



RESEARCH & DEVELOPMENT

# Estimation of the Retention of Menthol in the Respiratory Tract of Smokers: A Pilot Study

**Melissa Hagan Hughes, Kyle F. Lott, J. Daniel Heck**

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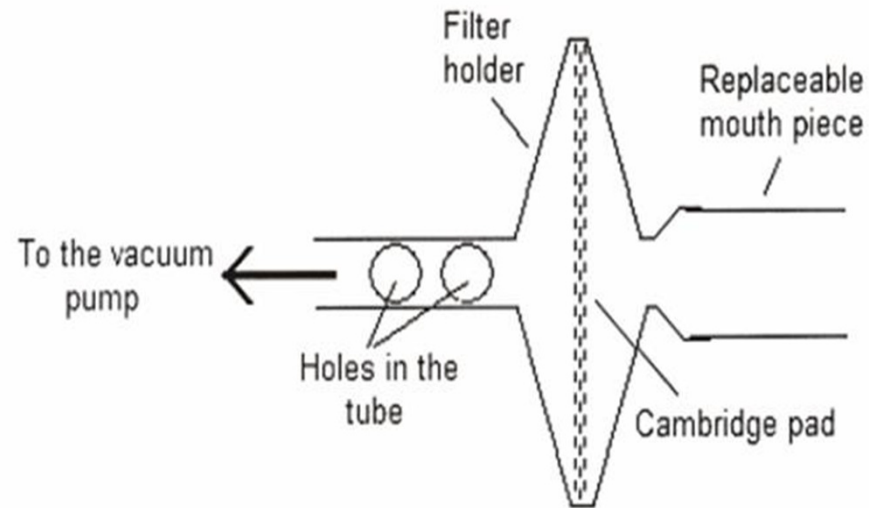
# Background

- To our knowledge, there are no known published retention values for menthol, nicotine and solanesol in a menthol cigarette as smoked by menthol smokers
  - Armitage, Dixon, Frost, Mariner, and Sinclair (2004)
    - n=10
    - 9.6 mg tar non-menthol cigarette
    - %Retained: solanesol ( $68.0 \pm 7.5$ ) nicotine ( $99.0 \pm 0.5$ )
  - Moldoveanu and St. Charles (2007)
    - n=8
    - 10.6 mg tar non-menthol cigarette
    - %Retained: nicotine ( $84.6 \pm NR$ )
  - Moldoveanu and Coleman (2008)
    - n=10
    - 16.2 mg tar non-menthol cigarette
    - %Retained: solanesol ( $71.3 \pm 7.2$ )

# Objective

- Evaluate the total retention of solanesol and nicotine by smokers of conventional mentholated cigarettes
- Compare retention values of solanesol and nicotine by smokers of mentholated cigarettes to retention values by smokers of non-mentholated cigarettes
- Evaluate the total retention of menthol by smokers of conventional mentholated cigarettes

# Overview



Moldoveanu and Coleman, 2008

Exhaled amount- collected from smoker

# Overview

- Collect smoke on a Cambridge pad using a smoking machine under different smoking conditions
- Analyze the level of analyte on the Cambridge pad
- Measure cigarette butt analyte from machine smoked cigarettes
- Establish machine curves (analyte on pad) vs. (analyte in cigarette butt)
- Measure the level of analyte from the butt of human smoked cigarettes
- Calculate the level of analyte delivered to the smoker from the butt analyte level using the previously established calibration charts



Delivered amount- determined from filter butt and compared to smoking machine curve results

# Overview

$$\text{Retained } (\mu\text{g/cig}) = \text{Delivered } (\mu\text{g/cig}) - \text{Exhaled } (\mu\text{g/cig})$$

$$\text{Retention } \% = \frac{\text{Delivered} - \text{Exhaled}}{\text{Delivered}} \times 100\%$$

# Outline

- Analysis of solanesol, nicotine, and menthol
- Smoking parameters and machine curve development
- Study protocol
- Results
- Discussion/conclusions

# Outline

- Analysis of solanesol, nicotine, and menthol



# High performance liquid chromatography

## Solanesol HPLC:

- Column  
(Waters Symmetry “3.5 $\mu$ m x 4.6mm x 75mm”)
- Wavelength (205nm)
- Injection volume (20 $\mu$ L)
- Mobile Phase (90% Acetonitrile:10% Methanol - Isocratic)
- Column Temp (40 $^{\circ}$ C)
- Flow Rate (1.5mL/min)
- Range = (0.00063-0.3258 mg/mL)



# Gas chromatography-FID methods



## Nicotine/menthol GC-FID:

- Column  
(Restek Rtx-5 “15m x 0.25mm x 0.25 $\mu$ m”)
- Retention Time  
(3.1 & 3.9 min for menthol and nicotine)
- Injection volume (1 $\mu$ L)
- Inlet & FID Temperature  
(250 & 300 $^{\circ}$ C)
- Initial Oven Temp (60 $^{\circ}$ C)
- Range (0.00052-3.232 mg/mL)

# Analysis methods

For both exhaled and machine methods:

## Solanesol

- Recovery values were  $\geq 93\%$
- Trapping efficiency was 100%
- $R^2 = 0.9996$

## Nicotine

- Recovery values were  $\geq 86\%$
- Trapping efficiency was 100%
- $R^2 = 0.9995$

## Menthol

- Recovery values were  $\geq 96\%$
- Trapping efficiency was 98%
- $R^2 = 0.9995$

# Outline

- Smoking parameters and machine curve development

# Development of calibration curves

## Cerulean SM450

- Measured smoke analytes delivered to Cambridge filter pads 11 different puffing parameters
  - 3 replicates and 3 cigarettes per replicate
- Measured analytes in cigarette butt
- Generated regression curves (analyte on pad) vs. (analyte in cigarette butt)



# Smoking machine methods

- Cigarette Butts

- Filter processed immediately after smoking
- 1cm portion cut from end
- 20mL of 0.1% anethole in methanol was added
- Wrist action shaker for 35min
- 1 $\mu$ L aliquot injected on GC-FID (nicotine and menthol)
- 20 $\mu$ L aliquot injected on HPLC (solanesol)

- Cambridge Pads

- Pad weighed
- 20mL of extraction solution was added to pads
- Wrist action shaker for 35min
- 1 $\mu$ L aliquot injected on GC-FID (nicotine and menthol)
- 20 $\mu$ L aliquot injected on HPLC (solanesol)

# Puffing parameters

Puff volume (mL)	Puff duration (s)	Puff interval (s)
35	2	60
35	1.5	75
35	1	120
35	1	180
35	1	240
45	2	30
45	1.5	40
50	2	30
55	2	80
60	2	30
60	2	60

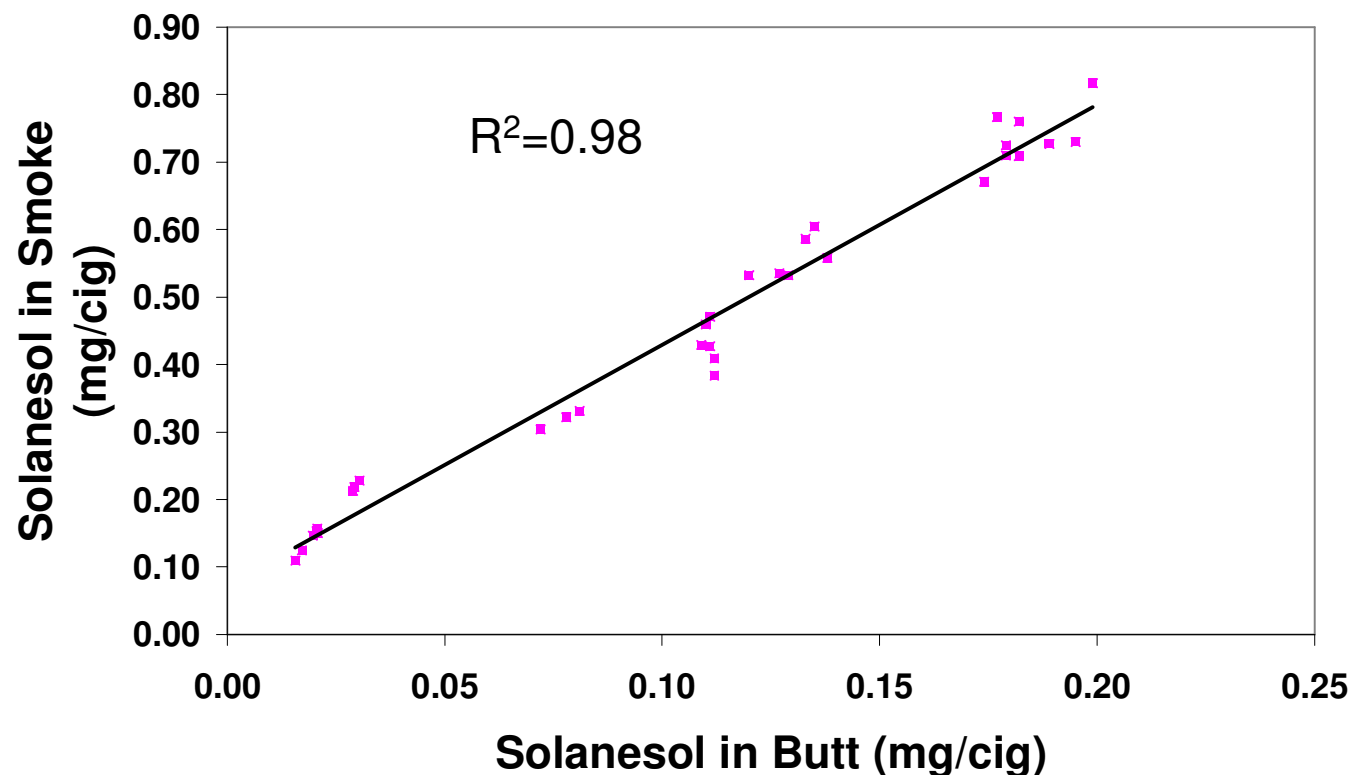
# Puffing parameters

Puff volume (mL)	Puff duration (s)	Puff interval (s)
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35	1	180
35	1	240
45	2	30
45	1.5	40
50	2	30
55	2	80
60	2	30
60	2	60



# Smoking machine curves: solanesol

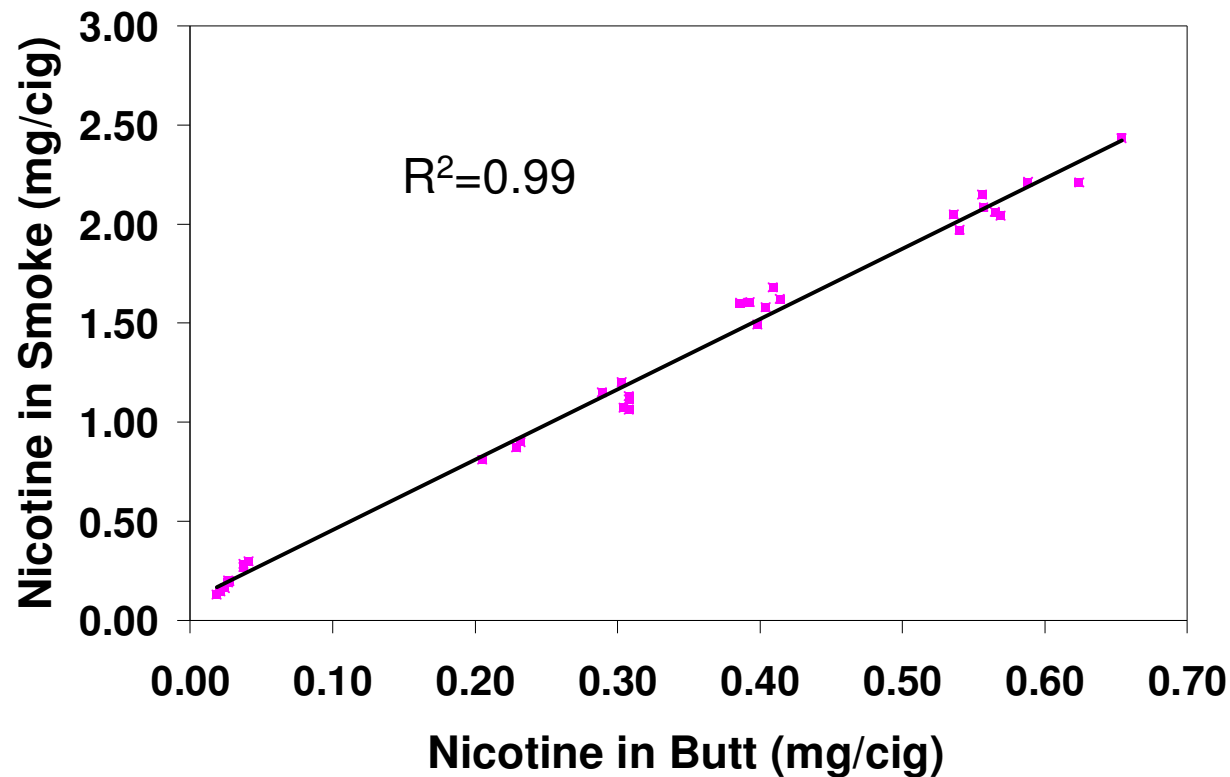
## Solanesol: Machine Regime Curve



Calibration line of solanesol level in smoke vs. solanesol in the cigarette butt

# Smoking machine curves: nicotine

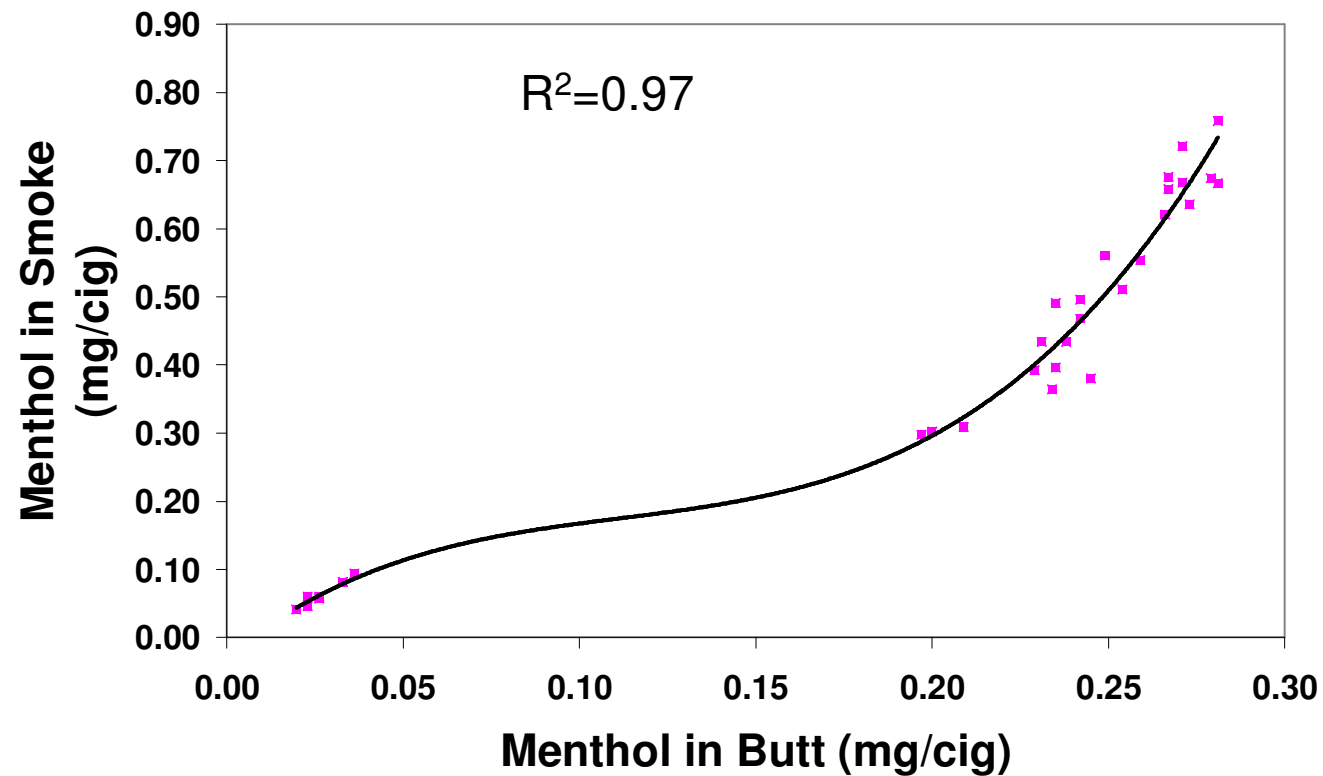
## Nicotine: Machine Regime Curve



Calibration line of nicotine level in smoke vs. nicotine in the cigarette butt

# Smoking machine curve: menthol

## Menthol: Machine Regime Curve



Calibration line of menthol level in smoke vs. menthol in the cigarette butt

# Outline

- Study protocol

# Study protocol

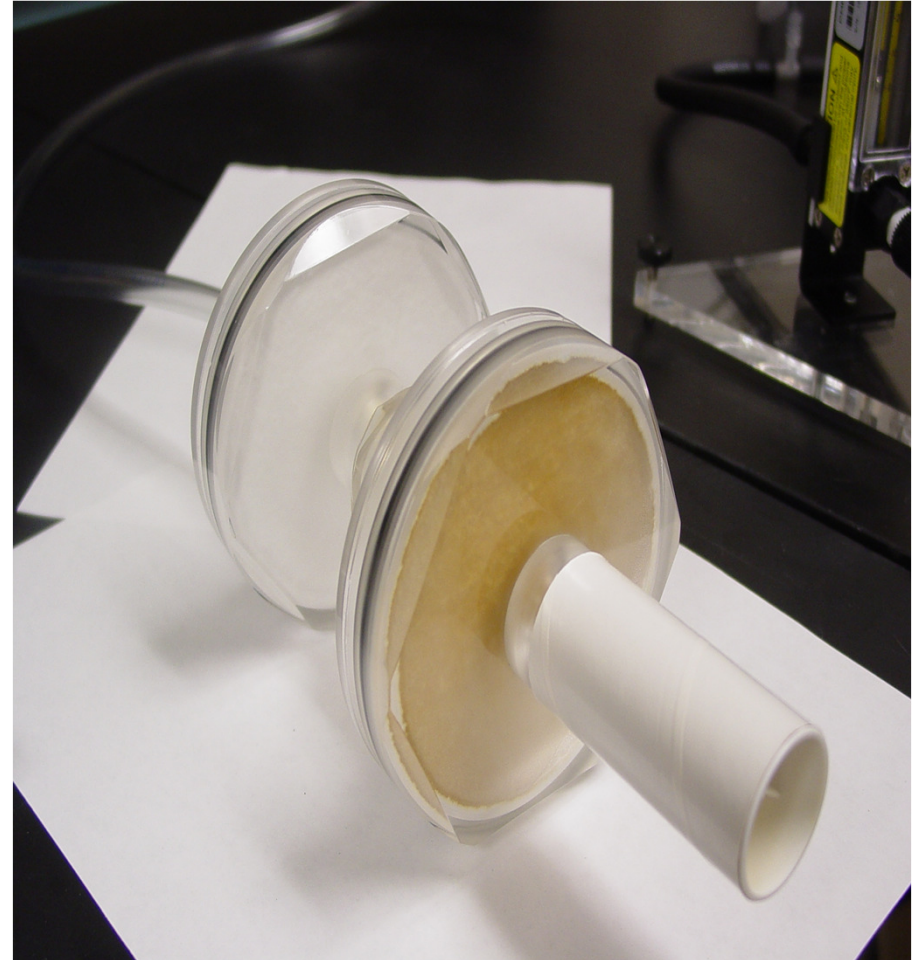
- Protocol was developed internally by Lorillard investigators and was approved by Quorum Review, Inc (Seattle, WA)
- Smokers were recruited from local research facility
- Menthol Smokers (n=15)
  - 20 cig/day
  - 12 black
  - 7 males
- Smoking was performed in a familiar environment for the smoker
- No physical parameters of the smoking process (i.e. puffing, inhalation or exhalation patterns) were measured

# Cigarettes

- Commercially available menthol cigarette
- Mainstream smoking data under ISO conditions:
  - 13.5 mg/cig “tar”
    - Nicotine-free dry particulate matter
  - 1.0 mg/cig Nicotine
  - 15.6 mg/cig CO

# Exhaled smoke determination

- Collected human exhaled smoke on two 92 mm Cambridge pads in series using a vacuum assisted apparatus (Moldoveanu and Coleman, 2008)
- Three cigarettes were smoked within one hour by each smoker
- Analyzed the analyte levels on the exhaled smoke Cambridge pad and used human-smoked cigarette butts
- Calculated exhaled amount



# Outline

- Results



# Results

Analyte	Exhaled ( $\mu\text{g}/\text{cig}$ ) Mean (Range)	Delivered ( $\mu\text{g}/\text{cig}$ ) Mean (Range)	Retained ( $\mu\text{g}/\text{cig}$ ) Mean (Range)	<b>Retention</b> (%) Mean (Range) RSD
Solanesol	107 (39-182)	302 (219-518)	195 (119-376)	<b>64.0</b> <b>(46-86)</b> <b>23.1</b>
Nicotine	13 (4-35)	488 (346-832)	474 (342-809)	<b>97.3</b> <b>(93-99)</b> <b>1.8</b>
Menthol	15 (9-22)	206 (168-271)	189 (147-256)	<b>92.7</b> <b>(87-96)</b> <b>2.5</b>

# Comparison to literature values

	<b>Lorillard (2010)</b>	Moldoveanu (2008)	Armitage (2004)	Moldoveanu (2007)	Moldoveanu (2008)
<b>Cigarette (ISO:TPM)</b>	<b>Menthol</b>	<b>Non- Menthol</b>	<b>Non- Menthol</b>	<b>Non- Menthol</b>	<b>Non- Menthol</b>
<b>Tar (ISO/FTC)</b>	13.5mg	5.0mg	9.6mg	10.6mg	16.2mg
<b># smokers</b>	13	10	10	8	10
<b>Solanesol</b>					
<b>retention (%)</b>	64.0 (±14.8)	59.0 (±5.9)	68.0 (±7.5)*	NR	71.3 (±7.2)
<b>Nicotine</b>					
<b>retention (%)</b>	97.3 (±1.8)	NR	99.0 (±0.5)*	84.6 (±NR)	NR
<b>Menthol</b>					
<b>retention (%)</b>	92.7 (±2.3)	NR	NR	NR	NR

NR= value not reported; \* 2s breath hold and 500ml inhalation of air

# Outline

- Discussion/conclusions

# Discussion and conclusions

- Solanesol and nicotine retention in the smokers of mentholated cigarettes were 64% and 97%, respectively
- Values for the retention of nicotine and solanesol in smokers of mentholated cigarettes were similar to published retention values obtained for smokers of non-mentholated cigarettes
  - Suggests that menthol does not influence retention efficiencies of solanesol and nicotine
- This is the first study to estimate menthol retention in smokers of mentholated cigarettes
  - Menthol retention 93%

# References

- Armitage AK, Dixon M, Frost BE, Mariner DC, Sinclair NM. (2004). The effect of inhalation volume and breath-hold duration on the retention of nicotine and solanesol in the human respiratory tract and on subsequent plasma and nicotine concentrations during cigarette smoking. *Beitr Tabakforsch Int* 21(4):240-249.
- Moldoveanu SC, Coleman WM (2008). A pilot study to assess solanesol levels in exhaled cigarette smoke. *Beitr Tabakforsch Int* 23(3):144-152.
- Moldoveanu SC, St Charles FK (2007). Differences in the chemical composition of the particulate phase of inhaled and exhaled cigarette mainstream smoke. *Beitr Tabakforsch Int* 22(4).