Supplemental Material

Characterization of Ambient PM_{10} Bioaerosols in a California Agricultural Town

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S1. Endotoxin and (1→3)-β-D-glucan Analyses

Endotoxin and (1→3)-β-D-glucan analyses used kinetic chromogenic limulus amebocyte lysate (LAL) assays with different activating factors: factor C for endotoxin (in Chromo-LAL assay) and factor G (in Glucatell assay) for (1→3)-β-D-glucan. The assays consist of a colorless substrate and a proenzyme extracted from amoebocyte cells in the blood of the horseshoe crab, *Limulus polyphemus*. The proenzyme is converted to an active enzyme with the presence of endotoxin or (1→3)-β-D-glucan. The enzyme then catalyzes the dissociation of the colorless substrate into a short peptide segment and a yellow organic compound (e.g., p-nitroaniline) that can be photometrically quantified. The speed of color development, measured by the time needed to attain a pre-specified optical density (i.e., onset time), is proportional to the concentration of endotoxin or (1→3)-β-D-glucan.

A Chromo-LAL endotoxin assay (Associates of Cape Cod Inc., East Falmouth, MA, USA) was run in duplicate on an incubating microplate reader (ELx808IU, BioTek Instrument Inc., Winooski, VT, USA) at 37 °C and absorbance wavelength (λ) of 405 nm, with an onset optical density (OD, i.e., absorbance) of 0.1 selected. A control endotoxin standard (*Escherichia coli* O113:H10; potency: 1 ng = 24 EU; Associates of Cape Cod Inc., East Falmouth, MA, USA) was diluted in series to 50, 5, 0.5, 0.05, and 0.005 EU mL^{-1}, three times each to establish a standard calibration curve. To eliminate the interference of (1→3)-β-D-glucan, the Chromo-LAL reagent was reconstituted with the Glucashield glucan-blocking buffer solution (Associates of Cape Cod Inc., East Falmouth, MA, USA). The possible inhibition or enhancement was tested by spiking a test sample with 10 μL of 5 EU mL^{-1} endotoxin standard, and a further dilution was conducted when the percent recovery of the spiked sample was > 200% or <50%. A sample was re-analyzed when the coefficient of variation (CV) between duplicates exceeded ±10%.

Glucatell (1→3)-β-D-glucan assay (Associates of Cape Cod Inc., East Falmouth, MA, USA) was run in duplicate on the incubating microplate reader at 37 °C and λ=405 nm, with an onset OD of 0.03 selected. A standard calibration curve was developed by diluting the stock standard (included in the Glucatell assay) to 100, 50, 25, 12.5, 6.25 and 3.125 pg mL^{-1} three times each. A similar quality assurance procedure, as that adopted for endotoxin analysis, was applied.

Both assays require the analyte concentration in filter extracts within the concentration range of calibration standards. Accordingly, the assays' minimum detection limits (MDLs) were calculated based on the lowest-concentration standard to be 0.046 EU m^{-3} and 0.029 ng m^{-3} for endotoxin and (1→3)-β-D-glucan, respectively. The precision of the assays was calculated by running replicates (>3 per microplate), following the method of Watson *et al.* (2001). The accuracy of the assays was primarily limited by the uncertainty in prepared calibration standards. Due to lack of a quality control standard, this uncertainty could not be reliably assessed. Thus, no estimation of the assays' accuracy was conducted.

Glassware and metal tools (e.g., tweezers and filter punchers) were baked at 250 °C for >4 hr prior to the experiment, and they were sterilized repeatedly on a micro-incinerator during the experiment to prevent cross-contamination among samples. Pipette tips, microplates and centrifuge tubes were certified by the suppliers to be pyrogen-free and were tested in the laboratory by running negative control samples. Laboratory and field blank samples were also tested, and they, as well as the negative control samples, contained endotoxin and (1→3)-β-D-glucan levels below the MDLs. Thus, no blank subtraction was conducted and all values reported were as-measured.

S2. Arabitol and Mannitol Analyses

Arabitol and mannitol in filter extracts were quantified using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). The system (Dionex ICS-3000, Sunnyvale, CA, USA) was equipped with a Dionex CarboPac MA1 column/guard column and a sample loop of 30 μL. The separation was run with an aqueous sodium hydroxide (NaOH) eluent (354 mM, in ultrapure water) at a flow rate of 0.4 mL min^{-1}. To prepare the calibration curve, arabitol (99% purity, Fluka) and mannitol (>98% purity, Acros Organics) were dissolved in ultrapure water and diluted in series to 5.0, 2.5, 1.0, 0.5, 0.2, 0.1, 0.05 μg mL^{-1}. A mixture solution of carbohydrates (including arabitol and mannitol, all diluted at 1.0 μg mL^{-1}; Absolute Standards, Inc., Hamden, CT, USA) was used as the quality control standard.

For both compounds, the MDL was calculated as three times the standard deviation of the lowest-concentration standard (0.05 μg mL^{-1}). The test of field blank samples showed that their arabitol and mannitol concentrations were below the MDLs (19 ng m^{-3} for arabitol and 18 ng m^{-3} for mannitol). Thus, all values reported were as-measured and were not adjusted for field blanks. The precision of the analyses was calculated by running replicates (>3 per batch), following the method of Watson *et al.* (2001). It was estimated to be <±3.2% for arabitol and <±2.6% for mannitol. The accuracy of the analyses (<±1.5%) was assessed by differences between measured and actual concentrations of the quality control standard.

Similar to endotoxin and (1→3)-β-D-glucan analyses, glassware and metal tools were baked at 250 °C for >4 hr prior to the experiment. Pipette tips and vials were tested by running negative control samples and no arabitol or mannitol was detected.

References

Watson, J.G., Turpin, B.J., and Chow, J.C. (2001). The Measurement Process: Precision, Accuracy, and Validity. In *Air Sampling Instruments for Evaluation of Atmospheric Contaminants, Ninth Edition*, Cohen, B.S. and McCammon, C.S.J. (Eds.), American Conference of Governmental Industrial Hygienists, Cincinnati, OH, p. 201-216.