Assessing Inflammatory Response to Air Pollution via VOCs in Exhaled Breath

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1. Introduction and Aims

In 2013, the International Agency for Research on Cancer determined that outdoor air pollution and one of its major components, particulate matter, are carcinogens and important causes of lung cancer. A recent report from the Global Burden of Disease estimated that 387,000 lung cancer deaths were attributable to outdoor air pollution in 2013 (24% of all lung cancer deaths).

Air pollution also increases the risk of death from ischaemic heart disease, cerebrovascular disease, chronic obstructive pulmonary disease, and lower respiratory infections. Specifically, the particle mass with an aerodynamic diameter of less than 2.5μm (PM2.5) portion of air pollution was the fifth-ranking mortality risk factor in 2015. Despite the importance of air pollution exposure as a risk factor for major causes of death at the population level, the effect of particulate matter exposure at the individual level has not been fully determined. Controlled exposure studies are one way to better understand the effects of particulate exposure and provide a controlled method of exploring acute inflammation in general.

Lung inflammation is a response to pollutants, releasing volatile organic compounds (VOCs) in breath, which could serve as non-invasive markers for assessing inflammation.The goal of the study is to find breath markers associated with acute lung inflammation induced via an exposure to 2.5 μm particulate matter (PM 2.5) in healthy individuals.

2. Methods

This study recruited 20 healthy individuals (10 males and 10 females, aged 18-45). Subjects were non-smokers. The study was a double-blinded cross-over design. Participants underwent two separate exposures in the UBC Air Pollution Exposure Lab booth: 300 μg/m^3 of PM2.5 from a diesel engine, and to filtered air (sham), spaced a minimum of six weeks apart. The participants were randomized and blinded as to which exposure happens first. The representative flowchart of the study design is shown in Figure 1.

Experimental Design

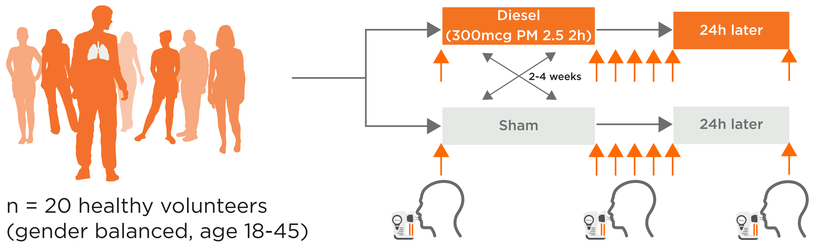

Figure 1. Study design diagram. The orange arrows represent the sampling points for exhaled breath (i.e. before the exposure, immediately after the exposure, and 30 minutes, 1 hour, 3 hours, 6 hours and 24 hours after exposure). In the case of respiratory droplets, the sampling will take place at 3 time points (before exposure, 3 hours after exposure, and 24 hours after exposure).

For each exposure, breath samples were collected using Breath Biopsy® at seven time points: baseline before exposure, immediately after exposure, 30 minutes post-exposure, 1 hour post-exposure, 3 hours post-exposure, 6 hours post-exposure, and 24 hours post-exposure. Respiratory droplet samples were collected at three time points: baseline before exposure, 3 hours post-exposure, and 24 hours post-exposure. Blood draws were collected at four time points: baseline before exposure, 1 hour post-exposure, 3 hours post-exposure, and 24 hours post-exposure. Samples were analysed by GC-MS, both untargeted and targeted analyses was conducted.

n-Hexane	2-Hexanone	Dodecane
2-Methylfuran	Hexanal	D-Limonene
Butanal	Undecane	2-Pentylfuran
3-Methylfuran	(E)-2-methyl-2-butenal	Octanal
Pentanal	2-vinylfuran	Nonanal
(E/Z)-1-methylthio-1-propene	3-Methyl thiophene	2,4-Heptadienal
Decane	E-2-Pentenal	2-Ethyl-hexanol
2-Ethylbutanal	p-Xylene	Pentadecane
(Z/E)-1-methylthio-1-propene	o-Xylene	Decanal
1-Propanol	Heptanal	Hexadecane
E-2-Butenal	Cyclopentanone	

Table 1: The list of 33 targeted VOCs

3. Results

Figure 2: PCA score plot of exhaled breath and blank samples: (A) consisting of 33 targeted VOCs (Table 1) and (B) 900 VOCs from untargeted analysis. Clear separation between breath samples and ambient blanks was demonstrated for both targeted and untargeted analysis

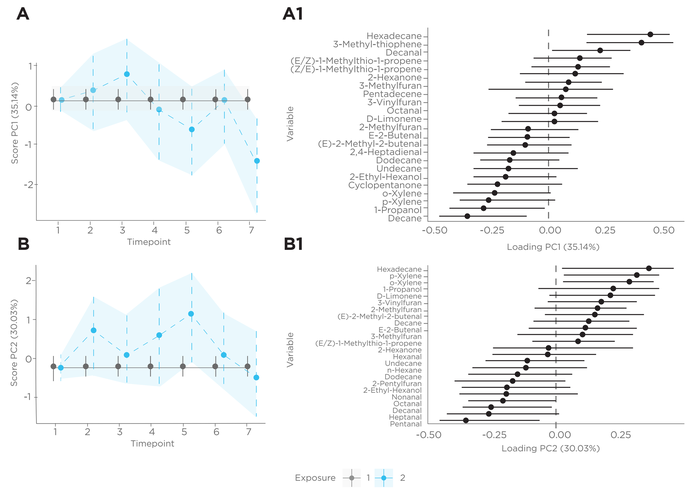

Figure 3: ANOVA Simultaneous Component Analysis of Targeted VOC Data in Exhaled Breath Following Exposure 1: Sham and Exposure 2: Diesel. (A) Score plot of the first principal component (PC1), explaining 35.16% of the variance, across seven time points. This plot highlights the temporal response of VOCs to sham (Exposure 1) and diesel, PM2.5 (Exposure 2) exposures. Key VOCs such as decane, hexadecane , 1-propanol, p-xylene, 3methylthiophene, and hexane have significant loadings on PC1, indicating their strong association with exposure effects. The importance of the VOCs contributing to the exposure effect is shown in A1. (B) Score plot of the second principal component (PC2), explaining 30.03% of the variance, over the same time course. This plot shows further differentiation between the exposures, with VOCs like pentanal, heptanal, and decanal contributing significantly to the observed variance. The importance of the VOCs contributing to the exposure effect is shown in B1.

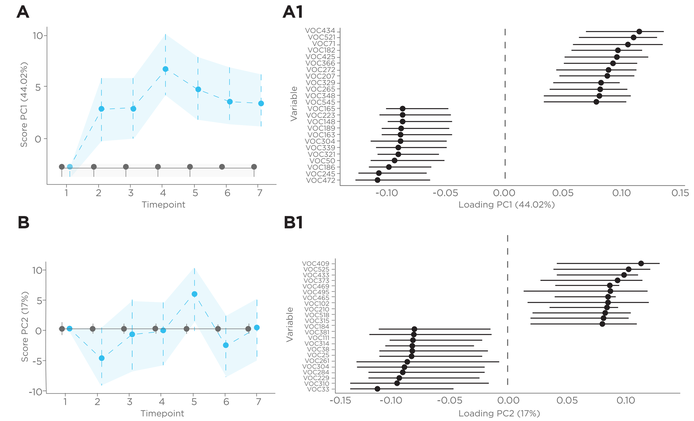

Figure 4: ANOVA Simultaneous Component Analysis of Untargeted VOC Data in Exhaled Breath Following Exposure 1: Sham and Exposure 2: Diesel. (A) Score plot of the first principal component (PC1), which explains 44.02% of the variance, across seven time points. This figure captures the primary response of VOC profiles to the sham (Exposure 1) and diesel (Exposure 2) with VOCs such as VOC472, VOC235, and VOC186 contributing heavily to this variance. The importance of the VOCs contributing to the exposure effect is shown in A1. (B) Score plot of the second principal component (PC2), explaining 17% of the variance, depicting additional differences between the exposures over time. VOCs such as VOC33, VOC310, and VOC229 are highlighted for their significant loadings on PC2, indicating their potential role in distinguishing the exposure effects. The importance of the VOCs contributing to the exposure effect is shown in B1.

4. Conclusions

- Data analysis revealed a significant effect of exposure type and a significant interaction between post-exposure time and exposure type.
- Targeted analysis found that inflammation-related compounds significantly increased in individuals exposed to PM2.5 compared to controls. Targeted analysis also found that 12 VOCs significantly increased in breath of the PM2.5 group, which diminished in samples collected 24 hours post-exposure.
- This study demonstrates that a distinct breath VOC profile is detectable in individuals exposed to PM2.5, suggesting that breath VOCs may detect acute airway inflammation and assess respiratory diseases secondary to air pollution.