BREATH® BIOPSY

Investigating Induced Acute Lung Inflammation Using Breath Biopsy®

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Aims

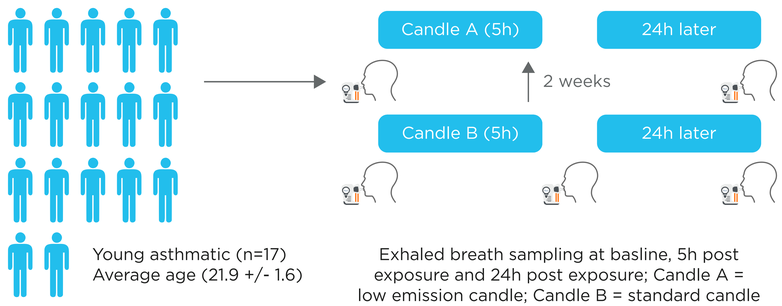
- Investigate whether exposure to burning candles can cause acute airway inflammation as reflected in the composition of exhaled breath.
- The hypothesis tested was that low-emission candles would induce less changes in the exhaled breath (and therefore less airway inflammation) compared to traditional candles.

1. Background and Objectives

Lung inflammation is a factor in several illnesses and is a response to pollution and other irritants. Inflammation is reported to release volatile organic compounds (VOCs) in breath that could be non-invasively detected and monitored to assess inflammation. One way of triggering lung inflammation is exposure to various hazardous emissions. Studies have investigated pollution of the air in residential homes and found that candle burning significantly elevates the indoor particulate concentration. Additionally, emissions from burning candles are of concern to our health as inhaled particles and gases can reach the deepest regions of the lungs. From here, they have the potential to diffuse into the blood stream, thereby accessing vital organs such as the heart and brain.
In this study burning candles were used as a possible source of hazardous emissions. This study investigated short-term exposure to particulates generated by candles in an at-risk population (asthmatics) by collecting and analyzing breath samples using ReCIVA® devices, in a randomized, cross-over, double-blind study. The project was conducted as part of the innovation project "Candle Development for Low Emissions". The aim was to investigate the VOCs associated with lung inflammation induced by acute exposure to emissions from new candles marketed as low-emission candles alongside standard candles.

2. Methods

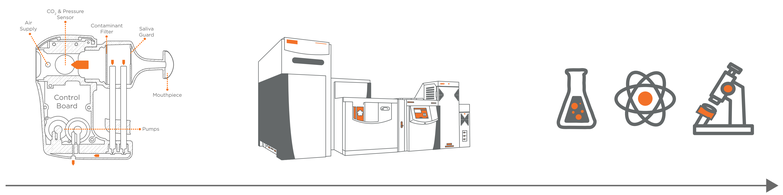
Experimental Design:



Young asthmatic (n=17)
Average age (21.9 +/- 1.6)

Exhaled breath sampling at basline, 5h post exposure and 24h post exposure; Candle A = low emission candle; Candle B = standard candle

Sample Processing



- Breath collected using Breath Biopsy Collection Station
- GC-MS processed on high-resolution accurate Q Exactive Orbitrap system
- VOC profiles at pre- (baseline) and post-exposure (5h and 24h) were compared
- Targeted analysis (Table 1), i.e. looking at absolute concentration of named VOCs in exhaled breath and untargeted analysis, i.e. any possible VOC detected in exhaled breath
- Principal Component Analysis (PCA) and statistical testing was used to find differences in VOC profiles.

n-Hexane	2-hexanone	Heptanal	Hexadecane	1-pentadecene
Butanal	Hexanal	Dodecane	o-xylene	2-ethyl-1-hexanol
Pentanal	Undecane	Octanal	1-propanol	Decanal
Decane	3-methyl thiophene	Nonanal	p-xylene	E-2-butenal
2-ethylbutanal	E-2-pentenal	2,4-heptadienal		

Table 1: The list of targeted VOCs

3. Results

The univariate analysis of targeted compounds (Table 1) did not show significant changes between baseline and post-exposure samples for both candles. Untargeted analysis indicated several significant changes in the content of exhaled air between baseline and post-5h exposure.

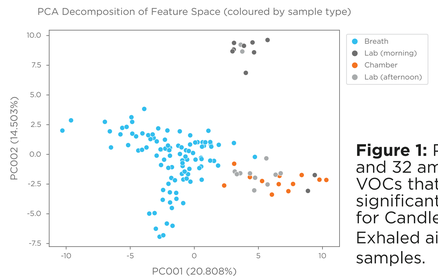


Figure 1: PCA score plot of 102 exhaled breath and 32 ambient blank samples, consisting of 21 VOCs that were considered statistically significant between baseline and post-exposure for Candle A and Candle B (i.e. both challenges). Exhaled air profile is different from ambient samples.

The univariate analysis by Wilcoxon signed rank test between baseline measurements (T0) and post-exposure measurements (T1) for low-emission and standard candle with corrected p-value <0.1 and log fold change (FC) ≥ 2, resulted in a set of 10 VOCs (Table 2).

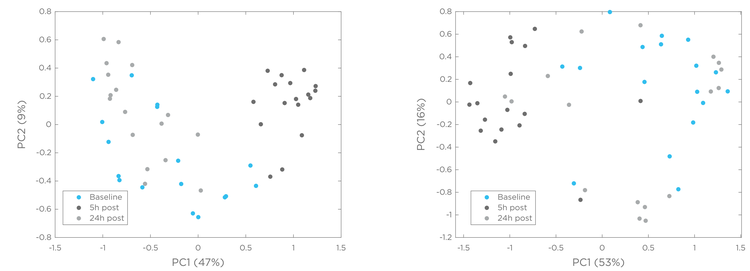


Figure 2: The PCA score plot of 51 exhaled breath samples collected during exposure to A) low emission candle (Candle A); B) standard candle (Candle B). In each case, a set of 21 VOCs was used. As can be seen the standard candle (Candle B) shows larger differences in exhaled breath profile between baseline and post-5h exposure. Interestingly, the VOCs profile post-24h candle exposure returned to baseline.

Compound name	Corrected p-values	Direction of change
2-methylfuran	0.022	+
3-methylfuran	0.022	+
1-(methylthio)-1-propene	0.05	-
2-methyl-2-butenal	0.02	+
3-vinylfuran	0.07	+
Cyclopentanone	0.06	-
Limonene	0.01	-
2-pentylfuran	0.01	+
Menthone	0.0016	-
Unknown	0.001	-

Table 2: The chemical identification of the significant VOCs (corrected with Benjamin-Hochberg p-value = <0.1) and their relative change in comparison to baseline samples. (+) indicates upregulation and (-) indicates downregulation.

4. Conclusions

- Significant differences in VOCs profile between baseline and post-5h candle exposure was found (p-value = <0.1).
- The present study shows that exposure to standard candles has a larger effect than the low-emission candles. Therefore, chronic airway effects during prolonged candle exposure cannot be precluded with our current knowledge.
- The exhaled breath profile after 24hr-post exposure came back to baseline, indicating that upregulation or downregulation is temporary.
- The statistically significant differences in compound abundance may be related to: (i) inflammation as seen with elevated aldehyde (ii) combustion and compounds created during burning processes and their accumulation in the lung (iii) assimilation of the products in the respiratory track.