Pickworth et al. (2002) examined the potential of menthol and nicotine delivery to interact in affecting physiological and subjective assessments of cigarette strength and satisfaction in menthol and non-menthol smoking volunteer subjects. Non-mentholated research cigarettes designed to deliver a low FTC smoke yield (0.2 mg nicotine/12.4 mg "tar") or a high yield (2.5 mg nicotine/20.9 mg "tar") were prepared; as were similar low- and high-yield mentholated cigarettes with FTC smoke deliveries of 0.2 mg nicotine/11.2 mg "tar" and 2.5 mg nicotine/20.8 mg "tar", respectively. Several commercial menthol and non-menthol cigarettes having FTC smoke deliveries ranging from 1.1 to 1.3 mg nicotine and 10.9–17 mg "tar" were also employed in the study. The carbon monoxide yields of the study cigarettes were not reported.

Study participants reporting themselves to be usual smokers of menthol or non-menthol cigarettes were instructed to smoke one cigarette each of the low-yield experimental, high-yield experimental and commercial cigarettes in a random order, 45 min apart, as a number of physiological and subjective responses were monitored. Participants were asked to smoke the type of cigarettes they normally preferred (menthol or non-menthol) to minimize the effect of smoke taste preference on smoking behavior. No formal smoking abstention period was enforced before the laboratory smoking; participants had typically smoked one of their own cigarettes about 45 min before the laboratory session. Physiological measurements were made before and after each cigarette smoking session, and subjective impressions of sensory effects were recorded after each cigarette.

No statistically significant differences in exhaled CO boost after smoking were seen between menthol and non-menthol cigarettes of any yield category. This endpoint also proved to be insensitive in detecting any differences between the low-yield, high-yield, and commercial cigarettes whatsoever. Menthol appeared to have no independent effect on changes in heart rate, systolic or diastolic blood pressure. However, these cardiovascular measures revealed a number of differences among cigarettes consistent with their differences in FTC nicotine yield. Menthol was found to have no measurable effect on number of puffs per cigarette, time to smoke the cigarette, or subjective evaluations of smoke "strength". The latter parameter was reported as a composite of subjective ratings of nose, tongue, throat, conducting airway, or chest impact/sensation. However, both menthol and non-menthol commercial cigarettes were smoked more rapidly than were the experimental cigarettes, and the experimental low-yield cigarette was subjectively rated as less strong than either the commercial or experimental high-yield cigarettes.

A final evaluation of the participants' subjective responses to the laboratory smoking sessions was performed by administration of the Duke Sensory Questionnaire and Cigarette Evaluation Scale. These instruments tabulate smokers' ratings of cigarettes in terms such as "puff satisfaction", "high in nicotine", "similarity to own brand", "craving relief", "negative effects", and "psychological reward". The commercial cigarettes and high-yield cigarettes were frequently preferred over the low-yield experimental cigarettes. No significant differences in subjective ratings of menthol and non-menthol cigarettes of similar smoke yield were recorded with the exception of "satisfaction" and "craving relief", in which the non-menthol cigarette was rated higher than the menthol cigarette of comparable yield.

The findings of Pickworth et al. (2002) that the physiological and sensory effects of smoking are closely related to nicotine smoke yield and independent of the presence of menthol are consistent with those of Pritchard and associates (1999), who found that menthol delivered in denicotinized cigarettes had no measurable physiological effect. The further demonstration by Pickworth and coworkers that measurement of exhaled CO boost is an insensitive means to compare among cigarettes having substantial differences in analytical smoke yield and physiologically-meaningful cardiovascular effects suggests that the utility of this technique

as a measure of human smoking behavior may be more limited than previously thought. This observation is tempered by the reality that the CO yields of the test cigarettes were not reported in the paper.

O'Connor et al. (2007)

O'Connor et al. (2007) collected data on CO boost by collecting exhaled CO before and after smoking, and recorded smoking topography parameters with a small pressure and flow transducer/recorder device to evaluate whether a sample of 20 college student smokers exhibited differences in the manner in which they smoked a conventional cigarette (Camel Lights) and a "flavored" cigarette (Camel Exotic Blends) product. While the menthol content of the latter commercial cigarette was not stated, the varieties employed included several brands having mint as a characterizing flavor (*e.g.* Dark Mint, Mandarin Mint, MochaMint), so it is reasonable to presume that they contained menthol or related flavorings.

The authors found no substantive differences in smoking intensity between the flavored and unflavored cigarettes, as indicated by similar CO boosts and total exposure, similar smoking topography, and similar subjective assessments of smoothness, harshness, mildness and irritation. The authors concluded that "*. . .we did not find evidence that adding flavors to Camel cigarettes dramatically altered exposures, nor how the cigarettes were smoked or rated compared to an unflavored cigarette of comparable nicotine yield. . .*" and that "*. . .adding flavors to cigarettes may not significantly impact how they are smoked by current smokers*".

Strasser et al. (2007)

Cytochrome P450 2A6 (CYP2A6) is the primary hepatic enzyme responsible for the metabolic conversion of nicotine to its biologically-inactive metabolite cotinine, as well as for some portion of the subsequent conversion of cotinine to other metabolites. Strasser et al. (2007) reported an investigation of three human phenotypes characterized as having slow, intermediate or normal CYP2A6 metabolic capacities, to determine whether such genetically-determined differences in nicotine-metabolizing efficiency were related to measurable differences in smoking topography (number of puffs, mean puff volume and total puff volume per cigarette). The potential of this phenotype to affect several parameters of cigarette preferences, including mentholation, was also explored. Although previous work had reported that smokers characterized as slow metabolizers of nicotine consumed fewer cigarettes per day and exhibited lower levels of smoke exposure biomarkers, this study was the first to explore the potential of differences in CYP2A6-mediated nicotine clearance efficiency to affect the manner in which cigarettes are smoked.

One hundred and nineteen participants entering a smoking cessation trial provided demographic and cigarette preference information at baseline; smoking topography data were recorded with the commercially-available Clinical Research Support System (CReSS) device. CYP2A6 phenotypic classification was performed with a PCR-based procedure that had previously been employed in a study reporting a high incidence of the slow-metabolizing genotype among African-American smoking subjects (Fukami et al., 2004).

Strasser and coworkers found no race-associated differences in the distribution of slow, intermediate and normal nicotine metabolizer phenotypes. Most smokers (79.3%) were categorized as normal nicotine metabolizers, while 13.8% were intermediate metabolizers and 6.9% exhibited the slow metabolizer phenotype. Smokers of the slow nicotine metabolizing phenotype exhibited statistically significantly reduced total and mean puff volumes relative to those having intermediate and normal nicotine metabolizing capacities. However, total puff volumes per cigarette and mean puff volumes were not significantly different between menthol and non-menthol cigarette smokers, consistent with a lack of any meaningful effect of menthol on these elements of smoking behavior via a mechanism relating to nicotine metabolism.

No significant differences among the genotypic classification assignments were evident for regular/lights cigarette preference, menthol/non-menthol preference, sex, or nicotine dependence measures. Thus, this report suggests that the normal pharmacogenetic variability that characterizes human nicotine metabolism may substantially limit the practical significance of the previous observation, as yet unconfirmed, that menthol may slow the metabolism of nicotine (Benowitz et al., 2004).

Ciftci et al. (2008a,b)

Ciftci et al. (2008a) sought to determine whether the smoking of menthol and non-menthol cigarettes may differentially affect coronary microvascular function in a clinical evaluation of 6 female and 14 male young adult smokers. The cigarettes employed in the study were closely matched in terms of machine-generated tar, nicotine, and carbon monoxide smoke yields. Echocardiographic evaluation was performed at baseline and again 20–30 min after subjects had smoked two menthol or non-menthol cigarettes. Impairment of coronary flow reserve did not differ ($p = 0.547$) between the cigarette types. The authors concluded that menthol and non-menthol cigarettes have similar detrimental effects on coronary microvascular functions.

A second investigation by these authors (Ciftci et al., 2008b) sought to determine whether menthol and non-menthol cigarettes induced different acute effects on left and right ventricular function. Eighteen healthy smokers and 20 non-smoking control subjects were subjected to baseline echocardiographic and tissue Doppler imaging evaluations; and the smoking subjects were again evaluated 20–30 min after having smoked two menthol or non-menthol cigarettes of similar machine-smoked tar, nicotine and carbon monoxide yield within 15 min in a closed room. The smoking procedure was repeated 15 days later with switching of the initial menthol and non-menthol cigarette assignments between the two groups of smoking subjects. No information on the smoking subjects' stated preferences for menthol or non-menthol cigarettes was reported. Both cigarette types were reported to induce a number of adverse effects on measures of left and right ventricular function. The menthol cigarette was reported to exert a significantly greater effect on systolic velocity and regional isovolumic contraction time (IVCTr) and on the myocardial performance index of the right ventricle (MPIR). The authors concluded that menthol cigarettes caused greater adverse effects on right ventricular tissue Doppler velocities than did non-menthol cigarettes in their experimental smoking subjects.

St.Charles et al. (2009)

An investigation of smokers' interpuff inhalation/exhalation depth and volume across a range of cigarettes having different machine-determined smoke yields was reported by St.Charles et al. (2009). The study was performed as an adjunct to an independently-reported investigation of smokers' biomarkers of exposure derived from the same commercial cigarettes (St.Charles et al., 2006). The biomarkers study found no substantive differences in smoke biomarkers levels between smokers of mentholated and non-mentholated cigarettes (St.Charles, personal communication). The breathing and smoke inhalation patterns were measured in 74 subjects by inductive plethysmography as they smoked one of their preferred brands of cigarettes on 2 days of a 5-day inpatient clinical study of smoking biomarkers. Cigarette smoke inhalation

volumes were normalized by resting tidal volumes to account for inter-individual differences in lung volume capacity. No significant differences in any respiratory measures were reported among the four different mainstream tar yield categories that were the primary focus of the investigation. The authors also analyzed their data for any apparent differences in inhalation patterns between the 18 participating smokers of menthol cigarettes and 56 smokers of non-menthol cigarettes, and concluded that "*...no difference in inhalation tidal ratio was found between smokers of mentholated and unmentholated products, consistent with the broader review of the effect of mentholation on smoking behavior by* Werley et al. (2007)". The study of St.Charles et al. (2009) is notable in that it was a relatively large study that assessed smoking topography with unobtrusive chest bands under normal smoking conditions rather than through the use of a topographic data capture device affixed to the cigarette.

5.1.1. Conclusions regarding menthol and smoking topography

The body of available studies on menthol and smoking topography do not provide convincing support for the hypothesis that menthol cigarette smoke is inhaled more intensely than is the smoke of regular cigarettes. The findings of studies to date are mixed, and the outcomes of diverse experimental attempts to measure human smoking topography may be method-dependent. A number of these investigations have found that smokers draw fewer and shallower puffs of mentholated smoke compared to regular smoke under comparable conditions, while others suggest more intense puffing of menthol cigarettes. Overall, these studies are consistent with the findings of more quantitative smoking biomarkers investigations, reviewed below, that do not support speculation that the absorption of smoke constituents from a given inhaled volume of smoke is meaningfully affected by menthol employed as a cigarette flavoring ingredient.

5.2. Menthol and smoke exposure biomarkers

A number of biomarkers of smoke exposure are presently in various stages of identification, refinement, and validation in support of efforts to determine whether differences in exposure to various smoke constituents of interest may result from the smoking of different types of cigarettes (Hatsukami et al., 2006; Zedler et al., 2006). Such studies typically employ analytical measurements of smoke constituents or their metabolites in exhaled breath, blood, saliva or excreted urine. This approach holds promise as a means to assess the net effect on internal exposures that may result from both cigarette type (*e.g.* blended-type *vs.* all flue-cured cigarettes, cigarettes of lower *vs.* higher machine-measured smoke yield, menthol *vs.* non-mentholated brands) as well as any effects on smoking behavior that may exist for different types of cigarettes. To the extent that smoke exposure biomarkers may be refined to a point that they may be compellingly associated with the risks and mechanisms of smoking-associated diseases, they hold promise as potential sentinels by which the chronic disease risks attending the smoking of different types of cigarettes may be estimated (Stratton et al., 2001; Hatsukami et al., 2006).

Nicotine and its metabolites are perhaps the most-studied among the available biomarkers employed in smoking research. A 2004 report by Benowitz and colleagues, discussed below, suggested that menthol may affect the metabolism of nicotine, and these authors explored the potential of such an effect to influence smoke constituent exposures accompanying menthol cigarette smoking. An earlier report by MacDougall et al., 2003 had reported inhibitory effects of menthol and similar compounds on the *in vitro* oxidation of nicotine by human microsomes, but the rather modest potency of menthol in affecting these enzyme activities does not suggest a likelihood of a meaningful effect *in vivo* at exposure levels resulting from menthol's use in cigarettes. Indeed, a number of subsequent studies in humans have not observed metabolic interactions between menthol and a number of drugs that are well-characterized as human Cytochrome P450 substrates (Gelal et al., 2003, 2005). A number of recent studies in this emerging field have attempted to develop information in regard to the potential of cigarette-delivered menthol to influence smokers' exposures to nicotine or to other smoke constituents that may have a role in the etiology of smoking-related disease. These studies are considered below in the chronological order of their appearance in the peer-reviewed literature.

Richie et al. (1997)

Richie et al. (1997) reported a study comparing urinary 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronide in 34 black and 27 white smokers to test hypotheses that racial differences in lung cancer risk may be related to differences in metabolism. These metabolites of the tobacco-specific nitrosamine NNK (4-(N-nitrosomethylamino)-1-(3-pyridinyl)-1-butanone) have been described as a sensitive and reliable measure of NNK exposure from tobacco sources. The authors adjusted excreted biomarkers values for numbers of cigarettes consumed, and reported higher levels of free NNAL in the urine of black smokers (1.22 ± 1.44 *vs.* 0.603 ± 0.345 pmol/mg creatinine for whites, $p < 0.05$). The NNAL-glucuronide/free NNAL ratio was also significantly lower for black smokers than for white smokers (3.11 ± 1.67 *vs.* 4.43 ± 2.60, $p < 0.01$). The authors suggested that their findings may indicate that black smokers are less efficient in detoxifying NNK. However, there was no evidence that this race-associated difference was due to menthol cigarette preference. Richie and coworkers suggested that race-associated differences in NNK detoxification may partially explain the higher risks for lung cancer reported for black smokers, while further concluding that "*...it is unlikely that the dissimilarities are due to racial differences in preference for mentholated cigarettes...*", with reference to their accompanying studies in rats that have been discussed above.

Rosenblatt et al. (1998)

Rosenblatt et al., 1998 investigated olfactory thresholds for nicotine and menthol in non-smoking subjects as well as in smoking subjects with and without a 1-day abstinence period. The study was pursued to develop information about the sensory role of nicotine in smoking behavior and acute and chronic sensory/olfactory tolerances to nicotine. However, exhaled breath carbon monoxide measurements were collected from five male menthol and five male non-menthol cigarette smokers in the course of the sensory experiments. The menthol cigarette-smoking subjects exhibited a mean exhaled breath carbon monoxide concentration of 16.2 ppm, which was statistically significantly lower ($p < 0.05$) than the 24.4 ppm carbon monoxide exhaled by the non-menthol cigarette smokers. While this small study was primarily intended to examine other endpoints, the reported findings are consistent with a lower smoke constituent exposure for menthol cigarette smokers.

Patterson et al. (2003)

Patterson et al. (2003) reported a study of 95 male and 95 female treatment-seeking smokers aged 18–75. Baseline plasma nicotine and cotinine levels were determined; as were pre- and post-smoking values, with differences between the latter reported as "boosts" in analyte values.

Black smokers exhibited higher baseline cotinine and "nicotine boost" than did whites in the authors' univariate analysis.

However, neither race nor menthol was a significant contributor to "nicotine boost" in the multivariate model analysis. Menthol smokers exhibited higher baseline cotinine levels, but there was no significant difference in "nicotine boost" per cigarette relative to those preferring non-menthol brands ($t = 0.49$, $p = 0.63$). The authors suggested that this may have been due to the relatively small numbers of study subjects.

"Lights" smokers exhibited statistically significant lower baseline nicotine and cotinine levels. This is consistent with lower long-term exposures from "lights" than "regular" cigarettes and with prior studies reporting the transient nature of "compensation" in forced-switching studies. "Lights" cigarettes smokers also exhibited a slightly (not statistically significant) lower "nicotine boost" from smoking one own-brand cigarette than did "regular" cigarette smokers (Patterson et al., 2003).

Benowitz et al. (2004)

Benowitz et al. (2004) reported a crossover study of 14 smokers, half of them African-American and half white, who were randomly assigned to groups smoking either a mentholated or non-mentholated cigarette of similar FTC smoke yield (Kool Kings or Marlboro Kings) for a 1-week period, at which time they were administered an intravenous dose of deuterated nicotine and cotinine. Urinary deuterated nicotine metabolites were collected to assess nicotine clearance under the mentholated or non-mentholated cigarette smoking condition. A second 1-week study interval was conducted after switching the subjects' menthol/non-menthol cigarette assignments. Carbon monoxide exposure data was collected as a general indicator of smoking intensity.

The authors reported no substantial differences in blood nicotine or carbon monoxide exposure, and no significant differences in urinary nicotine metabolite excretion. However, statistically significant differences in nonrenal clearance of nicotine (apparently representing deuterated nicotine metabolites not accounted for in excreted urine) led the authors to conclude that menthol inhibits nicotine metabolism. The authors stated that their findings "*... do not support the hypothesis that mentholated cigarette smoking results in a greater absorption of tobacco smoke toxins.*" The authors further concluded that "*...mentholated cigarette smoking enhances systemic nicotine exposure*". The findings of Benowitz et al. (2004) are consistent with the weight of epidemiological evidence, discussed above, which indicates that menthol cigarettes are no more harmful than are non-mentholated cigarettes.

Mustonen et al. (2005)

Mustonen et al. (2005) collected salivary cotinine and exhaled breath CO samples, recorded blood pressure, and gathered subjects' height and weight data from 307 smokers (256 White, 51 Black) recruited for a clinical smoking cessation trial employing transdermal nicotine replacement therapy (Mustonen et al., 2005). Women comprised 51.5% of the subjects and 28.7% of the subjects reported themselves to be smokers of mentholated cigarettes. Questionnaires were administered as a means to characterize smoking histories (duration, cigarettes per day [CPD]) and behaviors prior to the smoking cessation trial, and these data were analyzed with the pre-cessation biomarker and physiological data to determine any potential associations among sex, race and cigarette mentholation on the tobacco exposure measures.

The authors developed a 'cotinine/CPD ratio' for the subjects from the measured salivary cotinine values and self-reported smoking rate expressed as cigarettes per day. Exhaled breath CO measurements were corrected for ambient daily CO variations, but this adjustment can of course not fully account for the recent CO exposure history of individual study participants prior to their reporting to the clinic. The authors examined CPD and FTC nicotine yield as covariates, employing the term 'nicotine content level' to express the nicotine smoke yield for participants' reported cigarette brand preferences, with reference to the 2000 Federal Trade Commission report as the source of these data. Blood pressure and body mass index (BMI) were determined by standard methods.

Numerous statistical comparisons and correlations were reported; the present review will focus on the menthol-related findings. With regards to cigarette type (menthol *vs.* non-menthol) the authors found no difference in CPD ($F = 0.59$, $p = 0.44$). Cotinine levels were higher among the menthol smokers than the non-menthol smokers (mean = 476.1 ng/ml, S.D. = 218.7 *vs.* mean = 441.9 ng/ml, S.D. = 197.3) but this difference was not significant ($F = 1.8$, $p = 0.18$). Further analyses demonstrated that the cotinine/CPD ratio was higher among menthol smokers than non-menthol smokers (mean = 23.3, S.D. 13.6 *vs.* mean 19.4, S.D. = 9.4), $F = 8.2$, $p = 0.004$. The authors concluded that "*...these finding suggest that the relationship between number of cigarettes consumed and salivary cotinine is more complex that previously believed*".

Moolchan et al. (2006)

Benowitz and colleagues hypothesized in 2004 that menthol inhibition of nicotine metabolism by the hepatic enzyme Cytochrome P4502A6 may account for the frequently-reported observation that black smokers of mentholated cigarettes typically smoke fewer cigarettes per day than do age-matched white smokers (Benowitz et al., 2004). Moolchan et al. (2006) reported plasma *trans*-3′-hydroxy cotinine/cotinine ratios (a measure of CYP2A6 activity in metabolizing nicotine and cotinine) for 91 black and white 13–17-year old smokers who reported consuming at least 10 cigarettes/day and who had scored at least five on the six-item FTND instrument. Black participants exhibited significantly lower metabolite ratios, consistent with prior reports of slower cotinine clearance in black smokers (Perez-Stable et al., 1998). Separate analysis of menthol cigarette smokers in the study population (86% of blacks and 80% of whites in this urban Baltimore study setting) produced essentially identical results, leading the investigators to conclude that "*...the observed differences are due to factors other than menthol smoking*".

Williams et al. (2007)

Williams et al. (2007) reported an investigation of exhaled carbon monoxide and serum nicotine and cotinine in 89 schizophrenic smokers and 53 controls immediately after smoking their preferred brand of mentholated or non-mentholated cigarette in the afternoon. The report was a follow-up investigation to the authors' prior work that had reported that schizophrenics had 30% higher serum nicotine and cotinine relative to control subjects at similar rates of cigarette consumption.

Mean serum nicotine levels, a measure of recent smoking, were reported to be higher for the smokers of menthol cigarettes than non-menthol cigarettes, 27.2 (S.D. 10.9) ng/ml *vs.* 22.4 (S.D. 10.8) ng/ml, respectively, $p = 0.010$. Mean serum cotinine, a measure of smoking over approximately the last day, was similarly elevated in menthol smokers relative to non-menthol smokers, 294.3 (S.D. 172.2) ng/ml *vs.* 239.8 (S.D. 121.2) ng/ml, respectively, $p = 0.04$. Exhaled carbon monoxide was also higher for the menthol cigarette smokers, 25.1 (10.9) ppm *vs.* 20.6 (8.5) ppm, $p = 0.029$.

Although the group mean biomarker value comparisons above were adjusted for schizophrenia status, race and numbers of cigarettes smoked per day, the subjects reported smoking a variety of different full-flavor, lights and ultra-lights brands. The most frequently-smoked menthol brand among study subjects was Newport, which at the time of the study delivered 9% more nicotine

and 13% more carbon monoxide (FTC smoking protocol) than did the most frequently-reported non-menthol brand (Marlboro) in the study. Whether such yield differences among the cigarette brands and styles reportedly smoked by the study subjects may have contributed to the differences attributed to menthol cannot be determined from the published data.

Muscat et al. (2009)

Muscat et al. (2009) reported a community-based, cross-sectional study among 525 black and white smokers to examine levels of biomarkers of smoke exposure between smokers preferring mentholated and non-mentholated cigarette brands. The methods employed were generally similar to those reported in prior work by these investigators (Richie et al., 1997). Smokers of at least 5 cigarettes/day were requested to fast and abstain from smoking after midnight on the day before the study, and to report to the clinic for a spot urine collection at 9 a.m. Subsets of the study population were employed to develop comparative data on urinary and plasma cotinine, plasma thiocyanate, urinary 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronide (NNAL-Gluc). Detailed smoking histories were obtained from all subjects, and 278 participants were administered the Fagerström Test for Nicotine Dependence (FTND) to assess the degree of their self-described nicotine dependence. The smoking of mentholated cigarettes was not significantly associated with elevated FTND scores (OR 1.1; 95% CI, 0.6–2.0), but the menthol smokers in this study reported a marginally-increased propensity to smoke a cigarette within 30 min of waking OR 2.1 (95% CI, 1.0–3.8). This latter finding in regard to menthol cigarette smokers' reported shorter time to the first cigarette of the day is consistent with reports from the cessation clinic subjects studied by Foulds et al. (2006) and Gandhi et al. (2009), but is at odds with the report of Hyland et al. (2002), discussed below, that was developed from a substantially larger study population.

No significant differences were observed in the concentration of any of the measured smoke exposure biomarkers by menthol status in either black or white subjects when analyzed by age, sex and cigarettes smoked per day.

Although the total quantities of urinary NNAL and NNAL-Gluc were reported to be similar or slightly lower for black, white and all-subjects menthol cigarette smokers than for the respective non-mentholated cigarette smokers (authors' Table 2), the authors reported that the ratio of NNAL-Gluc to NNAL was significantly lower in menthol *vs.* non-menthol smokers (authors' Table 4). The NNAL-Gluc/NNAL ratio was a significant 34% lower for white menthol smokers ($p < 0.01$) and a marginal 22% lower for black menthol smokers. The authors hypothesized that menthol may inhibit or compete with the glucuronidation of NNAL in the human liver and thereby compromise the urinary clearance of this tobacco-specific nitrosamine metabolite from the body. However, the practical significance of NNAL-Gluc/NNAL metabolite ratio values in terms of human disease risk remains speculative.

An apparent inhibition of NNAL glucuronidation by a single human microsomal preparation incubated for 2 h in the presence of 0.5 mmol/l NNAL and 0–2.5 mmol/l menthol was described in a follow-up experiment. However, the authors acknowledged that neither the high experimental NNAL and menthol levels tested nor the microsomal preparation employed reflect *in vivo* events and called for further research on the topic. Although there are apparently no reliable published data on the systemic exposure to menthol that may result from its usage in cigarettes, an estimated delivery of 0.625 mg/cigarette developed by Benowitz et al. (2004) from measured quantities in commercial cigarettes suggests that those plasma concentrations may be on the order of about 1 μM, which is considerably below the levels tested by Muscat et al. (2009) in the microsomal incubation. Further investigation into the potential of menthol to inhibit NNAL glucuronidation does indeed seem warranted in light of the prior work by these authors that had suggested just the opposite effect in rats at very high levels of exposure (Richie et al., 1997), as well as the known high inter-individual variability (~49-fold) in NNAL glucuronidation capacities that have been reported among humans (Wiener et al., 2004).

Heck (2009)

One hundred and twelve male and female smokers participated in a parallel-arm study to determine whether the *ad libitum*, moderately heavy smoking of menthol or non-menthol cigarettes of similar machine-measured "tar" yield (~9-10 mg) may result in differences in smoke constituent exposure biomarkers in blood and urine (Heck, 2009). The commercial cigarettes employed in the study were provided to the subjects and were analyzed for mainstream smoke yields of "tar", nicotine, carbon monoxide and NNK. Study subjects smoked their preferred menthol or non-menthol cigarette types at home in their normal fashion, and both blood sampling and a 24-h urine collection were performed during two 24-h study intervals spaced 1 week apart. Blood carboxyhemoglobin levels were measured, as were six urinary nicotine metabolites (nicotine, cotinine, *trans*-3′-hydroxycotinine and respective glucuronides) and urinary 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronide conjugate. The cigarettes employed in the study were quite similar in terms of machine-measured yields of "tar" (8.5–9.8 mg), nicotine (0.6–0.8 mg), carbon monoxide (9.2–11.1 mg) and NNK (44.7–62.7 ng/cigarette). Neither blood carboxyhemoglobin values, nor total daily urinary NNAL nor urinary nicotine equivalents exhibited statistically significant differences between the menthol and non-menthol cigarette smokers. Some biomarkers levels (nicotine and cotinine glucuronides, total and glucuronide-conjugated NNAL) were statistically significantly lower ($p < 0.01$) in white menthol cigarette smokers compared to white non-menthol smokers. The author concluded that the smoking of menthol and non-menthol cigarettes of similar machine-generated smoke yield results in essentially identical levels of biomarkers of smoke constituent exposure.

5.2.1. Conclusions regarding menthol and smoke biomarkers of exposure

Studies intended to evaluate the potential of menthol employed as a cigarette ingredient to independently affect smokers' exposures to smoke constituents are challenging due to pharmacogenetic and smoking behavioral differences among smokers participating as study subjects in biomarkers investigations (Benowitz et al., 1999). Smokers' taste preferences for menthol or non-menthol cigarettes may constrain the use of crossover/forced switching study designs that might otherwise be preferred for such smoking biomarkers investigations. Some studies reported to date have reported differences between menthol and non-menthol smokers' biomarkers levels, most consistently as higher levels of nicotine or the nicotine metabolite cotinine among menthol smoking subjects. However, the weight of available evidence to date does not support an expectation that elevations in exposure to smoke constituents that are believed to be significant in the etiology of smoking-related diseases results from the use of menthol as a cigarette flavoring ingredient (Richie et al., 1997; Benowitz et al., 2004; Muscat et al., 2009; Heck, 2009). This conclusion is consistent with the body of complimentary epidemiological and smoking topography literature reviewed above. Further research into the potential of menthol to affect the metabolic disposition of biologically-significant smoke constituents is indicated.

6. Menthol cigarettes: smoking initiation and cessation

A number of authors have advanced speculation and hypotheses that the presence of menthol in cigarettes may facilitate the initiation of smoking, especially among youth, or render smoking cessation more difficult (Connolly, 2004; Giovino et al., 2004; Hersey et al., 2006). Controlled experimental study designs intended to explore any independent effects of menthol on smoking cessation outcomes may be rendered difficult by smokers' established taste and sensory expectations in regard to menthol. Some smokers strongly prefer menthol cigarettes, while others find them distasteful; a reality that complicates the interpretation of some crossover/forced brand switching cessation study designs (Rose and Behm, 2004). Despite the inherent limitations of self-reported, cross-sectional/observational questionnaire data that is typical in this area of study, a number of investigators have conducted and reported studies intended to address these questions in recent years. Recent findings from smoker survey instruments and open-ended discussions with menthol cigarette smokers have provided indications of complex social and cultural influences that may contribute to some smokers' preferences for mentholated cigarette brands (Allen and Unger, 2007; Richter et al., 2008). Differentiating the relative contribution of these social and economic factors from any independent effects of menthol on smoking initiation and cessation presents substantial challenges in the design of studies in this field.

A substantial number of the studies of the association of cigarette mentholation with smoking cessation outcomes that are discussed in the present review have been developed from subjects attending stop-smoking clinics. These subjects have been reported to comprise a self-selected population of smokers who perceive themselves to be more highly dependent than the typical smoker, so the extension of reported associations of cessation outcomes with demographic or cigarette characteristics from such clinical populations to other smokers may not be valid (Etter et al., 2009). The majority of smokers who achieve smoking cessation do so on their own rather than in a cessation clinic environment, so extrapolation of observations from clinical populations to smokers, generally, is best done with reservation (Ferguson et al., 2009; Chapman, 2008).

A final general cautionary note in regard to cessation clinic-based studies relates to the default assumption that outpatients lost to study follow-up have failed in cessation and resumed smoking (penalized imputation). Although penalized imputation is a common practice in the analysis of the efficacy of clinical treatment protocols that is represented as a conservative approach to those analyses (Gandhi et al., 2009), some authors have raised statistical issues with the practice. If smokers who prefer menthol cigarettes are less likely to successfully complete follow-up for reasons associated with socioeconomic status or other factors, then the relationship between menthol preference and cessation success may be distorted by penalized imputation. Other statistical alternatives to this approach exist, including multiple imputation methods for random missing data. However, these alternative statistical methods are seldom used due to their complexity (Nelson et al., 2009).

The use of observational data from cessation clinic outpatients' questionnaire responses to develop causal inferences relating to smoking cessation outcome is in any event a tenuous process. Potent statistical associations between various indices of socioeconomic status and smoking cessation success have been reported in many of the discussed studies. The sheer number of these associations is sufficient to indicate that particular caution should be exercised in informing causal inferences for menthol preference and cessation outcomes from observational or cross-sectional data.

Hyland et al. (2002)

A study by Hyland et al. (2002) explored possible associations between mentholated cigarette usage and a variety of indicators of nicotine dependence. While no smoking topography data were reported, the authors briefly mentioned menthol's purported effects on general smoking behaviors as the basis for their hypothesis that menthol cigarette smokers may differ from non-menthol smokers in measures of nicotine dependence. Approximately 80% of a smoker cohort originally included as participants in the large 1988 COMMIT (Community Intervention Trial for Smoking Cessation) study were re-interviewed in 1993 in regard to their smoking behavior. A total of 13,268 members of the original 1988 cohort met the criteria for inclusion in the follow-up study to assess whether those smoking mentholated cigarettes in the initial study differed from non-menthol smokers in terms of smoking cessation, time to first daily cigarette, and daily cigarette consumption. Analysis of data from the 24% of the original cohort who reported themselves to be menthol smokers did not indicate any differences from non-menthol smokers in terms of daily cigarette consumption rate, time to first daily cigarette, or subsequent success in smoking cessation. No consistent association between these indices of dependence and cigarette mentholation were observed in either overall or race-specific comparisons. Associations with menthol usage were found for female sex, 25–34 year age, African-American or Asian ethnicity, greater education, greater than 60 min until first daily cigarette, two or more past quit attempts, and use of premium brand cigarettes. It was clear from this relatively large study that menthol does not appear to have any meaningful effect on the evaluated behavioral indices of nicotine dependence.

Muscat et al. (2002)

These investigators conducted a cross-sectional analysis of data collected from 19,545 current and former smokers to explore the questions of whether smokers of menthol cigarettes exhibited differences in smoking rate or in smoking cessation success (Muscat et al., 2002). Subjects included 16,540 smokers of non-mentholated cigarettes and 3005 smokers of mentholated cigarettes. While black participants reported a significantly higher preference for menthol cigarettes than did whites (34.4% *vs.* 13.3%, $p < 0.01$), the study population's overall preference for menthol was lower than that reported in most recent surveys. Black participants in this study were more likely than whites to report current smoking (66.4% *vs.* 48.3%, $p < 0.01$).

Both black and white current smokers of menthol cigarettes were found to be less likely to be heavy smokers of 21 or more cigarettes per day than were smokers of non-mentholated cigarettes [prevalence OR of 0.7 (0.5–0.9, 95% CI) and 0.9 (0.8–1.0, 95% CI) , respectively]. Cigarette mentholation showed no significant relationship to smoking cessation success in either racial group. In summary, the cross-sectional analysis of Muscat et al. (2002) showed a statistically significant association of menthol with lower smoking rates among black current smokers and no relationship of menthol to prior cessation success in either white or black former smokers.

Okuyemi et al. (2003)

Okuyemi et al. (2003) examined differences in smoking characteristics and cessation rates in a cohort of 600 African-American (AA) smokers who were enrolled in a clinical trial of the efficacy of bupropion for smoking cessation. Of the 600 smokers, 471 smoked menthol and 129 smoked non–mentholated cigarettes. The authors reported that although menthol and non-menthol

smokers had similar scores on the Fagerström Test for Nicotine Dependence (FTND), menthol smokers were more likely to smoke their first cigarette within 30 min of waking up (81.7% *vs.* 69.8%, $p = 0.003$). At 6 weeks follow-up, 28.3% of menthol smokers *vs.* 41.5% of non-menthol smokers were abstinent ($p = 0.006$), and among those younger than 50 years, non-menthol smokers were more likely to have successfully quit smoking (OR = 2.0; 95% CI: 1.03–3.95). However, this reduced short-term smoking cessation success for younger menthol smokers was not statistically significant at the terminal 6-month follow-up nor in the placebo control group who were not receiving active bupropion treatment. The authors concluded that "*. . .AA menthol smokers had lower smoking cessation rates after 6 weeks of treatment with bupropion thereby putting menthol smokers at greater risk from the health effects of smoking*".

Okuyemi et al. (2004)

Okuyemi et al. (2004) reported findings from a cross-sectional survey study of 480 African-American smokers attending an inner-city health center in Kansas City, KS that provides services primarily to low-income patients. Demographic and smoking behavioral characteristics were collated and analyzed from responses to a 186-question questionnaire, in addition to the Fagerström Test for Nicotine Dependence (FTND) and other instruments previously employed to characterize smoking behavior. The study population comprised 407 smokers of mentholated cigarettes and 73 smokers of non-mentholated brands.

The authors mentioned the collection of exhaled carbon monoxide from study participants at the time of the completion of the survey instrument, but did not further mention or discuss the results of these tests. Exhaled CO has previously been employed as a biomarker of smoking exposure and a measure of puffing intensity, and these data could have provided some perspective on the accuracy of the self-reported survey responses from which the authors developed their conclusions.

The investigators reported that smokers of mentholated cigarettes were younger (median 40 *vs.* 45 years, $p < 0.001$), more likely to smoke cigarettes with longer rod length (54.1% *vs.* 46.5%, $p < 0.05$), and having higher FTC smoke yields ("tar": 16 *vs.* 14 mg; nicotine: 1.2 mg *vs.* 1.0 mg; CO: 16.0 mg *vs.* 15.0 mg) than those preferring non-mentholated brands. This sample of smokers of menthol cigarettes was also found to be significantly more likely to be uninsured and to have reported recent marijuana use than the regular cigarette smoker group.

The authors reported that their study "*. . .did not find differences in addiction between menthol and non-menthol smokers*", presumably in reference to the FTND scores. The menthol and non-menthol smoker groups reported similar numbers of smoking cessation attempts, but menthol smokers reported more recent cessation attempts and marginally shorter duration of prior cessation episodes. The authors reported only surrogate indices of smoking cessation rather than primary smoking cessation data. Despite having acknowledged that "*. . .being a cross-sectional study, causal relationship between menthol and smoking cessation cannot be implied. . .*", the authors concluded that their data "*. . .suggest that African-American menthol smokers are less successful with smoking cessation*".

Harris et al. (2004)

A study by Harris et al. (2004) investigated the characteristics of black smokers that were associated with success in smoking cessation in a placebo-controlled trial of the anti-depressant drug bupropion SR (300 mg administered daily for 7 weeks), with a 27-week follow-up to document successful cessation. Five hundred and thirty-five participants completed the 7-week pharmacologic cessation regimen, 65 of whom were lost during follow-up. Seventy percent of the study participants were women. While bupropion increased cessation success 2.5-fold regardless of individual characteristics, several subject characteristics were significantly associated with less successful abstinence at the end of the buproprion dosing, including reported smoking within 30 min of waking (26.43% abstinence *vs.* 48.70%, $p < 0.0001$) and a preference for menthol cigarettes (28.30% abstinence *vs.* 41.53%, $p = 0.0062$). Other characteristics showing statistically significant associations with successful abstinence were older age, fewer cigarettes smoked per day and lower salivary cotinine levels. The authors urged that "[*c*]*aution should be used in generalizing our results to other groups of smokers*" since the report "*. . .constitutes a secondary analysis for which the original study was not primarily designed*". Study particulars mentioned included the over-representation of middle-aged black female subjects (70% of participants) and the "*. . .inadequate assessment of pertinent psychosocial factors that are particularly relevant to African-Americans*". Additionally, univariate comparisons between abstinent and continuing smokers, including that for menthol preference, were developed at the end of the 7-week buproprion treatment rather than at the end of the 27-week follow-up. The persistence of the reported association between menthol cigarette smoking and less successful cessation can therefore not be determined from the data reported. The authors offered speculation that "*. . .smoking of menthol cigarettes could be a marker for some other yet undetermined factor that are*[*sic*]*also associated with cessation such as gender or age*".

Moolchan et al. (2004)

Moolchan et al. (2004) reported physiological responses and smoking topography measurements in a study of 128 black and white menthol cigarette-smoking adolescents enrolled in a smoking cessation program in Baltimore, MD. Subjects were asked to smoke one mentholated cigarette of their preferred brand for assessment of racial differences in smoking topography, acute cardiovascular response parameters and exhaled carbon monoxide. The authors reported no race-related differences in blood pressure, heart rate or smoking topography measures, concluding that "*. . .[t]he present study found no statistically or clinically significant differences to support the hypotheses of early ethnic differences in acute physiological responses to menthol cigarette smoking and puff topography measures among teenage smokers seeking cessation treatment*". However, due to the young age and self-selected nature of the subjects participating in the survey (about 15 years), the authors urged caution in generalizing their findings of no race-related differences in the measured parameters to other adolescent populations or to adults.

Moolchan (2004)

Moolchan (2004) briefly reported findings from a telephone survey of 593 Baltimore-area adolescent smokers applying to a smoking cessation program. Participants had a mean age of 15.5 years, were 51% female and 45% black. The overwhelming majority of the sample population (93% total; 98.5% blacks and 89.8% whites) preferred menthol cigarettes, including both males and females. Although the author suggested that his "*. . .findings of overwhelming menthol preference in a treatment-seeking sample of adolescents warrant further research on the developmental trajectory, cessation, and health-related impact of menthol smoking by youth*", it would seem likely that the reported high preference for mentholated cigarette brands among the treatment-seeking study population was simply a reflection of the brands that are currently most

popular among peers and family members, both young and old, in this urban community study setting.

Rose and Behm (2004)

A study examining the effectiveness of several behavioral treatments in promoting the pharmacologically-aided extinction of reward responses to cigarettes was reported by Rose and Behm (2004). Among the smoking behavioral modifications evaluated was the forced switching to or from menthol cigarettes by participating subjects preferring either menthol or non-menthol brands. The switching was imposed over a 2-week cessation treatment period employing combinations of nicotine replacement therapy, the nicotinic antagonist mecamylamine, denicotinized cigarettes, and low tar/nicotine cigarettes. The authors reported that forced changes to one sensory component of smoking, menthol, profoundly influenced the participating smokers' ratings of smoking reward. Smokers preferring both menthol and non-menthol cigarettes were similarly responsive to the active pharmacologic treatments, and smokers of each cigarette type reported that the addition or deletion of the menthol sensory cues reduced the rewarding aspects of smoking. Interestingly, the forced switching of menthol smokers to a non-menthol brand during the active pharmacological cessation treatment appeared to prevent a decrease in reward ratings for subjects' usual brand cigarettes; in contrast, the switching of non-menthol smokers to menthol brands did not appear to affect the course of reward extinction by cessation pharmacotherapy. The 2004 study of Rose and Behm provides evidence for the substantial role of the familiar sensory components of the smoking experience, including either the presence or absence of menthol, in contributing to smokers' perceptions of smoking rewards.

DiFranza et al. (2004)

DiFranza et al. (2004) performed a prospective longitudinal study of the development of nicotine dependence among a cohort of 237 smoking public school students in three annual interviews over a period of three years. This report appears to be the first to have investigated adolescents' subjective responses to their first lifetime cigarette. The participating adolescents' subjective recollections of their first cigarette smoking experience were recorded and compared to responses provided to the Hooked on Nicotine Checklist, an instrument intended to characterize 10 measures of nicotine dependence, over the course of the study. The investigators' analysis sought to determine whether cigarette characteristics or any of the subjective responses to the first inhaled cigarette were correlated to later development of nicotine dependence among these novice smokers. Subjectively-perceived relaxation, nausea and dizziness, possible indicators of nicotine sensitivity, were reported to be significantly associated with subsequent development of nicotine dependence. These dependence-associated subjective responses were not significantly related to the sex of the subjects or to cigarette brand "strength" (regular, lights, ultra-lights). Additionally, the dependence-predictive subjective responses to the first inhaled cigarette were not significantly related to cigarette mentholation. The results of this study do not support speculation that menthol flavoring facilitates the acquisition of nicotine dependence among youth.

Li et al. (2005)

A presentation by Li et al. at the 2005 National Conference on Tobacco or Health comprised a detailed analysis of cigarette mentholation and dependence data collected in the large Community Intervention Trial for Smoking Cessation (COMMIT). The presentation represents a further analysis of the potential of menthol cigarette preference to affect smoking cessation success among 4488 subjects from the COMMIT cohort further to that previously explored in the published report of Hyland et al. (2002). COMMIT included telephone data collection and follow-up between 1988 and 1993 in 22 communities, with terminal follow-up completed for over 13,000 of the over 20,000 original survey respondents. The 4488 participants analyzed by Li et al. (2005) comprised those subjects who had been successfully contacted in all three prior surveys as well as the authors' additional 2001 survey; and for whom complete menthol or non-menthol brand preference information was available. Outcome variables included long-term (6 months or more) smoking cessation, changes in daily cigarettes smoked between 1988 and 2001 or changes over the study interval in time to first daily cigarette after waking. No statistically significant differences between smokers preferring menthol and non-menthol brands were found in daily smoking rates or time to first daily cigarette among continuing smokers, nor were any differences in successful smoking cessation outcomes reported. The authors concluded that there was no consistent relationship between cigarette mentholation and indicators of nicotine dependence, and called for further studies to reconcile the conflict between theories and actual findings for menthol in regard to nicotine dependence.

Foulds et al. (2006)

Survey data collected from 1021 consecutive outpatients attending a New Jersey smoking cessation clinic were analyzed and discussed by Foulds et al. (2006) to identify demographic and smoking behavior variables associated with cessation success at 4-week and 6-month follow-up contacts. These subjects represented an earlier sampling of the same self-selected clinical population reported on subsequently by Bover et al. (2008) and Gandhi et al. (2009); these later papers, discussed below, explore the associations noted by Foulds et al. (2006) in additional detail.

Factors identified by Foulds et al. (2006) to be associated with significantly greater success in achieving sustained smoking abstinence included greater age, married/cohabiting marital status, higher educational attainment, white race, stable employment, health insurance coverage, later age of smoking initiation and current smoking of a light/low-tar brand. Subjects having a preference for menthol cigarettes were significantly less likely to report sustained, 6-month cessation success (24.9%) than were smokers of non-mentholated cigarettes (35.8%; $p < 0.0001$).

Collins and Moolchan (2006)

Collins and Moolchan (2006) reported data gathered in a telephone survey of 572 adolescents applying to a smoking cessation program in Baltimore, MD. Participants had a mean age of 15.6 years, and included 55.1% females and 46.9% blacks. The authors reported that 92.8% of the survey participants smoked menthol cigarettes, and that 45% of these menthol smokers reported smoking a cigarette within 5 min of waking, as opposed to 29% of the 41 non-menthol smoking participants ($p < 0.05$). However, no differences in cigarettes per day or in Fagerström Test for Nicotine Dependence scores were reported between smokers of menthol and non-menthol cigarettes.

Hersey et al. (2006)

An analysis of questionnaire data from the nationally-representative 2000 and 2002 National Youth Tobacco Surveys was reported by Hersey et al. (2006) in an investigation of whether mentholated cigarette brands may be favored by younger,

beginning smokers. While survey response inconsistencies introduced a certain degree of uncertainty in the determination of cigarette preference, the authors' definitions identified 36.9% of survey respondents as menthol smokers and 40.9% as smokers of non-mentholated brands. Smoking cessation and dependence measures were extracted from survey data through application of the Nicotine Dependence Scale for Adolescents, a six-question battery of subjective self-assessments of smoking behaviors.

The authors reported that, overall, adolescent smokers who had been smoking for less than a year expressed a higher (51%) preference for menthol cigarettes than those who had been smoking for over a year (43.6%). The authors suggested that this observation "*. . .may indicate that menthol cigarettes are a starter tobacco product that adolescents smoke before they move on to other types of tobacco products*". The authors speculated that this observation was consistent with "*. . .the possibility that because menthol cigarettes are perceived as less harsh to smoke, they may serve as a starter cigarette for adolescents*". However, no data on participants' subjective perceptions in regard to cigarette harshness is found in the report. Whether novice smokers truly perceive that mentholated cigarettes are less harsh, or rather that the abandonment of menthol cigarette smoking by some beginning smokers reflects a rejection of mentholated brands or a changing taste preference for non-mentholated brands will require additional study.

The authors reported that adolescents who are regular smokers of mentholated cigarettes are 45% more likely than regular smokers of non-mentholated cigarettes to be above the median on a Nicotine Dependence Scale for Adolescents ($p = 0.006$).

Hersey and colleagues reported mixed findings in regard to mentholated cigarettes and smoking cessation. While menthol and non-menthol smokers reported a similar frequency of quit attempts overall, menthol smokers were 50% more likely to have accessed cessation program resources despite the fact that significantly ($p = 0.05$) fewer menthol smokers reported "seriously thinking about quitting".

Pletcher et al. (2006)

Pletcher et al. (2006) reported an investigation of success in smoking cessation among smokers of regular and mentholated cigarettes as part of a larger study of smoking-related disease risks (Pletcher et al., 2006). The paper's disease-related findings are discussed above. Study subjects comprised 1535 African-American and European-American smokers from a cohort of 5115 males and females in four major US cities participating in the Coronary Artery Risk Development in Young Adults (CARDIA) study. Study subjects provided questionnaire and clinical data at enrollment and 2, 5, 7, 10 and 15 years thereafter (calendar years 1985–2000).

The 1535 smoking study subjects included 563 non-mentholated cigarette smokers and 972 mentholated cigarette smokers. The 63% preference for mentholated brands recorded for smokers participating in the CARDIA study is considerably higher than that previously reported for the US population as a whole. The African-American study subjects stated a significantly higher preference for menthol brands than did European-American subjects (89% *vs.* 29%, $p < 0.001$). The stated preference for mentholated cigarettes among African-American smokers in this study was somewhat higher than had been previously reported for this demographic group (approximately 70%) (Giovino et al., 2004), but it is consistent with the findings from several more recent surveys. The authors stated that adjustment for ethnic, demographic and social factors, as well as for smoking rate (cigarettes per day) had been performed in their data analyses.

The authors found a statistically-insignificant trend ($p = 0.06$) toward less successful sustained smoking cessation by menthol cigarette smokers (OR 0.71; 0.49–1.02, 95% CI) relative to smokers of non-mentholated brands, when cessation success was defined as two self-reports of non-smoking status in follow-up interviews of subjects who had described themselves as smokers at study initiation. The authors also reported a statistically-insignificant trend toward fewer quit attempts by smokers of mentholated cigarettes relative to those smoking regular brands (OR 0.77; 0.56–1.06, 95% CI; $p = 0.11$). Smokers of mentholated brands reported a greater incidence of smoking relapse (OR 1.89; 1.17–3.05, 95% CI; $p = 0.009$) which the authors defined as self-reported smoking by baseline smoking subjects at the final 15-year follow-up interview after having reported themselves to be non-smokers at any one of the four previous follow-up interviews.

The findings of Pletcher et al. (2006) in regard to cigarette menthol and smoking cessation experience are summarized below in OR and 95% CI extracted from the authors' Table 2. These values were reportedly adjusted for age, sex, ethnicity and social factors.

Not currently smoking	0.90 (0.68–1.19)
Recent quit attempts	0.77 (0.56–1.06)
Cessation if recent attempt	1.00 (0.71–1.42)
Sustained cessation	0.71 (0.49–1.02)
Documented relapse	1.89 (1.17–3.05) $p = 0.009$

Four of the authors' five metrics of smoking cessation showed no significant differences between mentholated and non-mentholated cigarettes. The "documented relapse" measure that was reported as the only significant statistical finding in the adjusted analyses appeared to be less affected by the adjustment factors (age, sex, ethnicity, social factors, baseline cigarettes per day) than were the other four metrics. This metric may be particularly sensitive to bias, as subjects qualifying as having experienced a "documented relapse" by virtue of having reported themselves to be non-smokers at any one of the four prior follow-up visits may have had a variety of reasons for providing that single self-report other than a decision to permanently quit smoking. The final footnote in the table legend also noted that a higher number of CPD at baseline was associated with several of the other metrics, but not with "documented relapse".

Notwithstanding the fact that four of their five metrics of smoking cessation revealed no statistically-significant menthol – non-menthol differences, the authors concluded from their analyses that "*. . .[m]enthol and non-menthol cigarettes seem to be equally harmful per cigarette smoked in terms of atherosclerosis and pulmonary function decline, but menthol cigarettes may be harder to quit smoking*".

Allen and Unger (2007)

The strong preference for menthol cigarettes that has been consistently reported among black smokers was attributed to taste preference, psychosocial and cultural factors in the report of Allen and Unger (2007) that presented survey data and limited clinical findings from 432 black smokers from Los Angeles. No correlation between exposure to menthol cigarette advertising and menthol preference was found, and neither exhaled breath carbon monoxide nor salivary cotinine was found to differ between the menthol and non-menthol study participants (Allen and Unger, 2007). Menthol cigarette preference was associated with parental menthol smoking and with peer group menthol preferences. Similar peer influence may have accounted for the strong menthol preferences stated for both black and white smokers in the previously-discussed study by Moolchan (2004) in another large urban community.

Ashare et al. (2007)

Ashare et al. (2007) reported findings from a www-based survey instrument for which 424 (240 females, 57%) undergraduate college student participants received course credits in experimental

introductory psychology. Since the survey was cross-sectional in nature, its findings cannot be generalized as representing the opinions of other populations. Survey participants viewed randomly-ordered advertisements for 12 brands of cigarettes, including Camel (Exotics, Lights, and Turkish Blend), Marlboro (Red, Lights, and Ultra Lights), Salem (Regular, Silver Label), Kool Smooth Fusion, Basic, Quest, and Eclipse. The subjects rated the advertisements on 28 parameters relating to appeal, likeliness to try the different products and other measures relating to positive or negative expectancies generated by the advertisements.

The authors reported that their study "*. . .demonstrated that flavored cigarettes increased positive expectancies and decreased negative expectancies for smoking, that positive expectancies predicted greater intentions to smoke, and that none of these relationships was specific to regular smokers. This pattern seems consistent with the view that flavored cigarettes serve as "starter" products (*Carpenter et al., 2005*), rather than as specialty products for regular smokers.*" However, the study of Ashare and coworkers actually provides no useful information on the inherent taste appeal of menthol or other flavoring substances to the non-random college-age population sample, as the survey instrument was merely an assessment of the responses of the study participants to printed advertisements.

Murray et al. (2007)

The 2007 report of Murray and associates, discussed above in the Epidemiology of Menthol Cigarette Smoking section, described findings developed from 5887 adult smokers who were followed for 14 years as participants in the Lung Health Study (LHS). Data on health status with an ipratropium inhaler or placebo, as well as smoking cessation histories, were gathered at annual follow-ups. Chi-square tests were used to compare sex-specific relationships among smoking categories (sustained quitter, intermittent smoker, and continuing smoker) and use of menthol or non-menthol cigarettes. The authors reported no significant differences in sustained smoking cessation or in the intermittent or regular continuation of smoking for either sex in relation to the use of menthol cigarettes (chi-square, p value 0.80 in men and 0.57 in women). The study of Murray et al., 2007 is consistent with similar rates of cessation and similar persistence of smoking in smokers of either menthol or non-menthol cigarettes.

Okuyemi et al. (2007a)

Okuyemi et al. (2007a) reported an investigation and comparison of nicotine dependence between African-American light smokers (1–5 cigarettes/day) and moderate smokers (6–10 cigarettes/day) who were enrolled in a clinical smoking cessation study. While cigarette mentholation was not a major topic of the investigation, 567 participants (81.2%) smoked menthol cigarettes. However, the participants' preference for mentholated cigarettes was not different ($p = 0.26$) between the light-smoking group (83.8%) and the moderate-smoking group (80.2%).

Several notable differences, some statistically-significant, between light and moderate smoker groups were inconsistent with the "nicotine titration" hypothesis that would otherwise suggest that light smokers (1–5 cigarettes/day) might consciously or unconsciously smoke higher-yielding cigarettes or smoke their chosen cigarettes more intensely. Light smokers exhibited lower mean exhaled CO levels (11.1 *vs.* 14.79 ppm, $p < 0.0001$) and lower serum cotinine levels (176.4 *vs.* 271.4, $p < 0.001$). Light smokers also reported a lower incidence of smoking within 30 min of waking (50.3% *vs.* 69.7%, $p < 0.0001$), a reduced preference for high nicotine/tar cigarettes (18.1% *vs.* 43.3%, $p = 0.26$), and a reduced self-report of deep smoke inhalation (14.8% *vs.* 40.1%, $p = 0.14$).

Okuyemi et al. (2007b)

Okuyemi et al. (2007b) investigated whether black subjects who were light smokers of mentholated cigarettes ($n = 615$) had lower cessation rates than those who smoke non-mentholated ($n = 140$) cigarettes following treatment with nicotine gum and counseling. They found that at an 8-week follow-up, abstinence rates were not significantly different between non-menthol and menthol smokers ($p = 0.29$). However, at a 26-week follow-up, non-menthol smokers were more likely to have quit than were smokers of mentholated brands ($p = 0.015$). Comparisons among the evaluated cessation regimes at the 26-week follow-up indicated that non-menthol smokers who received nicotine gum had a significantly higher abstinence rate than menthol smokers who received counseling only ($p = 0.037$). No other significant findings in regard to differences in treatment outcome in association with cigarette mentholation were noted. The authors concluded that "*. . .*[a]*mong* [African-American] *light smokers, use of mentholated cigarettes is associated with lower smoking cessation rates*".

Wackowski and Delnevo (2007)

An evaluation of menthol cigarette smoking and subjective measures of nicotine dependence was developed from data collected from a nationally-representative sampling of 1345 smoking adolescents in grades 9–12 who participated in the 2004 National Youth Tobacco Survey (NYTS) (Wackowski and Delnevo, 2007). Odds ratios were developed by logistic regression for the 46% of current self-reported smokers who reported a preference for mentholated cigarettes in order to compare them to smokers of non-mentholated cigarettes, controlling for demographic characteristics and smoking patterns.

The authors' reporting of the respondents' stated preferences for mentholated cigarettes (as either exclusive, usual or regular menthol cigarette use) showed considerably more variability in all demographic categories than is typically reported for adult smokers. It would appear that among these high-school age smokers, menthol/non-menthol preferences may not be firmly established, or possibly that the under-age respondents have only irregular access to different cigarette types (menthol/non-menthol). Overall, black smokers indicated a substantially higher preference for mentholated cigarettes than did participating smokers of other racial/ethnic groups.

Adjusted ORs for menthol smokers' subjective self-reported measures related to dependence were reported, with two of the four measures showing statistically-significant differences:

Self-reported measure	Adjusted Odds Ratio, menthol *vs.* non-menthol (95% confidence interval)
"Need a cigarette within 1 h".	2.6 (1.6–4.3)
"Experience cravings"	1.6 (1.1–2.2)
"Irritable when deprived"	1.2 (0.9–1.5)
"Think I can't quit"	1.2 (0.8–1.6)

The authors acknowledged that "*. . .*[*t*]*he NYTS was not designed to test hypotheses related to menthol use and dependence*", but nevertheless concluded that "*. . .*[m]*enthol use was associated with two dependence measures and may be more addictive than regular cigarettes in young smokers*".

O'Connor et al. (2007)

The 2007 report of O'Connor and colleagues was discussed above in the context of the smoking topography elements of their investigation. The study design also included subjective assessments of flavored cigarette brands relative to conventional

commercial cigarettes by young (college-aged) smokers in addition to the laboratory smoking topography measurements.

The authors recorded broadly similar subjective assessments of various descriptive elements of cigarette liking, but overall 70% of the young smokers preferred the conventional Camel Lights cigarette over the flavored Camel Exotic Blends products. No significant sex or demographic preferences for flavored *vs.* unflavored cigarettes were evident in the study. These findings suggest that notions and presumptions about the appeal to younger smokers of cigarettes that incorporate menthol or other flavorings are not necessarily justified.

Fu et al. (2008)

Fu et al. (2008) hypothesized that menthol cigarette smoking would be associated with lower abstinence rates in a multi-ethnic sample of smokers ($n = 1343$) making a pharmacotherapy-aided quit attempt (nicotine replacement therapy or bupropion). Their outcome was a self-reported 7-day point prevalence smoking abstinence. The authors reported no significant effects on smoking abstinence for cigarette mentholation (OR = 1.31, 95% CI: 0.9–1.82), or ethnicity (black *vs.* white); (OR = 1.06, 95% CI: 0.66–1.71), concluding that "*. . .this study suggests that smoking menthol cigarettes does not decrease smoking cessation among older smokers during a quit attempt aided with pharmacotherapy*".

Ruskin et al. (2008)

Ruskin et al. (2008) reported an experimental study of the effects of menthol on nicotine-induced hypothermia, a well-characterized physiological response that is believed to be elicited by the alkaloid's action at central hypothalamic nicotinic receptors. The authors hypothesized that differences in this previously-described interaction (Ruskin et al., 2007) between adolescent and adult rats could provide information relevant to differential responses to menthol cigarettes between adolescent and adult human smokers that could be related to smoking initiation and cessation.

The authors referred to their 5-day course of intraperitoneal injections of rats with menthol at 100 mg/kg bw as "chronic" menthol treatment, although that term is more typically applied to dosage regimens of longer duration. The rats' body temperatures were measured following the subcutaneous administration of nicotine at 0.5 mg/kg bw on days 4 and 5 of menthol administration. An additional single-dose experiment employing a subcutaneous 400 mg/kg dose of menthol and a 0.5 mg/kg dose of nicotine administered 30 min later was also reported, with temperature responses recorded for adolescent rats (34–36 days of age), young adult rats (53–56 days old) and mature rats (9–10 months old).

The authors reported a more significant attenuating effect of menthol on nicotine-induced hypothermia among adolescent rats than among young adult or mature rats. Ruskin and colleagues acknowledged the complexities introduced by the larger surface area-to-volume ratios and greater thermal inertia of older, heavier rats; as well as the previously-reported age-dependence of nicotine's effects on body temperature in the rat model. They hypothesized that the observed attenuation of nicotine hypothermia by menthol could be manifested through an action of menthol on the central nervous system.

Although a plausible mechanism relating the nicotine-evoked body temperature responses of rats to human smoking initiation is not immediately apparent, the authors suggested that "*. . .*[a]*lthough youthful smokers might choose menthol cigarettes based on sociocultural influences or youth-targeted marketing campaigns, the age-specific biological interaction of menthol with nicotine might lead young menthol smokers to become more rapidly or more deeply nicotine-dependent. . .*".

Bover et al. (2008)

Bover et al. (2008) analyzed questionnaire responses from 2312 consecutive outpatients from a New Jersey smoking cessation clinic to assess the value of subjects' reporting of awaking at night to smoke as a measure of dependence and cessation success. These subjects were from the same clinical population reported on previously by Foulds et al. (2006) and subsequently by Gandhi et al. (2009).

A number of demographic and personal characteristics showed statistically-significant associations both with less successful 26-week smoking cessation and with awaking at night to smoke, including black or Latino/Hispanic race, disability or unemployment, single or divorced marital status, longer history of smoking, shorter time to first daily cigarette and heavier daily cigarette smoking rates. Mentholated cigarettes were preferred by a significantly greater percentage of subjects who reported awaking from sleep to smoke (57.9%) than those who did not (42.1%; $p < 0.0001$). Mentholated cigarette preference was also associated with a lower rate of successful 26-week smoking cessation (20.1%) than was reported by smokers of non-mentholated brands (29.3%; $p < 0.0001$). The associations of minority race identity, unemployment and menthol cigarette preference with less successful cessation in this population was explored further in the follow-up report of Gandhi et al. (2009) discussed below.

Gandhi et al. (2009)

A report by Gandhi et al. (2009) described a retrospective study of 1688 consecutive patients participating in a New Jersey outpatient smoking cessation program employing multidisciplinary counseling and nicotine replacement strategies. Smoking cessation outcomes and their associations with various characteristics of this same clinical population had been the subject of two prior reports by these investigators (Foulds et al., 2006; Bover et al., 2008). The association of cigarette mentholation with cessation success and subjects' characteristics was explored in detail in the 2009 Gandhi paper. Four-week and 6-month cessation outcomes were analyzed by race, cigarette smoking habits and demographic variables to identify factors associated with smoking cessation success.

Although biomarker confirmation of reported smoking cessation was not available for all patients, a sampling of patients attending the clinic at the 4-week and 6-month time points reportedly showed good correlation between exhaled CO levels and stated smoking abstention.

Mentholated cigarettes were preferred by 46% of all subjects, by 81% of blacks, 66% of Latinos and 32% of whites. Although white participants reported smoking similar numbers of mentholated or non-mentholated cigarettes daily, black and Latino smokers of menthol cigarettes reported smoking fewer cigarettes per day than did their race-matched non-menthol smoking counterparts.

Similar success in smoking cessation was reported at the 4-week follow up for white, black and Latino subjects who preferred non-mentholated cigarettes (50%, 54% and 50%, respectively). However, after adjustment for age, socioeconomic factors and smoking habits, black and Latino smokers of menthol cigarettes were reported to be significantly less successful in quitting than were their matched counterparts who smoked non-menthol cigarettes. No such difference was apparent for white subjects. Adjusted ORs and 95% CI for 6-month (end-of-study) success in quitting menthol cigarettes relative to non-menthol brands were 1.0 (0.8–1.4) for whites, 0.48 (0.25–0.9) for blacks and 0.64 (0.2–1.80) for Latinos.

Some race-specific, unadjusted smoking cessation parameters were also partially reported by Gandhi et al. (2009). White subjects who were employed full-time had identical success in quitting both menthol and non-menthol cigarettes at the 4-week interim assessment, while those who were unemployed displayed a trend toward reduced cessation success when smoking menthol cigarettes ($p = 0.07$). Unemployed black smokers of menthol cigarettes had significantly reduced success in quitting ($p = 0.03$) relative to those preferring non-menthol brands, while those having full-time employment showed no significant differences between cigarette types at the 4-week time point.

The substantial difference in the reported association of cigarette mentholation with unsuccessful smoking cessation that was observed for the white subjects relative to the black subjects at the 6-month study termination was notable. It suggests that either the reported effects of menthol on long-term smoking cessation are not manifested in white smokers; or that an occult, race-associated mediating factor is affecting the outcome of this and perhaps other such analyses (Cropsey et al., 2009). That this latter possibility may be the case is suggested by the numerous indices of socioeconomic status that have been consistently reported to be associated with unsuccessful smoking cessation in this and other studies derived from this same clinical population (Foulds et al., 2006; Bover et al., 2008; Gandhi et al., 2009). For example, Gandhi et al. found that full-time employment status was highly and negatively associated with the 2009 study populations' reported preference for menthol cigarettes (menthol: 20% unemployed, non-menthol: 12.6% unemployed; $p < 0.001$). Other negative indicators of socioeconomic status among the study subjects (lower educational attainment, unmarried/divorced status, lack of private medical insurance) showed similar strong statistical associations with a stated preference for menthol cigarettes.

The authors further observed that menthol cigarette smoking subjects (combined racial groups) reported shorter time to the first daily cigarette after waking and more frequent awakenings at night to smoke than did the non-menthol cigarette smokers. These observations had previously been reported by these authors (Bover et al., 2008). Interestingly, the menthol cigarette smoking participants in the report of Gandhi et al. (2009) were significantly less likely to report themselves as having a disease condition caused or aggravated by smoking ($p = 0.007$).

The 6-month follow-up rate for the study subjects was 58%, and the authors assumed that all subjects not reporting at the 6-month time point had been unsuccessful in quitting and resumed smoking (penalized imputation). Although the follow-up rates for menthol and non-menthol smokers were not presented in the paper, the authors stated that this assumption was unlikely to be a major cause of bias.

Cropsey et al. (2009)

Cropsey et al. (2009) compared and reported success in smoking cessation among black and white female prisoners enrolled in a 10-week program of group therapy supplemented by nicotine replacement medication, with follow-up at 6 weeks and 1 year. Two hundred and eighty-nine control subjects and 116 subjects receiving the intervention treatment completed the study. Generalized estimation equations were used to model smoking cessation over the study interval. The authors reported that both black and white subjects benefited from the treatment protocol relative to race-matched control subjects. However, white subjects had significantly greater success in achieving cessation than did black subjects (30% *vs.* 24% abstinence at 6 weeks, respectively). A similar significant racial difference in cessation success was recorded 1 year later (13% and 10% abstinence for white and black subjects, respectively). The authors' analysis also included consideration of cigarette mentholation in the model, and concluded that menthol preference did not account for the observed race-associated differences in smoking cessation in this incarcerated female population.

6.1. Conclusion regarding the potential of menthol to influence smoking initiation and cessation

Investigation of the influence of cigarette type and composition on smoking initiation and cessation are hampered by the inherently subjective nature of the questionnaire-based survey and opinion data that comprises the basis for hypothesis testing in regard to smoking attitudes and behaviors. The development of causal inferences from observational studies is in any event tenuous. Social, demographic and peer influence mediators and confounders are difficult to fully account for, rendering conclusive confirmation or refutation of hypotheses about cigarette type difficult. Furthermore, many of the studies of the association of cigarette mentholation with smoking cessation outcomes have been developed from outpatient populations enrolled in formal cessation programs employing various pharmacological and counseling strategies to assist in cessation. These subjects differ from the general population of smokers and their experiences may not necessarily be representative of those of other smokers (Chapman, 2008; Etter et al., 2009).

The findings of investigations of the potential of cigarette mentholation to affect smoking initiation or success in smoking cessation are mixed. Some studies report statistically-significant associations of these complex behaviors with menthol, and others do not. The development of a conclusive judgment of causation in regard to the potential role of menthol to independently affect rates of smoking initiation or cessation is not possible from survey data collected in observational/cross-sectional study designs.

Conflict of interest

The author is an employee of the Lorillard Tobacco Company, which manufactures and markets menthol and non-menthol cigarettes in the USA.

SUPPLEMENTARY INFORMATION

R. J. Reynolds Tobacco Company (2000). Chemical and biological analyses of the smoke of menthol and non-menthol cigarettes

Appendices A & B comprise heretofore unpublished cigarette smoke chemistry and toxicology data from comparative studies performed by the R.J. Reynolds Tobacco Company on experimental cigarettes manufactured with and without menthol added at 1.03% w/w tobacco.

The original internal technical reports summarized below are publicly available for review and may be accessed online at http://www.rjrtdocs.com

Appendix A. Smoke chemistry studies of menthol and non-menthol cigarettes

A.1. Comparison of selected smoke constituent yields for menthol and non-menthol cigarettes

A comparative study was conducted to examine the mainstream cigarette smoke yields of a non-menthol control cigarette and an identically-constructed test cigarette containing 1.03%

menthol w/w tobacco. A typical 'American-style' tobacco blend was used in both the mentholated and non-mentholated cigarettes of conventional, filtered construction.

This study compared mainstream smoke from the menthol and non-menthol cigarettes generated according to the US Federal Trade Commission (FTC) smoking protocol. Analyses included "tar", nicotine, CO, puff count, menthol, ammonia, benzo[*a*]pyrene, formaldehyde, acetaldehyde, acetone, acrolein, hydrogen cyanide, hydroquinone, catechol, phenol, *m*- +*p*-cresol, N-nitrosoanatabine (NAT), N-nitrosonornicotine (NNN), 4-methylnitrosoamino-1-(3-pyridyl)-1-butanone (NNK), carbon, hydrogen, nitrogen, as well as sidestream smoke "tar" and nicotine yields.

Some smoke constituents were found to differ slightly or to a statistically significant degree, but these differences did not result in any meaningful differences in biological activity under the conditions of these studies (Appendix B). These constituent yields were within the range obtained from a US composite market sample from 1995 (Chepiga et al., 2000), 1998 (unpublished) and 2000 (unpublished). In conclusion, the addition of menthol to cigarettes did not meaningfully alter smoke chemistry in a manner consistent with an expectation of increased risk to health compared to similar cigarettes without added menthol.

Table A1. Selected Mainstream Smoke Constituent (MSC) Yields from Menthol and Non-Menthol cigarettes

MSC	Menthol (1.03% w/w tobacco blend)	Non-menthol
Menthol		
Mean (mg/cigarette)	0.41 (mg/cigarette)	–
SD	0.022	–
Count	6	–
Ammonia		
Mean (μg/cigarette)	4.6	4.49
SD	0.178	0.53
Count	6	6
Benzo(a)pyrene		
Mean (ng/cigarette)	2.65	2.87
SD	0.338	0.213
Count	6	6
Carbonyls		
Formaldehyde (μg/cigarette)	**4.2**[a]	**3.4**[b]
SD	0.59	0.27
Count	6	6
Acetaldehyde (μg/cigarette)	284.6	275.8
SD	16.18	20.14
Count	6	6
Acetone (μg/cigarette)	114.8	117.1
SD	5.28	8.01
Count	6	6
Acrolein (μg/cigarette)	22.1	21.4
SD	1.24	2.15
Count	6	6
HCN		
Mean (μg/cigarette)	40.8	43.3
SD	6.65	5.16
Count	6	6
Carbon, hydrogen and nitrogen		
% Carbon	**69**[c]	**65.25**[d]
SD	3.145	0.661
Count	6	6
% Hydrogen	**9.71**[e]	**8.57**[f]
SD	0.308	0.633
Count	6	6
% Nitrogen	**3.7**[g]	**5.35**[h]
SD	0.461	0.664
Count	6	6
FTC nicotine, tar and CO		
TPM (mg/cigarette)	4.6	4.1
SD	0.33	0.32
Count	5	5
Nicotine (mg/cigarette)	0.35	0.35
SD	0.016	0.027
Count	5	5
Water (mg/cigarette)	0.3	0.3
SD	0.05	0
Count	5	5
Tar (mg/cigarette)	3.9	3.5
SD	0.3	0.27
Count	5	5
CO (mg/cigarette)	5.2	5.3
SD	0.33	0.41
Count	5	5
CO_2 (mg/cigarette)	22.1	22.5
SD	1.01	0.99
Count	5	5
Hydroxybenzenes (Phenols)		
Hydroquinone μg/cig	17.36	17.56
SD	1.551	0.686
Count	6	6
Catechol μg/cig	20.98	21.38
SD	1.303	0.739
Count	6	6
Phenol μg/cig	2.56	2.42
SD	0.275	0.114
Count	6	6
p- + *m*-Cresol μg/cig	2.5	2.34
SD	0.211	0.079
Count	6	6
Nitrosamines		
NNN ng/cig	29	35
SD	7.9	12.7
Count	6	6
NAT ng/cig	30	33
SD	8.1	11.2
Count	6	6
NNK ng/cig	22	28
SD	7.3	11.1
Count	6	6

[a,b,c,d…,h]Means without a common superscript letter differ ($p < 0.05$).

Source: Bodnar, J.A., and M.F. Borgerding. (2000). Comparison of selected smoke constituent yields for menthol and non-menthol cigarettes that primarily heat tobacco and menthol and non-menthol cigarettes that burn tobacco. Research and Development Report, RJRT, Document No.: ACD-MJAB2000-242.

Chepiga, T.A., Morton, M.J., Murphy, P.A., Avalos, J.T., Bombick, B.R., Doolittle, D.J., Borgerding, M.F. and Swauger, J.E. (2000). A comparison of the mainstream smoke chemistry and mutagenicity of a representative sample of the US cigarette market with two Kentucky reference cigarettes (K1R4F and K1R5F). *Food and Chemical Toxicology* 38: 949-962.

A.2. Determination of 1,3-butadiene, acrylonitrile, isoprene, benzene, and toluene in the mainstream vapor phase from menthol and non-menthol cigarettes

A comparative analysis was conducted to evaluate the potential impact of menthol addition on the mainstream cigarette smoke yields of 1,3-butadiene, acrylonitrile, isoprene, benzene, and toluene. The two cigarettes were identical with the exception of the addition of menthol. The test cigarettes were smoked using the US Federal Trade Commission (FTC) smoking method regimen (35 ml puff volume, 2-s duration, once per minute).

No significant difference in mainstream yields of selected products was observed between the menthol and non-menthol test cigarettes.

Table A2. Comparison of concentrations of mainstream vapor phase compounds.

Sample	1,3-Butadiene (μg/cigarette)	Acrylonitrile (μg/cigarette)	Isoprene (μg/cigarette)	Benzene (μg/cigarette)	Toluene (μg/cigarette)	WTPM[a] (μg/cigarette)
Menthol	26.2	4.0	212.4	20.0	26.4	4.92
	23.9	4.0	221.2	19.1	23.5	4.66
	21.8	3.7	205.7	19.4	24.2	4.85
	17.5	4.0	159.8	21.3	27.8	5.00
	18.8	3.9	166.3	19.7	26.5	4.74
	22.5	3.5	189.2	17.8	21.8	3.19
Average	**21.8**	**3.9**	**192.4**	**19.6**	**25.0**	**4.56**
S.D.	3.2	0.2	25.1	1.1	2.2	0.68
CV	15%	5%	13%	6%	9%	15.0%
Non-menthol	21.5	4.3	190.1	22.0	31.5	4.16
	17.8	3.9	181.8	20.2	26.9	4.03
	17.2	3.5	164.1	19.1	26.6	3.93
	15.1	3.9	152.3	21.4	29.8	4.05
	23.1	3.9	194.1	20.1	26.7	3.96
	21.5	3.7	187.2	18.8	24.1	3.78
Average	**19.4**	**3.9**	**178.3**	**20.3**	**27.6**	**3.99**
S.D.	3.1	0.3	16.5	1.3	2.6	0.13
CV	16%	7%	9%	6%	10%	3.2%

Source: Steelman, D.T., and B.W. Dawson. (2000). Quantitative determination of 1,3-butadiene, acrylonitrile, isoprene, benzene, and toluene in the mainstream vapor phase smoke from prototypes SP111999AA, SP111999AB, PD8609, PD8610, Merit Ultra Light, Kentucky Reference 1R4F and Kentucky Reference 1R5F Cigarettes. Research and Development Report, RJRT, Document No.: ACD-MTDS2000-024.

[a] WTPM = Wet Total Particulate Matter.

A.3. Determination of quinoline in mainstream smoke total particulate matter (TPM)

A comparative study was conducted to evaluate quinoline yields in mainstream cigarette smoke from menthol and non-menthol test cigarettes. A standard commercial blend was used in both the mentholated and non-mentholated cigarettes. The two filtered cigarettes were identical with the exception of the addition of menthol. The test cigarettes were smoked using the US Federal Trade Commission (FTC) smoking method regimen (35 ml puff volume, 2-s duration, once per minute).

The average quinoline content of mainstream smoke for both the menthol and non-menthol cigarettes was 69 ng TPM/cigarette (see table below). No significant difference was found in the amount of quinoline in the mainstream smoke TPM in the menthol and non-menthol test cigarettes.

Table A3. Comparison of quinoline concentrations in mainstream smoke TPM[a] from menthol cigarettes versus non-menthol cigarettes.

Sample	Menthol (ng/cigarette)	Non-menthol (ng/cigarette)
1	71	68
2	63	57
3	73	76
4	69	68
5	66	71
6	70	71
Avg.	**69**	**69**
S.D.	3.7	6.5
RSD	5.3	9.5
Avg. TPM (mg/cigarette)	4.4	4.0

Source: Clapp, W. L., and P. Martin. (2000). Determination of quinoline in mainstream smoke TPM from GN19502 Products. Research and Development Report, RJRT, Document No.: ACD-MWLC2000-060.

[a] TPM: Total Particulate Matter.

A.4. Quantitative determination of 2-furancarboxaldehyde in mainstream cigarette smoke from menthol and non-menthol cigarettes

A comparative study was conducted to evaluate the potential impact of menthol addition on the 2-furancarboxaldehyde (2-furfural) in the mainstream cigarette smoke from menthol and non-menthol test cigarettes. A standard commercial blend was used to produce menthol and non-menthol filtered test cigarettes that were identical with the exception of the addition of menthol. The test cigarettes were smoked using the US Federal Trade Commission (FTC) smoking method regimen (35 ml puff volume, 2-s duration, once per minute). Vapor phase, particulate phase and total 2-furancarboxaldehyde content were quantified (see table below).

When mainstream cigarette smoke vapor phase from menthol cigarettes was compared with that of non-menthol cigarettes, menthol cigarettes were found to have a slightly, but significantly higher amount of 2-furfural (0.22 μg/cigarette) than non-menthol cigarettes (0.09 μg/cigarette), ($p < 0.05$). No difference was observed in 2-furfural in the mainstream cigarette particulate phase between menthol and non-menthol cigarettes.

Table A4. Determination of 2-furancarboxaldehyde in mainstream cigarette smoke.

Sample	Vapor phase (μg/cigarette)	Particulate phase (μg/cigarette)	Total (μg/cigarette)	WTPM[a] (μg/cigarette)
Menthol	0.29	0.16	0.45	5.50
	0.19	0.14	0.33	5.30
	0.19	0.13	0.32	4.60
	0.22	0.18	0.40	5.50
	0.19	0.15	0.34	4.90
	0.25	0.14	0.39	5.10
Average	**0.22**[a]	**0.15**	**0.37**	**5.15**
S.D.	0.04	0.02	0.05	0.36
C.V.	19%	12%	14%	6.9%
Non-menthol	0.09	0.16	0.25	4.60
	0.11	0.16	0.27	4.50
	0.07	0.14	0.21	4.40
	0.07	0.14	0.21	4.40
	0.08	0.13	0.21	4.90
	0.09	0.15	0.24	4.50
Average	**0.09**[b]	**0.15**	**0.23**	**4.55**
S.D.	0.02	0.01	0.03	0.19
C.V.	18%	8%	11%	4.1%

[a,b]Means without a common superscript letter differ ($p < 0.05$).

Source: Steelman, D., and B. Smith. (2000). Quantitative determination of 2-furancarboxaldehyde in mainstream cigarette smoke from Eclipse SP111999AA, Eclipse SP111999AB (menthol), PD8609 (menthol), PD8610, Merit Ultra Light Box, Kentucky Reference 1R4F, Kentucky Reference 1R5F and Industry Monitor 15 Cigarettes. Research and Development Report, RJRT, Document No.: ACD-MDTS 2000-61.

[a] WTPM: Wet Total Particulate Matter.

A.5. Yields of vapor phase radicals in mainstream smoke from menthol and non-menthol cigarettes

A comparative analysis was conducted to evaluate the vapor phase yields of chemical radical species in the mainstream smoke of menthol and non-menthol filtered test cigarettes, using the US Federal Trade Commission (FTC) smoking method regimen (35 ml puff volume, 2-s duration, once per minute). A standard commercial blend was used in both the mentholated and non-mentholated cigarettes. The two cigarettes were identical with the exception of the addition of menthol.

No significant difference was found in the yields of vapor phase free radicals in the mainstream smoke of menthol and non-menthol cigarettes (see table below).

Table A5. Comparison of vapor phase free radicals in mainstream smoke from menthol and non-menthol cigarettes.

Sample	# Cigarette	WTPM (mg/cigarette)	Puffs/ cigarette	10^{15} Spins/ cigarette
Menthol	20	4.6	9.1	0.824
	20	4.8	8.9	0.772
	20	4.5	9.0	0.689
	20	4.5	8.9	0.742
	20	5.0	8.7	0.825
	20	4.6	8.8	0.841
Average	20	**4.7**	**8.9**	**0.782**
S.D.		0.2	0.1	0.059
CV		4.2	1.4	7.5
Non-menthol	20	5.3	9.4	0.956
	20	2.4	9.5	0.544
	20	3.6	8.7	0.827
	20	3.8	8.7	0.838
	20	4.4	8.9	0.807
	20	3.9	8.9	0.786
Average	20	**3.9**	**9.0**	**0.793**
S.D.		1.0	0.3	0.136
CV		24.7	3.8	17.1

Source: Blakley, R. L., and D. D. Henry. (2000). Yields of Vapor Phase Radicals in Mainstream Smoke from SP111999AA, SP111999AB, PD8609, PD8610, Merit Ultra Light Box, Kentucky Reference 1R4F and 1R5F Cigarettes and a Smoke Blank. Research and Development Report, RJRT, Document No.: ACD-MRLB2000-33.

A.6. Comparison of mainstream smoke from menthol and non-menthol cigarettes

A comparative study was conducted to further characterize the mainstream smoke of menthol and non-menthol filtered test cigarettes, using the US Federal Trade Commission (FTC) smoking method puffing regimen (35 ml puff volume, 2-s duration, once per minute). A standard commercial blend was used in both the mentholated and non-mentholated cigarettes. The two cigarettes were identical with the exception of the addition of menthol.

These compounds were evaluated by gas chromatography with mass selective detection and the total chromatographic response, number of peaks representing concentrations at or above 0.5 μg/cigarette. Both vapor phase (MSVP) and particulate phase (MSPP) mainstream smoke were evaluated. Mainstream particulate phase smoke for menthol cigarettes provided 90 ± 16 peaks (peak chromatographic response (PCR) of 940 ± 212 μg/cigarette and total chromatographic response (TCR) of 1440 ± 135 μg/cigarette), for non-menthol cigarettes 86 ± 15 peaks (PCR 579 ± 130 μg/cigarette and TCR 984 ± 108 μg/cigarette). No significant difference was found in the vapor phase mainstream smoke evaluated from the menthol versus the non-menthol cigarettes. On the other hand, particulate phase mainstream smoke from non-menthol cigarettes was significantly ($p \leqslant 0.05$) lower in PCR and TCR than the menthol cigarettes.

Table A6. Comparison of number of chromatographic peaks and chromatographic response (Vapor Phase based on m/z 136 ISTD).

Cigarette configuration	Smoke ID number	Number of peaks	Peak chromatographic response (μg/cigarette)	Total chromatographic response (μg/cigarette)	WTPM (mg/cigarette)
Menthol	Replicate 1	82	796	1475	4.83
	Replicate 2	90	949	1326	4.78
	Replicate 3	92	1304	1373	5.01
	Replicate 4	97	1175	1461	4.58
	Replicate 5	92	1132	1309	4.63
	Replicate 6	95	1189	1400	4.52
	Average	**91**	**1091**	**1391**	**4.72**
	STD	5	185	68	0.18
	CV	6%	17%	5%	3.9%
Non-menthol	Replicate 1	93	780	1343	4.03
	Replicate 2	90	815	1437	4.18
	Replicate 3	98	1092	1538	4.14
	Replicate 4	97	1148	1581	3.86
	Replicate 5	96	986	1434	4.04
	Replicate 6	93	915	1435	3.80
	Average	**95**	**956**	**1469**	**4.01**
	STD	3	148	95	0.15
	CV	3%	15%	6%	3.8%

Source: Brooks, C.O., D.D. Henry, and H. Chung. (2000). Comparison of Mainstream Smoke from SP111999AA, SP111999AB, PD8609, PD8610, Merit ULT Box, and 1R4F and 1R5F Kentucky Reference Cigarettes by Gas Chromatography with Mass Selective Detection. Research and Development Report, RJRT, Document No. ACD-MCOB 2000-315.

Table A7. Comparison of number of chromatographic peaks and chromatographic response (Particulate Phase based on m/z 136 ISTD).

Cigarette	Smoke ID number	Number of peaks	Peak chromatographic response (μg/cigarette)	Total chromatographic response (μg/cigarette)	WTPM (mg/cigarette)
Menthol	Replicate 1	117	1201	1573	4.83
	Replicate 2	99	1128	1563	4.78
	Replicate 3	95	1057	1546	5.01
	Replicate 4	78	763	1343	4.58
	Replicate 5	75	733	1339	4.63
	Replicate 6	78	755	1275	4.52
	Average	**90**	**940**[a]	**1440**[c]	**4.72**
	STD	16	212	135	0.18
	CV	18%	23%	9%	3.9%
Non-menthol	Replicate 1	77	477	1116	4.03
	Replicate 2	78	467	1088	4.18
	Replicate 3	68	444	1023	4.14

(*continued on next page*)

Table A7 (*continued*)

Cigarette	Smoke ID number	Number of peaks	Peak chromatographic response (μg/cigarette)	Total chromatographic response (μg/cigarette)	WTPM (mg/cigarette)
	Replicate 4	108	721	913	3.86
	Replicate 5	99	717	915	4.04
	Replicate 6	87	647	848	3.80
	Average	**86**	**579**[b]	**984**[d]	**4.01**
	STD	15	130	108	0.15
CV	17%	23%	11%	3.1%	

[a,b,c,d]Means without a common superscript letter differ ($p < 0.05$).

Table A8: Comparison of number of chromatographic peaks and chromatographic response (particulate phase based on *m/z* 136 ISTD and excluding major components).

Cigarette	Smoke ID number	Number of peaks	Peak chromatographic response (μg/cigarette)	Total chromatographic response (μg/cigarette)
Menthol	Replicate 1	112	249	620
	Replicate 2	93	212	646
	Replicate 3	89	177	666
	Replicate 4	72	157	737
	Replicate 5	69	142	749
	Replicate 6	72	161	681
	Average	**85**	**183**[a]	**383**[c]
	STD	17	40	51
	CV	20%	22%	7%
Non-menthol	Replicate 1	72	165	607
	Replicate 2	73	161	575
	Replicate 3	63	142	555
	Replicate 4	104	212	601
	Replicate 5	94	204	609
	Replicate 6	82	180	546
	Average	**81**	**177**[b]	**582**[d]
	STD	15	27	28
	CV	20%	15%	5%

[a,b,c,d]Means without a common superscript letter differ ($p < 0.05$).
Source: Brooks, C.O., D.D. Henry, and H. Chung. (2000). Comparison of Mainstream Smoke from SP111999AA, SP111999AB, PD8609, PD8610, Merit ULT Box, and 1R4F and 1R5F Kentucky Reference Cigarettes by Gas Chromatography with Mass Selective Detection. Research and Development Report, RJRT, Document No. ACD-MCOB 2000-315.

Appendix B. In vitro toxicology tests of menthol and non-menthol cigarettes

A series of biological assays was performed to compare the responses elicited by preparations of smoke particulate material from a filtered reference cigarette containing no menthol to those from a matched test cigarette containing menthol added at 1.03% menthol w/w tobacco (6.68 mg/cigarette).

B.1. Salmonella/Mammalian-microsome reverse mutation assay (Ames Assay)

A comparative study was conducted to assess the potential impact of menthol addition on the mutagenic activity of cigarette smoke total particulate material (TPM) from menthol and non-menthol cigarettes. A standard commercial blend was used to produce test cigarettes for the purpose of evaluation. The two test cigarettes were identical with the exception of the addition of menthol. Menthol was added at 1.03 % (6.68 mg/cigarette) to the appropriate test cigarette. The smoke menthol yield was 0.41 mg/cigarette. TPM was prepared by smoking the cigarettes on a smoking machine under standard Federal Trade Commission (FTC) conditions (35 ml puff volume, 2-s duration, once per minute).

Ames bacterial mutagenesis activity was evaluated in the genome of several strains of *Salmonella typhimurium*, including TA98, TA100, TA1535, TA1537, and TA1538 in the presence and absence of a standard S9 mix. There were no statistically significant differences observed when the mutagenicity of TPM from menthol (4.8 ± 0.18 mg TPM/cigarette) was compared to that of TPM from non-menthol (4.2 ± 0.16 mg TPM/cigarette) cigarettes with TA98, TA100, TA1537 and TA1538. There was no evidence that the addition of menthol increases the mutagenicity of smoke particulate material.

Table B1. Ames activity in *S. typhimurium* Strain TA98, TA100, TA1535, TA1537, and TA1538 following exposure to TPM from menthol and non-menthol cigarettes.

Sample	S9 %	TA98 Revs./ mg TPM	TA100 Revs./ mg TPM	TA1535 Revs./ mg TPM	TA1537 Revs./ mg TPM	TA1538 Revs./ mg TPM
Menthol	0	194	NEG	NEG	NEG	NEG
	5	1280	738	NEG	77	1175
Non-menthol	0	200	NEG	NEG	NEG	NEG
	5	1592	637	NEG	107	1024

Source: Avalos, J.T., K.W. Fowler, and J.E. Swauger. (2000). A comparison of Ames activity on smoke condensates derived from menthol and non-menthol Eclipse cigarette prototypes and menthol and non-menthol tobacco-burning cigarettes. Research and Development Report, RJRT, Document No.: EMT000113.

B.2. Neutral red cytotoxicity assay

A comparative study was conducted to assess the potential impact of menthol addition on the cytotoxicity of cigarette smoke condensate (CSC) in Chinese Hamster Ovary (CHO) cells in the neutral red assay. A standard commercial blend was used to produce filtered test cigarettes for the purpose of evaluation. The two test cigarettes were identical with the exception of the addition of menthol. Menthol was added at 1.03% (6.68 mg/cigarette) to the appropriate test cigarette. The smoke menthol yield was 0.41 mg/cigarette. CSC was prepared by smoking the cigarettes on a smoking machine under standard Federal Trade Commission (FTC) conditions (35 ml puff volume, 2-s duration, once per minute).

CHO cells were exposed to 10, 25, 50, 75, 100, and 150 μg/ml of cigarette smoke condensate (CSC) from either menthol or non-mentholated test cigarette. The initial plating density was 10,000 cells per well in 96 well microtiter tissue culture plates. There was no statistical difference in cytotoxicity at any of the CSC concentrations tested. There was no evidence that menthol addition increases the cytotoxic potential of CSC.

Table B2. Cytotoxicity of cigarette smoke condensates from menthol and non-menthol cigarettes.

Sample	EC_{50} Value* (μg/ml)	Initial Conc. Where cytotoxicity was observed (μg/ml)	Slope
Menthol	58.8	25	−0.806
Non-menthol	47.0	25	−0.848

EC_{50} = effective concentration of the test article causing a 50% reduction in cell viability relative to control cell cultures.
Source: Putnam, K.P., 2000. Use of neutral red cytotoxicity assay to determined the cytotoxic potential of cigarette smoke condensate from menthol and non-menthol Eclipse and tobacco-burning cigarettes. Research and Development Report, RJRT, Document No. EMT000223.

B.3. Sister chromatid exchange assay (SCE)

The potential of menthol addition to cigarettes to impact the genotoxicity of cigarette smoke total particulate material (TPM) was evaluated in the SCE assay. A comparative SCE study was conducted in Chinese Hamster Ovary (CHO) cells in the presence and absence of S9 metabolic activation. A standard commercial blend was used in both the mentholated and non-mentholated cigarettes. The two filtered test cigarettes were identical with the exception of the addition of menthol. Menthol was added at an inclusion level of 1.03% (6.68 mg/cigarette) to the appropriate test cigarette. The smoke menthol yield was 0.41 mg/cigarette. TPM was prepared by smoking the cigarettes on a smoking machine under standard Federal Trade Commission (FTC) conditions (35 ml puff volume, 2-s duration, once per minute). CHO cells were exposed to multiple concentrations of cigarette smoke TPM from menthol and non-menthol cigarettes.

In the absence of S9 metabolic activation, duplicate CHO cell cultures were exposed to concentrations 0, 10, 25, 37.5, 50, and 75 μg TPM/ml. In the presence of S9 metabolic activation, CHO cells were exposed to 150, 200, 250, 275, 300 μg TPM/ml from mentholated cigarettes and to 0, 150, 175, 200, 225, 250, 275, and 300 μg TPM/ml from non-menthol cigarettes. Due to observed toxic effects, some concentrations were not scored (see table below).

The results showed a significant linear dose response effect on SCE counts for cigarette smoke TPM from menthol and non-menthol cigarettes in the absence ($p < 0.02$) of S9 metabolic activation (see table below). Cigarette smoke TPM from menthol cigarettes was not significantly different from that of non-menthol cigarettes either in the absence or presence of S9 activation under the conditions of this study.

Table B3. SCE assay of menthol and non-menthol cigarette smoke TPM without S9 metabolic activation.

Sample	TPM dose (μg /ml)	SCE/ Cell ± S.E.	Time in BrdU	% M1	% M1+	% M2	% M2+	% Confluency
Menthol	10	8.76 ± 0.60	27	0.5	3.5	93.5	2.5	95
	25	14.04 ± 0.72	27.5	0.5	14.5	85.0	0.0	90
	37.5	18.70 ± 0.46	28	4.0	34.0	62.0	0.0	90
	50	20.25 ± 0.63	29	21.0	47.0	32.0	0.0	90
	75	Too toxic to score	29					80
Non-menthol	10	10.70 ± 0.30	27	0.5	4.5	91.5	3.5	90
	25	13.88 ± 1.32	27.5	0.0	12.0	88.0	0.0	85
	37.5	19.16 ± 0.00	28	6.5	37.0	56.5	0.0	85
	50	21.60 ± 0.060	29	28.0	46.5	25.5	0.0	80
	75	Too toxic to score	30					70

SCE assay of menthol and non-menthol cigarette smoke TPM with metabolic activation.

Sample	TPM dose (μg /ml)	SCE/ Cell ± S.E.	Time in BrdU	% M1	% M1+	% M2	% M2+	% Confluency
Menthol	150	12.78 ± 1.74	27.5	2.5	7.5	87.5	2.5	95
	200	15.88 ± 0.32	29	9.5	19.5	69.5	1.5	90
	250	17.16 ± 0.92	30	42.0	47.0	11.0	0.0	80 & 70
	275	14.24 ± 0.93	31	66.0	27.5	6.5	0.0	55 & 70
	300	Too toxic to score	31					60
Non-menthol	150	13.84 ± 0.24	29	2.0	20.0	78.0	0.0	95
	175	16.24 ± 0.56	30	11.5	32.0	56.5	0.0	90
	200	15.50 ± 0.74	31	9.5	23.0	67.5	0.0	85-90
	225	13.52 ± 0.65	31	58.0	34.0	8.0	0.0	75-80
	250	Too toxic to score	31					50
	275	Too toxic to score	31					35 & 55
	300	Too toxic to score	31					35

Source: Bombick, B.R., D.L. Bowman, J.B. Mabe, and W.T. Morgan. (2000). A comparative study of sister chromatid exchange frequencies in Chinese Hamster Ovary cells exposed to cigarettes, smoke condensate from Eclipse menthol and non-menthol cigarettes, tobacco-burning menthol and non-menthol cigarette, and Kentucky Reference 1R4F. Research and Development Report, RJRT, Document No.: EMT000717.

References

Adams, T.B., Hallagan, J.B., Putnam, J.M., Gierke, T.L., Doull, J., Munro, I.C., Newberne, P., Portoghese, P.S., Smith, R.L., Wagner, B.M., Weil, C.S., Woods, L.A., Ford, R.A., 1996. The FEMA GRAS assessment of alicyclic substances used as food flavour ingredients. Food and Chemical Toxicology 34, 763–828.

Adams, P.F., Schoenborn, C.A., 2006. Health behaviors of adults: United States, 2002–04. Vital Health Statistics 10 (230), 1–140 [Data from the National Health Survey].

Ahijevych, K.L., Wewers, M.E., 1994. Patterns of cigarette consumption and cotinine levels among African-American women smokers. American Journal of Respiratory and Critical Care Medicine 150, 1229–1233.

Ahijevych, K., Gillespie, J., Demirci, M., Jagadeesh, J., 1996. Menthol and non-menthol cigarettes and smoke exposure in black and white women. Pharmacology Biochemistry and Behavior 53, 355–360.

Ahijevych, K., Parsley, L.A., 1999. Smoke constituent exposure and stage of change in black and white women cigarette smokers. Addictive Behaviors 24, 115–120.

Ahijevych, K., Garrett, E., 2004. Menthol pharmacology and its potential impact on cigarette smoking behavior. Nicotine and Tobacco Research 6 (Suppl. 1), S17–S28.

Alakayak, J., Knall, C., 2008. Mentholated and non-mentholated cigarettes alter the transepithelial electrical resistance of Calu-3 human bronchial epithelial cells. Ethnicity and Disease 18 (S1), 45–46.

Ale, S.I., Hostynek, J.J., Maibach, H.I., 2002. Menthol: a review of its sentizazation potential. Exogenous Dermatology 1 (2), 74–80.

Alberg, A.J., Horner, M.-J.D., Daguise, V.G., Carpenter, M.J., Mosley, C.M., Vincent, B., Silvestri, G., Reed, C.E., Hebert, J.R., 2006. Lung and bronchus cancer disparities in South Carolina: epidemiology and strategies for prevention. The Journal of the South Carolina Medical Association 102, 183–190.

Alberg, A.J., Ford, J.G., Samet, J.M., 2007. Epidemiology of lung cancer: ACCP evidence-based clinical practice guidelines (2nd Edition). Chest 132 (3), S29–S55.

Allen Jr., B., Unger, J.B., 2007. Sociocultural correlates of menthol cigarette smoking among adult African-Americans in Los Angeles. Nicotine and Tobacco Research 9 (4), 447–451.

Andersen, P.H., Jensen, N.J., 1984. Mutagenic investigation of peppermint oil in the Salmonella/mammalian-microsome test. Mutation Research 138, 17–20.

Anderson, K.E., Maibach, H.I., 1980. Allergic reaction to drugs used topically. Clinical Toxicology 16, 415–465.

Ashare, R.L., Hawk Jr., L.W., Cummings, K.M., O'Connor, R.J., Fix, B.V., Schmidt, W.C., 2007. Smoking expectancies for flavored and non-flavored cigarettes among college students. Addictive Behaviors 32, 1252–1261.

Atzl, G., Bertel, M., Daxenbichler, G., Gleispach, H., 1972. Determination of etheral oils from the urine by gas–liquid chromatography. Chromatographia 5, 250–255.

Azzi, C., Zhang, J., Purdon, C.H., Chapman, J.M., Nitcheva, D., Hebert, J.R., Smith, E.W., 2006. Permeation and reservoir formation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and benzo[*a*]pyrene (B[*a*]P) across porcine esophageal tissue in the presence of ethanol and menthol. Carcinogenesis 27 (1), 137–145.

Baker, R.R., Bishop, L.J., 2004. The pyrolysis of tobacco ingredients. Journal of Analytical and Applied Pyrolysis 71, 223–311.

Baker, R.R., Pereira da Silva, J.A., Smith, G., 2004. The effect of tobacco ingredients on smoke chemistry. Part I: flavourings and additives. Food and Chemical Toxicology 42 (S1), 3–37.

Baker, R.R., 2006. The generation of formaldehyde in cigarettes – overview and recent experiments. Food and Chemical Toxicology 44, 1799–1822.

Bass, R.T., Comes, R.A., Jenkins, R.W., 1975. The use of carbon-14 labeled compounds in smoke precursor studies: a review. In: 29th Tobacco Chemists Research Conference.

Belsito, D., Bickers, D., M. Bruze, M., Calow, P., Greim, H., Hanifin, J.M., Rogers, A.E., Saurat, J.H., Sipes, I.G., Tagami, H., 2008. A toxicologic and dermatologic assessment of cyclic and non-cyclic terpene alcohols when used as fragrance ingredients. Food and Chemical Toxicology 46, S1–S71.

Benowitz, N.L., 1996. Cotinine as a biomarker of environmental tobacco smoke exposure. Epidemiologic Reviews 18, 188–204.
Benowitz, N.L., Perez-Stable, E.J., Fong, I., Modin, G., Herrera, B., Jacob III, P.J., 1999. Ethnic differences in *N*-glucuronidation of nicotine and cotinine. Journal of Pharmacology and Experimental Therapeutics 291 (3), 1196–1203.
Benowitz, N.L., Herrera, B., Jacob, P., 2004. Mentholated cigarette smoking inhibits nicotine metabolism. Journal of Pharmacology and Experimental Therapeutics 310 (3), 1208–1215.
Best, F.W., 1993. Effects of some cigarette construction parameters on menthol migration and transfer. Recent Advances in Tobacco Science 19, 155–201.
Bhatia, S.P., McGinty, D., Letizia, C.S., Api, A.M., 2008a. Fragrance material review on menthol. Food and Chemical Toxicology 46 (11), S209–S214.
Bhatia, S.P., McGinty, D., Letizia, C.S., Api, A.M., 2008b. Fragrance material review on d,l-menthol. Food and Chemical Toxicology 46 (11), S224–S227.
Borschke, A.J., 1993. Review of the technologies relating to menthol use in cigarettes. Recent Advances in Tobacco Science 19, 47–70.
Bover, M.T., Foulds, J., Steinberg, M.B., Richardson, D., Marcella, S.W., 2008. Waking at night to smoke as a marker for tobacco dependence: patient characteristics and relationship to treatment outcome. International Journal of Clinical Practice 62 (2), 182–190.
Burleigh-Flayer, H.D., 1988. Evaluation of the Sensory Irritation Potential and Assessment of the Respiratory Response During Exposure to Menthol Vapor. Bushy Run Research Center. p. 34.
Brooks, D.R., Palmer, J.R., Strom, B.L., Rosenberg, L., 2003. Menthol cigarettes and risk of lung cancer. American Journal of Epidemiology 158, 609–616.
Camarasa, G., Alomar, A., 1978. Menthol dermatitis from cigarettes. Contact Dermatitis 4, 169–170.
Carmines, E.L., 2002. Evaluation of the potential effects of ingredients added to cigarettes. Part 1: cigarette design, testing approach, and review of results. Food and Chemical Toxicology 40, 77–91.
Carpenter, C.L., Jarvik, M.E., Morgenstern, H., McCarthy, W.J., London, S.J., 1999. Mentholated cigarette smoking and lung-cancer risk. Annals of Epidemiology 9, 114–120.
Carpenter, C.M., Wayne, G.F., Pauly, J.L., Koh, H.K., Connolly, G.N., 2005. New cigarette brands with flavors that appeal to youth: tobacco marketing strategies. Health Affairs 24 (6), 1601–1610.
Caskey, N.H., Jarvik, M.E., McCarthy, W.J., Rosenblatt, M.R., Gross, T.M., Carpenter, C.L., 1993. Rapid smoking of menthol and non-menthol cigarettes by black and white smokers. Pharmacology Biochemistry and Behavior 46, 259–263.
CDC, 2008. Centers for disease control and prevention cigarette smoking among adults – United States. Morbidity and Mortality Weekly Report 57 (45), 1221–1226.
Celebucki, C.C., Wayne, G.E., Connolly, G.N., Pankow, J.F., Chang, E.I., 2005. Characterization of measured menthol in 48 US cigarette sub brands. Nicotine and Tobacco Research 7 (4), 523–531.
Chapman, S., 2008. The inverse impact law of smoking cessation. The Lancet 373, 701–703.
Chepiga, T.A., Morton, M.J., Murphy, P.A., Avalos, J.T., Bombick, B.R., Doolittle, D.J., Borgerding, M.F., Swauger, J.E., 2000. A comparison of the mainstream smoke chemistry and mutagenicity of a representative sample of the US cigarette market with two Kentucky reference cigarettes (K1R4F and K1R5F). Food and Chemical Toxicology 38 (10), 949–962.
Chrisman, B.B., 1978. Menthol and dermatitis. Archives of Dermatology 114, 286.
Ciftci, O., Topcu, S., Caliskan, M., Gullu, H., Erdogan, D., Yildirim, E., Yildirir, A., Muderrisoglu, H., 2008a. Smoking mentholated cigarettes impairs coronary microvascular function as severely as does smoking regular cigarettes. Acta Cardiologica 63 (2), 135–140.
Ciftci, O., Caliskan, M., Güllü, H., Yildirir, A., Müderrisoglu, H., 2008b. Mentholated cigarette smoking induced alterations in left and right ventricular functions in chronic smokers. Anadolu Kardiyol Derg 8 (2), 116–122.
Clark, G.S., 2007. Aroma chemical profile: menthol. Perfumer and Flavorist 32, 38–47.
Clark, P.I., Gautam, S., Gerson, L.W., 1996. Effect of menthol cigarettes on biochemical markers of smoke exposure among black and white smokers. Chest 110, 1194–1198.
Clark, P.I., Gardiner, P.S., Djordjevic, M.V., Leischow, S.J., Robinson, R.G., 2004. Menthol cigarettes: setting the research agenda. Nicotine and Tobacco Research 6, S5–S9.
Cochran, E.W., 1995. Menthol analyses of tar and cigarette samples. Unpublished Internal Report. Lorillard Tobacco Company Research Center, Greensboro, North Carolina. Bates # 89812079/2083.
Collins, C.C., Moolchan, E.T., 2006. Shorter time to first cigarette of the day in menthol adolescent cigarette smokers. Addictive Behaviors 31, 1460–1464.
Connolly, G.N., 2004. Sweet and spicy flavours: new brands for minorities and youth. Tobacco Control 13, 211–212.
Cook, C.J., Lauterbach, J.H., Pannell, W.T., Price, B.F., Bowser, W.M., Pinion, D.O., Spencer, A.K., 1999. Transfer of Semi-volatile Components from Tobacco to Smoke. CORESTA Smoke and Technology Groups Meeting. CORESTA Bulletin, p. 8.
Cropsey, K.L., Weaver, M.F., Eldridge, G.D., Villabos, G.C., Best, A.M., Stitzer, M.L., 2009. Differential success rates in racial groups: results of a clinical trial of smoking cessation among female prisoners. Nicotine and Tobacco Research 11, 690–697.
Derfer, J.M., Derfer, M.M., 1983. Terpenoids, third ed.. In: Mark, H.F., Othmer, D.F., Overberger, C.G., Seaborg, G.T., Grayson, N. (Eds.), Kirk-Othmer Encyclopedia of Chemical Technology, vol. 22 John Wiley and Sons, New York, pp. 709–762.
DiFranza, J.R., Savageaua, J.A., Fletcher, K., Ockene, J.K., Rigotti, N.A., McNeill, A.D., Coleman, M., Wood, C., 2004. Recollections and repercussions of the first inhaled cigarette. Addictive Behaviors 29, 261–272.
Eccles, R., 1994. Menthol and related cooling compounds. Journal of Pharmacy and Pharmacology 46, 618–630.
Ernst, A., Zibrak, J.D., 1998. Carbon monoxide poisoning. New England Journal of Medicine 339, 1603–1608.
Etter, J.-F., Le Houzec, J., Huguelet, P., Etter, M., 2009. Testing the cigarette dependence scale in 4 samples of daily smokers: psychiatric clinics, smoking cessation clinics, a smoking cessation website and in the general population. Addictive Behaviors 34 (5), 446–450.
Etzel, C.J., Kachroo, S., Liu, M., D'Amelio, A., Dong, O., Cote, M.L., Wenzlaff, A.S., Hong, W.K., Greisinger, A.J., Schwartz, A.G., Spitz, M.R., 2008. Development and validation of a lung cancer risk prediction model for African–Americans. Cancer Prevention Research 1 (4), 255–265.
Ewing, P., Blomgren, B., Ryrfeldt, A., Gerde, P., 2006. Increasing exposure levels cause an abrupt change in the absorption and metabolism of acutely inhaled benzo(a)pyrene in the isolated, ventilated, and perfused lung of the rat. Toxicological Sciences 91 (2), 332–340.
FCC, 1996. Food Chemicals Codex. National Academy of Sciences, Washington, DC. 882pp.
FDA, 1973. Teratologic evaluation of FDA-71-57 (Menthol, natural, Brazilian). Food Drug Research Laboratories.
Ferguson, S.G., Shiffman, S., Gitchell, J.G., Sembower, M.A., West, R., 2009. Unplanned quit attempts – results from a US sample of smokers and ex-smokers. Nicotine and Tobacco Research 11, 827–832.
FEMA, 1992. Database printout on menthol. FEMA 2665. 87-78-1. The Flavor and Extract Manufacturers Association of the US.
Foulds, J., Gandhi, K.K., Steinberg, M.B., Richardson, D.L., Williams, J.M., Burke, M.V., Rhoads, G.G., 2006. Factors associated with quitting smoking at a tobacco dependence treatment clinic. American Journal of Health Behavior 30 (4), 400–412.
Friedman, G.D., Sadler, M., Tekawa, I.S., Sidney, S., 1998. Mentholated cigarettes and non-lung smoking related cancers in California, USA. Journal of Epidemiology and Community Health 52, 202.
Fu, S.S., Okuyemi, K.S., Partin, M.R., Ahluwalia, J.S., Nelson, D.B., Clothier, B.A., Joseph, A.M., 2008. Menthol cigarettes and smoking cessation during an aided quit attempt. Nicotine and Tobacco Research 10 (3), 457–462.
Fukami, T., Nakajima, M., Yoshida, R., Tsuchiya, Y., Fujiki, Y., Katoh, M., McLeod, H.L., Yokoi, T., 2004. A novel polymorphism of human CYP2A6 gene CYP2A6*17 has an amino acid substitution (V365M) that decreases enzymatic activity in vitro and in vivo. Clinical Pharmacology and Therapeutics 76, 519–527.
Gandhi, K.K., Foulds, J., Steinberg, M.B., Lu, S.-E., Williams, J.M., 2009. Lower quit rates among African-American and Latino menthol cigarette smokers at a tobacco treatment clinic. International Journal of Clinical Practice 63 (3), 360–367.
Garten, S., Falkner, R.V., 2003. Continual smoking of mentholated cigarettes may mask the early warning symptoms of respiratory disease. Preventive Medicine 37 (4), 291–296.
Garten, S., Falkner, R.V., 2004. Role of mentholated cigarettes in increased nicotine dependence and greater risk of tobacco-attributable disease. Preventive Medicine 38 (6), 793–798.
Gaworski, C.L., Dozier, M.M., Gerhart, J.M., Rajendran, N., Brennecke, L.H., Aranyi, C., Heck, J.D., 1997. 13-Week inhalation study of menthol cigarette smoke. Food and Chemical Toxicology 35, 683–692.
Gaworski, C.L., Heck, J.D., Bennett, M.B., Wenk, M.L., 1999. Toxicologic evaluation of flavor ingredients added to cigarette tobacco: skin painting bioassay of cigarette smoke condensate in SENCAR mice. Toxicology 139, 1–17.
Gelal, A., Jacob III, P.J., Yu, L., Benowitz, N.L., 1999. Disposition kinetics and effects of menthol. Clinical Pharmacology and Therapeutics 66, 128–135.
Gelal, A., Guven, H., Balkan, D., Artok, L., Benowitz, N.L., 2003. Influence of menthol on caffeine disposition and pharmacodynamics in healthy female volunteers. European Journal of Clinical Pharmacology 5, 417–422.
Gelal, A., Balkan, D., Ozzeybek, D., Kaplan, Y.C., Gurler, S., Guven, H., Benowitz, N.L., 2005. Effect of menthol on the pharmacokinetics and pharmacodynamics of felodipine in healthy subjects. European Journal of Clinical Pharmacology 6, 785–790.
Giovino, G.A., Sidney, S., Gfroerer, J.C., O'Malley, P.M., Allen, J.A., Richter, P.A., Cummings, K.M., 2004. Epidemiology of menthol cigarette use. Nicotine and Tobacco Research 6 (S1), S67–S81.
Goldstein, E., Cooper, A.D., Tarkington, B., 1976. Effect of inhaling medication vapors from a cold preparation on murine pulmonary bacterial defense systems. Journal of Toxicology and Environmental Health 2, 371–388.
Green, B.G., McAuliffe, B.L., 2000. Menthol desensitization of capsaicin irritation: evidence of a short-term anti-nociceptive effect. Physiology and Behavior 68, 631–639.
Green, M.D., King, C.D., Mojarrabi, B., Mackenzie, P.I., Tephly, T.R., 1998. Glucuronidation of amines and other xenobiotics catalyzed by expressed human UDP-glucuronosyltransferase 1A3. Drug Metabolism and Disposition 26, 507–512.
Hall, R.L., Oser, B.L., 1965. Recent progress in the consideration of flavoring ingredients under the food additives amendment. III. GRAS substances. Food Technology 19, 151–197.
Harris, K.J., Okuyemi, K.S., Catley, D., Mayo, M.S., Ge, B., Ahluwalia, J.S., 2004. Predictors of smoking cessation among African-Americans enrolled in a randomized controlled trial of bupropion. Preventive Medicine 38, 498–502.

Hatsukami, D., Benowitz, N.L., Rennard, S.I., Oncken, C., Hecht, S.S., 2006. Biomarkers to assess the utility of potential reduced exposure tobacco products. Nicotine and Tobacco Research 8 (4), 599–622.
Hebert, J.R., 2003. Invited commentary: menthol cigarettes and risk of lung cancer. American Journal of Epidemiology 158, 609–616.
Hebert, J.R., Kabat, G.C., 1988. Menthol cigarettes and esophageal cancer. American Journal of Public Health 78 (8), 986–987.
Hebert, J.R., Kabat, G.C., 1989. Menthol cigarette smoking and oesophageal cancer. International Journal of Epidemiology 18, 37–44.
Heck, J.D., 2009. Smokers of menthol and nonmenthol cigarettes exhibit similar levels of biomarkers of smoke exposure. Cancer Epidemiology Biomarkers and Prevention 18 (2), 622–629.
Hersey, J.C., Ng, S.W., Nonnemaker, J.M., Mowery, P., Thomas, K.Y., Vilsaint, M.C., Allen, J.A., Haviland, M.L., 2006. Are menthol cigarettes a starter product for youth? Nicotine and Tobacco Research 8 (3), 403–413.
Hilliard, C.A., Armstrong, M.J., Bradt, C.I., Hill, R.B., Greenwood, S.K., Galloway, S.M., 1998. Chromosome aberrations in vitro related to cytotoxicity of nonmutagenic chemicals and metabolic poisons. Environ. Mol. Mutagen 31 (4), 316–326.
Hoffmann, D., Hoffmann, I., 1998. Tobacco smoke components (letter to the Editors). Beiträge zur Tabakforschung International/Contributions to Tobacco Research 18 (1), 49–52.
Hopp, R., 1993. Menthol: its origins, chemistry, physiology and toxicological properties. Recent Advances in Tobacco Science 19, 3–46.
Hyland, A., Giovino, S., Garten, G.A., Cummings, K.M., 2002. Mentholated cigarettes and smoking cessation: findings from COMMIT. Tobacco Control 11, 135–139.
Ishidate, M., Sofuni, T., Yoshikawa, K., Hayashi, M., Nohmi, T., Sawada, M., Matsuoka, A., 1984. Primary mutagenicity screening of food additives currently used in Japan. Food and Chemical Toxicology 22, 623–636.
Ivett, J.L., Brown, B.M., Rodgers, C., Anderson, B.E., Resnick, M.A., Zeiger, E., 1989. Chromosomal aberrations and sister chromatid exchange tests in Chinese hamster ovary cells in vitro. IV. Results with 15 chemicals. Environmental and Molecular Mutagenesis 14, 165–187.
Jarvik, M.E., Tashkin, D.P., Caskey, N.H., McCarthy, W.J., Rosenblatt, M.R., 1994. Mentholated cigarettes decrease puff volume of smoke and increase carbon monoxide absorption. Physiology and Behavior 56, 563–570.
JECFA, 1998. Menthol. In: 51st Meeting of the Joint FAO/WHO Expert Committee on Food Additives, FAS 42-JECFA 51/57, p. 381.
Jenkins, R.W., Newman, R.H., Chavis, M.K., 1970. Cigarette smoke formation studies II. Smoke distribution and mainstream pyrolytic composition of added 14C-menthol. Beiträge zur Tabakforschung International/Contributions to Tobacco Research 5, 299–302.
Jing, Y., Gong, C., Xian, K., Wang, C., Lu, P., 2005. The effects of filter ventilation on flavor constituents in cigarette smoke. Beiträge zur Tabakforschung International/Contributions to Tobacco Research 21 (5), 280–285.
Jöckel, K.-H., Pohlabeln, H., Jahn, I., 2004. Use of menthol cigarettes and risk of lung cancer. Biometrical Journal 46 (S1). S15.2,33.
Kabat, G.C., Hebert, J.R., 1991. Use of mentholated cigarettes and lung cancer risk. Cancer Research 51, 6510–6513.
Kabat, G.C., Hebert, J.R., 1994. Use of mentholated cigarettes and oropharyngeal cancer. Epidemiology 5, 183–188.
Kaffenberger, R.M., Doyle, M.J., 1990. Determination of menthol and menthol glucuronide in human urine by gas chromatography using an enzyme-sensitive internal standard and flame ionization detection. Journal of Chromatography 527, 59–66.
Kreslake, J.M., Wayne, G.F., Alpert, H.R., Koh, H.K., Connolly, G.N., 2008. Tobacco industry control of menthol in cigarettes and targeting of adolescents and young adults. American Journal of Public Health 98 (9), 1685–1692.
Leung, A.Y., Foster, S., 1996. Menthol. In: Encyclopedia of Common Natural Ingredients. John Wiley and Sons, New York, pp. 368–371.
Li, Q., Hyland, A., Giovino, G., Bauer, J., Cummings, M., 2005. Nicotine dependence: comparing menthol and non-menthol cigarette smokers. In: National Conference on Tobacco or Health, Chicago, IL, May 4–6, Abstract #12437.
Litton Bionetics Inc., 1975. Mutagenic evaluation of compound FDA 71-57, menthol. NTIS, PB-245 444, 152pp.
Luke, E., 1962. Addiction to mentholated cigarettes. Lancet 279 (7220), 110–111.
MacDougall, J.M., Fandrick, K., Zhang, X., Serafinand, S.V., Cashman, J.R., 2003. Inhibition of human liver microsomal (S)-nicotine oxidation by (−)-menthol and analogues. Chemical Research in Toxicology 16, 988–993.
McCarthy, W.J., Caskey, N.H., Jarvik, M.E., Gross, T.M., Rosenblatt, M.R., Carpenter, C., 1995. Menthol vs. non-menthol cigarettes: effects on smoking behavior. American Journal of Public Health 85, 67–72.
Miller, G.E., Jarvik, M.E., Caskey, N.H., Segerstrom, S.C., Rosenblatt, M.R., McCarthy, W.J., 1994. Cigarette mentholation increases smokers' exhaled carbon monoxide levels. Experimental and Clinical Psychopharmacology 2, 154–160.
Moolchan, E.T., 2004. Adolescent menthol smokers: will they be a harder target for cessation? Nicotine and Tobacco Research 6 (S1), S93–S95.
Moolchan, E.T., Hudson, D.L., Schroeder, J.R., Sehnert, S.S., 2004. Heart rate and blood pressure responses to tobacco smoking among African-American adolescents. Journal of the National Medical Association 96 (6), 767–771.
Moolchan, E.T., Franken, F.H., Jaszyna-Gasior, M., 2006. Adolescent nicotine metabolism: ethnoracial differences among dependent smokers. Ethnicity and Disease 16 (1), 239–243.
Munro, I.C., Kennepohl, E., 2001. Comparison of estimated daily per capita intakes of flavoring substances with no-observed-effect levels from animal studies. Food and Chemical Toxicology 39, 331–354.
Murray, R.P., Connett, J.E., Skeans, M.A., Tashkin, D.P., 2007. Menthol cigarettes and health risks in lung health study data. Nicotine and Tobacco Research 9 (1), 101–107.
Muscat, J.E., Richie Jr., J.P., Stellman, S.D., 2002. Mentholated cigarettes and smoking habits in whites and blacks. Tobacco Control 11, 368–371.
Muscat, J.E., Chen, G., Knipe, A., Stellman, S.D., Lazarus, P., Richie Jr., J.P., 2009. Effects of menthol on tobacco smoke exposure, nicotine dependence, and NNAL glucuronidation. Cancer Epidemiology Biomarkers and Prevention 18 (1), 35–41.
Mustonen, T.K., Spencer, S.M., Hoskinson Jr., R.A., Sachs, D.P.L., Garvey, A.J., 2005. The influence of gender, race, and menthol content on tobacco exposure measures. Nicotine and Tobacco Research 7 (4), 581–590.
Myhr, B.C., Caspary, W.J., 1991. Chemical mutagenesis at the thymidine kinase locus in L5178Y mouse lymphoma cells. Environmental and Molecular Mutagenesis 18, 51–83.
NCI, 1979. Bioassay of dl-menthol for possible carcinogenicity. National Cancer Institute, DHEW/PUB/NIH-79-1348, NCI-CG-TR-98, 112pp.
Nelson, D.B., Partin, M.R., Fu, S.S., Joseph, A.M., An, L.C., 2009. Why assigning ongoing tobacco use is not necessarily a conservative approach to handling missing tobacco cessation outcomes. Nicotine and Tobacco Research 11 (1), 77–83.
Newell, M.P., Latimer, P.H., Haefle, R.J., 1968. The fate of menthol in cigarette smoke. In: 22nd Tobacco Chemists' Research Conference Proceedings, p. 18.
Nil, R., Battig, K., 1989. Separate effects of cigarette smoke yield and smoke taste on smoking behavior. Psychopharmacology 99, 54–59.
NIOSH, 1979. Health hazard evaluation determination report no. HE 77-66-531, Sucrets Department, Merch, Sharp and Dohme, West Point, Pennsylvania. National Institute for Occupational Safety and Health.
Nishino, T., Tagaito, Y., Sakurai, Y., 1997. Nasal inhalation of l-menthol reduces respiratory discomfort associated with loaded breathing. American Journal of Respiratory and Critical Care Medicine 155, 309–313.
O'Connor, R.J., Ashare, R.L., Cummings, K.M., Hawk Jr., L.W., 2007. Comparing smoking behaviors and exposures from flavored and unflavored cigarettes. Addictive Behaviors 32 (4), 869–874.
OECD, 2003. Screening Information Dataset for High Production Volume Chemicals: Menthols. The Organization foe Economic Cooperation and Development. <www.inchem.org/documents/sids/sids/MENTHOLS.pdf>.
Okuyemi, K.S., Ahluwalia, J.S., Ebersole-Robinson, M., Catley, D., Mayo, M.S., Resnicow, K., 2003. Does menthol attenuate the effect of bupropion among African-American smokers? Addiction 98, 1387–1393.
Okuyemi, K.S., Ebersole-Robinson, M., Nazir, N., Ahluwalia, J.S., 2004. African-American menthol and non-menthol smokers: differences in smoking and cessation experiences. Journal of the National Medical Association 96 (9), 1208–1211.
Okuyemi, K.S., Pulvers, K.M., Cox, L.S., Thomas, J.L., Kaur, H., Mayo, M.S., Nazir, N., Etter, J.-F., Ahluwalia, J.S., 2007a. Nicotine dependence among African-American light smokers: a comparison of three scales. Addictive Behaviors 32, 1989–2002.
Okuyemi, K.S., Faseru, B., Sanderson, C.L., Bronars, C.A., Ahluwalia, J.S., 2007b. Relationship between menthol cigarettes and smoking cessation among African-American light smokers. Addiction 102, 1979–1986.
Orani, G.P., Anderson, J.W., Sant'Ambrogio, G., Sant'Ambrogio, F.B., 1991. Upper airway cooling and l-menthol reduce ventilation in the guinea pig. Journal of Applied Physiology 70, 2080–2086.
Paschke, T., Scherer, G., Heller, W.-D., 2002. Effects of ingredients on cigarette smoke composition and biological activity: a literature overview. Beiträge zur Tabakforschung International/Contributions to Tobacco Research 20, 107–247.
Patel, T., Ishiuji, Y., Yosipovitch, G., 2007. Menthol: a refreshing look at this ancient compound. Journal of the American Academy of Dermatology 57 (5), 873–878.
Patterson, F., Benowitz, N., Shields, P., Kaufmann, V., Jepson, C., Wileyto, P., Kucharski, S., Lerman, C., 2003. Individual differences in nicotine intake per cigarette. Cancer Epidemiology Biomarkers and Prevention 12, 468–471.
Perez-Stable, E.J., Herrera, B., Jacob III, P., Benowitz, N.L., 1998. Nicotine metabolism and intake in black and white smokers. Journal of the American Medical Association 280 (2), 152–156.
Pickworth, W.B., Moolchan, E.T., Berlin, I., Murty, R., 2002. Sensory and physiologic effects of menthol and non-menthol cigarettes with differing nicotine delivery. Pharmacology, Biochemistry and Behavior 71, 55–61.
Pletcher, M.J., Hulley, B.J., Houston, T., Kiefe, C.I., Benowitz, N., Sidney, S., 2006. Menthol cigarettes, smoking cessation, atherosclerosis, and pulmonary function: the coronary artery risk development in young adults (CARDIA) study. Archives of Internal Medicine 166 (17), 1915–1922.
Pritchard, W.S., Houlihan, M.E., Guy, T.D., Robinson, J.H., 1999. Little evidence that "denicotinized" menthol cigarettes have pharmacological effects: an EEG/heart rate/subjective-response study. Psychopharmacology 143, 273–279.
Rabinoff, M., Caskey, M., Rissling, A., Park, C., 2007. Pharmacological and chemical effects of cigarette additives. American Journal of Public Health 97 (11), 1–11.
Reid, J., 1993. A history of mentholated cigarettes: this Spud's for you. Recent Advances in Tobacco Science 19, 71–84.
Richardson, T.L., 1997. African-American smokers and cancers of the lung and of the upper respiratory and digestive tracts. Is menthol part of the puzzle? West J Med. 166 (3), 189–194.
Richie Jr., J.P., Carmella, S.G., Muscat, J.E., Scott, D.G., Akerkar, S.A., Hecht, S.S., 1997. Differences in the urinary metabolites of the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in black and white smokers. Cancer Epidemiology Biomarkers and Prevention 6, 783–790.

Richter, P., Beistle, D., Pederson, L., O'Hegarty, M., 2008. Small-group discussions on menthol cigarettes: listening to adult African-American smokers in Atlanta, Georgia. Ethnicity and Health 13 (2), 171–182.

Riechelmann, H., Brommer, C., Hinni, M., Martin, C., 1997. Response of human ciliated respiratory cells to a mixture of menthol, eucalyptus oil and pine needle oil. Drug Research 47, 1035–1039.

RJRT, 2000. Chemical and biological analyses of the smoke of menthol and non-menthol cigarettes. R.J. Reynolds Tobacco Company, Winston-Salem, North Carolina. Appended as Supplementary Information.

Rodgman, A., 2002. Some studies of the effects of additives on cigarette mainstream smoke properties. I. Flavorants. Beiträge zur Tabakforschung International/ Contributions to Tobacco Research 20 (2), 83–102.

Rodgman, A., Perfetti, T.A., 2009. The Chemical Components of Tobacco and Tobacco Smoke. CRC Press, Taylor and Francis Group, New York. 1784pp.

Roemer, E., Rustenmeier, K., Vanscheeuwijck, P.M., Meisgen, T.J., Veltel, D.J., Haussmann, H., Carmines, E.L., 2000. Effects of the addition of flavor ingredients to tobacco on the chemical composition and biological activity of cigarette smoke (abstract #77). The Toxicologist 54, 16.

Roemer, E., Tewes, F.J., Meisgen, T.J., Veltel, D.J., Carmines, E.L., 2002. Evaluation of the potential effects of ingredients added to cigarettes. Part 3: in vitro genotoxicity and cytotoxicity. Food and Chemical Toxicology 40, 105–111.

Rose, J.E., Behm, F.M., 2004. Extinguishing the rewarding value of smoke cues: pharmacological and behavioral treatments. Nicotine and Tobacco Research 6 (3), 523–532.

Rosenblatt, M.R., Olmstead, R.E., Iwamoto-Schapp, P.N., Jarkik, M.E., 1998. Olfactory thresholds for nicotine and menthol in smokers (abstinent and nonabstinent) and nonsmokers. Physiology and Behavior 65 (3), 575–579.

Ruskin, D.N., Anand, R., LaHoste, G.J., 2007. Menthol and nicotine oppositely modulate body temperature in the rat. European Journal of Pharmacology 559, 161–164.

Ruskin, D.N., Anand, R., LaHoste, G.J., 2008. Chronic menthol attenuates the effect of nicotine on body temperature in adolescent rats. Nicotine and Tobacco Research 10 (12), 1753–1759.

Russin, W.A., Hoesly, J.D., Elson, C.E., Tanner, M.A., Gould, M.N., 1989. Inhibition of rat mammary carcinogenesis by monoterpenoids. Carcinogenesis 10, 2161–2164.

Rustenmeier, K., Sabbert, R., Haussmann, H.-J., Roemer, E., Carmines, E.L., 2002. Evaluation of the potential effects of ingredients added to cigarettes. Part 2: chemical composition of mainstream smoke. Food and Chemical Toxicology 40, 93–104.

Scanlon, P.D., Connett, J.E., Waller, L.A., Altose, M.D., Bailey, W.C., Buist, A.S., Tashkin, D.P., 2000. Smoking cessation and lung function in mild-to-moderate chronic obstructive pulmonary disease – the lung health study. American Journal of Respiratory and Critical Care Medicine 161, 381–390.

Schievelbein, H., 1969. Risks to health in smoking menthol cigarettes? [in German]. Munchener Medizinische Wochenschrift 111, 2457.

Schmeltz, I., Schlotzhauer, W.S., 1968. Benzo(a)pyrene, phenols, and other products from the pyrolysis of menthol. Nature 219, 370–371.

Schwartzstein, R.M., Lahive, K., Pope, A., Weinberger, S.E., Weiss, J.W., 1987. Cold facial stimulation reduces breathlessness induced in normal subjects. American Review of Respiratory Disease 136 (1), 58–61.

Sidney, S., Tekawa, I.S., Friedman, G.D., Sadler, M.C., Tashkin, D.P., 1995. Mentholated cigarette use and lung cancer. Archives of Internal Medicine 155, 727–732.

St.Charles, F.K., Krautter, G.R., Dixon, M., Mariner, D.C., 2006. A comparison of nicotine dose estimates in smokers between filter analysis, salivary cotinine, and urinary excretion of nicotine metabolites. Psychopharmacology 189, 345–354.

St.Charles, F.K., Krautter, G.R., Mariner, D.C., 2009. Post-puff respiration measures on smokers of different tar yield cigarettes. Inhalation Toxicology 19, 2009 (open access published February).

Stellman, D., Chen, Y., Muscat, J.E., Djordjevic, M.D., Richie Jr., J.P., Lazurus, P., et al., 2003. Lung cancer risk in white and black Americans. Annals of Epidemiology 13, 294–302.

Stoner, G.D., Shimkin, M.B., Kniazeff, A.J., Weisburger, J.H., Weisburger, E.K., Gori, G.B., 1973. Test for carcinogenicity of food additives and chemotherapeutic agents by the pulmonary tumor response in strain A mice. Cancer Research 33, 3069–3085.

Strasser, A.A., Malaiyandi, V., Hoffmann, E., Tyndale, R.F., Lerman, C., 2007. An association of CYP2A6 genotype and smoking topography. Nicotine and Tobacco Research 9 (4), 511–518.

Stratton, K., Shetty, P., Wallace, R., Bondurant, S., 2001. Clearing the Smoke. Assessing the Science Base for Tobacco Harm Reduction. National Academy Press, Washington, DC.

Technical Resources Inc., 1993. Menthol and other isomers. NCI Contract NO1-CP-05619, 31pp.

USDHHS, 1998. Tobacco Use Among US Racial/Ethnic Minority Groups – African-Americans, American Indians and Alaska Natives, Asian Americans and Pacific Islanders, and Hispanics: A Report of the Surgeon General. US Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health.

Vanscheeuwijck, P.M., Teredesai, A., Terpstra, P.M., Verbeeck, J., Kuhl, P., Gerstenberg, B., Gebel, S., Carmines, E.L., 2002. Evaluation of the potential effects of ingredients added to cigarettes. Part 4: subchronic inhalation toxicity. Food and Chemical Toxicology 40, 113–131.

Wackowski, O., Delnevo, C.D., 2007. Menthol cigarettes and indicators of tobacco dependence among adolescents. Addictive Behaviors 32, 1964–1969.

Wagenknecht, L., Cutter, G., Haley, N., Sidney, S., Manolio, T., Hughes, G., Jacobs, D., 1990. Racial differences in serum cotinine levels among smokers in the coronary artery risk development in (young) adults study. American Journal of Public Health 80, 1053–1056.

Wattenberg, L.W., 1991. Inhibition of azoxymethane-induced neoplasia of the large bowel by 3-hydroxy-3,7,11-trimethyl-1,6-10-dodecatriene (nerolidol). Carcinogenesis 12, 151–152.

Werley, M.S., Coggins, C.R.E., Lee, P.N., 2007. Possible effects on smokers of cigarette mentholation: a review of the evidence relating to key research questions. Regulatory Toxicology and Pharmacology 47, 189–203.

Wiener, D., Doerge, D.R., Fang, J.L., Upadhyaya, P., Lazarus, P., 2004. Characterization of the N-glucuronidation of the lung carcinogen 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanol (NNAL) in human liver: importance of UDP-glucuronosyltransferase 1A4. Drug Metabolism and Disposition 32 (1), 72–79.

Williams, J.M., Gandhi, K.K., Steinberg, M.L., Foulds, J., Ziedonis, D.M., Benowitz, N.L., 2007. Higher nicotine and carbon monoxide levels in menthol cigarette smokers with and without schizophrenia. Nicotine and Tobacco Research 9 (8), 873–881.

Wilson, S.A., 1993. Theoretical aspects of menthol migration and transfer. Recent Advances in Tobacco Science 19, 129–153.

Yamaguchi, T., Caldwell, J., Farmer, P.B., 1994. Metabolic fate of [3H] – l-menthol in the rat. Drug Metabolism and Disposition 22 (4), 616–624.

Zedler, B.K., Kinser, R., Oey, J., Nelson, B., Roethig, H.J., Walk, R.A., Kuhl, P., Rustemeier, K., Schepers, G., Von Holt, K., Tricker, A.R., 2006. Biomarkers of exposure and potential harm in adult smokers of 3–7 mg tar yield (Federal Trade Commission) cigarettes and in adult non-smokers. Biomarkers 11 (3), 201–220.