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2026

Document Version:
Early version, also known as pre-print

ROS from indoor sources – comparison of online and offline measurements

Jonas Enarsson[1,*], Vilhelm Malmborg[1], Branka Miljevic[2], Axel Eriksson[1,3], Hao Wang[4], Zoran Ristovski[2], Aneta Wierzbicka[1]

[1] Ergonomics and Aerosol Technology, Lund University, Lund, Sweden
[2] ILAQH, Queensland University of Technology, Brisbane, Australia
[3] Nanolund, Lund University, Lund Sweden
[4] Institute for Environmental and Climate Research, Jinan University, China
[*] *Corresponding email: jonas.enarsson@design.lth.se*

SUMMARY

Reactive oxygen species (ROS) are considered to be a major contributor to particle-induced toxicity. There are several methods of measuring ROS, including both online and offline methods, as well as different acellular assays. In this study, we measured ROS from common indoor sources and compared the results from an online method (PINQ) and an offline method (DCFH assay). The results show differences between the two methods, where SOA gave the highest response for online measurements, and candle smoke gave the highest response from offline methods. The difference can be explained by the degradation of short-lived ROS before detection in the offline analysis, and different sensitivity to particles containing black carbon between the assays. For health-relevant particle assessment, we suggest using multiple assays and both online and offline methods.

KEYWORDS

Aerosols, particulate matter, oxidative potential, reactive oxygen species, assay

1 INTRODUCTION

Reactive oxygen species (ROS) are a group of chemicals considered to be a major contributor to particle-induced toxicity. ROS can be either present on the surface of particles or generated through reactions between particles and cells after inhalation. Therefore, measuring particle-bound ROS may be used to assess the harmful effects of inhaling particles. For ROS detection, cellular and acellular assays are used. Acellular assays are commonly used to assess particle-derived ROS, since they are technically simpler than cellular assays. There are several different acellular assays available, and they can be used in both offline (more common) and online methods (Bates et al., 2019). Short half-life of ROS has been recently highlighted (Campbell et al., 2025), which emphasizes the need for rapid detection that is only possible in online methods. Acellular assays have been extensively used for over a decade, but ROS from indoor sources remains largely unknown. This study aimed to determine and compare ROS from indoor sources generated in laboratory settings, using online and offline methods based on acellular assays.

2 METHODS

In laboratory settings, we generated emissions from five indoor sources: frying of hamburgers, candle smoke, incense smoke, and two types of secondary organic aerosol (SOA), formed from reactions between α-pinene and ozone, and squalene and ozone). The Particle Into Nitroxide Quencher (PINQ) (Brown et al., 2019) was used for rapid online detection of ROS. It is based on the 9,10-bis (phenylethynyl) anthracene-nitroxide (BPEAnit) ROS assay. Off-line methods entail collection of particles on filters, storage, extraction from filters, drying and analysis. In the offline methods, we used the 2,7-dichlorofluorescein diacetate DCFH-DA assay. During the

generation of the emissions, the physicochemical properties of the particles were characterized by several instruments, including Scanning mobility particle sizer (SMPS) and aethalometer.

3 RESULTS

Based on the online method, the highest ROS per particle mass was measured for SOA from α-pinene, while the lowest ROS was measured from frying. Insoluble carbonaceous particles, such as soot, have been shown to decrease the response in the online method, leading to an underestimation of the result for candle smoke and incense smoke. For the offline method, candle smoke and incense smoke had the highest ROS generation potential, while frying had the lowest. SOA from squalene had significantly higher ROS generation potential than SOA from α-pinene in offline analysis.

4 DISCUSSION

The differences between the online and offline methods that we observed could have multiple explanations. The online method detects short-lived ROS, while the offline method detects oxidative reactions that take time to occur, hence the different results regarding SOA could be that α-pinene creates more short-lived ROS, while squalene creates ROS that are slower to react. Furthermore, the two probes (BPEAnit and DCFH-DA) detect different ROS with varying sensitivity, and the assays rely on different measurement techniques, which may contribute to the observed differences. This is likely the reason that candle smoke and incense smoke give the highest response in the offline method, while they are underestimated in the online method. The underestimation in the online method is further increased due to presence of black carbon.

5 CONCLUSIONS

Our results show that ROS levels differ depending on the method used. Considering the processes that occur in the respiratory tract, both online and offline measurements, for capturing oxidative reactions at different time scales, are relevant for health effect assessment. Based on these findings, using multiple assays and combining online and offline approaches is recommended when evaluating particle-induced ROS. Ways of correcting for the underestimation of the ROS signal in the online method at high black carbon concentration are being explored.

ACKNOWLEDGEMENT

Extraction and offline analysis performed by Fredric Ahrling
This work was funded by: A Swedish Research Council for Sustainable Development, Formas (Dnr 2020-01370), The Swedish Research Council (Dnr 2021-05049) and Nanolund (p09-2021).

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