I. Exposure Concentration and Duration Dependent Cell Viability

- Viability was measured to determine the optimal PM exposure time and concentration for measurement of ROS production. Exposure conditions that decreased cell viability below 80% were considered unsuitable for further experimentation (Figure 2: black points).
- No significant decreases in viability were found for all carbon black exposure times and concentrations (data not shown). However, significant decreases in viability were seen at 12 h, 120 µg mL⁻¹ TiO₂, and at 16 h for 80, 120, and 160 µg mL⁻¹ TiO₂ (p < 0.05). Therefore, exposure parameters will be limited to 40 µg mL⁻¹ and 8 h.

II. PM induced ROS Production Over Extended Exposures

- Intra-cellular ROS production, as indicated by levels of DCFH oxidation, was not significantly enhanced by exposure time or carbon black concentrations of TiO₂ and carbon black (5, 20, 80, and 160 µg mL⁻¹), or over multi-hour incubation periods (4, 12, and 20 h).

III. Real-time PM induced ROS Production

- TiO₂ induced a rapid increase in ROS production (i.e. DCF fluorescence) in the first 50 min of the exposure.
- Carbon black, at the same exposure concentration, caused no significant ROS production.
- Photobleaching and background corrections resulted in a decrease in fluorescence signal over the exposure.

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- Exhaust samples are collected using particle impingers positioned at three sampling ports along the exhaust (Figure 4). These units allow direct collection of particles from the exhaust stream into aqueous media, and have been evaluated by examining particle retention over the size range of interest (Figures 5 & 6). Cell culture media (D-PBS and SFM) achieved the maximum collection efficiency, over deionized water, between 10 and 200 nm.
- Cultured cells will be exposed to an aliquot of these particle impinged samples for toxicity testing. Use of this sampling setup eliminates the need for filters, which require laborious extraction procedures and are susceptible to losses of semi-volatile compounds.
- Parallel sample collection will enable toxicity of exhaust pollutants to be contrasted across fuel types, engine operating modes, emission control systems.

Summary of Conclusions

- Elevated TiO₂ exposures concentrations above 80 µg mL⁻¹ and over 12 h decreased Calu-3 cell viability. DEP exposure times and concentrations will be limited to a max of 40 µg mL⁻¹ and up to 8 h.
- TiO₂ induced peak Calu-3 ROS generation in the first hour of exposure. No response was observed with carbon black. Real-time DEP induced intra-cellular ROS production will be assessed over the first 1.5 s of exposure.
- Maximum DEP collection efficiencies into D-PBS and SFM will allow us to investigate these responses further with actual DEP. In these future studies, TiO₂ and carbon black will be used as positive and negative PM controls, respectively.

Acknowledgements

The authors would like to acknowledge the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Canadian Foundation for Innovation (CFI) for funding the EMITTED Study.

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