ADDITIONAL FILE 2

Ambient $PM_{2.5}$ and Risk of Emergency Room Visits for Myocardial Infarction: Impact of Regional $PM_{2.5}$ Oxidative Potential: A Case-Crossover Study

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Supplemental Methods

Laboratory Analysis of Regional Oxidative Potential

Integrated $PM_{2.5}$ samples were equilibrated for 24 hours in a weighing room with temperature (18-22 °C) and humidity (45-50%) controls. Filters were weighed pre- and post-extraction using a Sartorius model MC5 microbalance and PM was extracted from filter samples into HPLC grade methanol through sonication with a titanium probe sonicator operated at an amplitude of 5 microns for 30 seconds. Filters were then rinsed with additional methanol and sample extracts were dried with nitrogen gas in a water bath heated to 37 °C. Samples were re-suspended in a 5% methanol/ultrapure water solution to 75 μg/mL. The extraction efficiency for each filter sample was calculated by comparing station- reported filter masses with the net loss of filter mass from the filters, pre- to post-extraction. The median extraction efficiency was 97%.

Re-suspended $PM_{2.5}$ samples were incubated with a synthetic human respiratory tract lining fluid for 4 hours at 37 °C (1-2). This fluid was a 200 μM composite solution of physiologically-relevant antioxidants including ascorbate (AA), urate, and glutathione (GSH). Glutathione and ascorbate depletion were used to measure regional $PM_{2.5}$ oxidative potential expressed on a per mass basis (% depletion/μg). This operationally defined metric described the portion of the initial 200 μM reacted over four hours normalized by the 75 μg/mL $PM_{2.5}$ used in the assay. Urate was not included as PM does not have an important impact on urate depletion (2-3). The plate was incubated at 37 °C in a UV-vis plate reader (Molecular Devices, SpectraMax 190). Ascorbate-related oxidative potential was measured using absorbance spectra and percent ascorbate depletion was measured as the percent change in absorbance at a wavelength of 260 nm over the 4-hour incubation period.

After the 4-hour incubation period, the remaining reduced glutathione concentration was determined using the oxidized glutathione -reductase-5,5′-dithio-bis(2-nitrobenzoic acid)

recycling assay (4). Glutathione-related oxidative potential was expressed as the percent change between each sample and a 4-hour incubated water blank. $PM_{2.5}$ samples and controls were analyzed in triplicate on a 96-well plate at a final concentration of 75 μg PM/mL. Known positive (non-ferrous dust, NRC PD-1) and negative (carbon black, Arosperse 15B) controls were run in parallel with $PM_{2.5}$ samples to evaluate inter-experimental standardization .

Supplemental References

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