calculated and algorithmic measurements tending to exclude extremes of larger or small nm sized particles at given agglomerate density, and 3) lack of detail regarding the metal constituents of emitted particles. Novel WUI emission mixtures require additional approaches for complete particle characterization. Therefore, it is crucial to analyze particle morphology in two-(2D) and three-(3D) dimensions for sufficient interpretations within given exposure conditions. To better study these WUI emissions, this work assessed the morphological features of particles emitted during combustion of materials representing WUI fires using transmission (TEM for 2D) and scanning (SEM for 3D) electron microscopy. SEM coupled with energy dispersive X-Ray spectroscopy (EDS) was also used to provide metal constituent analysis of the particles. **Methods:** We have previously established a novel combustion generator based on a modified pellet stove to assess multiple parameters of open-air combustion, characterize the resultant emissions, and perform inhalation exposures. Four wood types from distinct regions of the United States were studied: 1) Northeast: Red Oak [RO]; 2) Northwest: Douglas Fir [DF]; 3) Southwest: Ponderosa Pine [PP]; 4) Southeast: Loblolly Pine [LP]. Three types of widely used synthetic building materials were also examined: 1) Foam Board Insulation [FB]; 2) Vinyl Flooring [VF]; 3) Oriented Strand Board Plywood [OSB]. Custom-made pellets were created from different proportions of the wood varieties and the synthetic building materials. Five pellet types were created: 100% wood, 97% wood + 3% FB, 83% wood + 17% VF, 75% wood + 25% OSB, and 55% wood + 3% FB + 17% VF + 25% OSB [Mix]. Cumulatively, 20 different compositions of pellets (5 types within each wood type * 4 types of regional wood varieties) were combusted and their emissions were characterized. Wood alone simulated regional wildfires and the wood mixed with synthetic material(s) mimicked WUI fire emissions. Pellets were combusted at a fixed rate (~ 6 g/minute) during a trial to uniformly compare the measurement assays across the different types of pellets. Particles were characterized using multiple sizer instruments: 1) Scanning Mobility Particle Sizer - Aerodynamic Particle Sizer (SMPS-APS) combination (TSI Inc., Shoreview, MN), 2) Electric Low-Pressure Impactor (ELPI+: Dekati Ltd., Finland), and 3) Micro Orifice Uniform Deposit Impactor (MOUDI: TSI Inc., Shoreview, MN). The count (CMD using ELPI+ and SMPS-APS) or mass median diameter (MMAD using MOUDI) and geometric standard deviation (GSD) was derived from the PSD. Results are provided as the range of CMD-MMAD for wood alone and wood mixed with synthetic materials. Electron microscopy samples were collected by drawing air through filters using a pre-calibrated vacuum pump at 1 liter per minute for 45 seconds according to the modified NIOSH 7300 method. Samples were collected on 200 mesh copper grid (Electron Microscopy Science, Hatfield, PA) and analyzed for TEM (JEM 1400; JEOL Ltd., Peabody, MA), and on 25 mm diameter, 0.2 µm pore size track-etched polycarbonate filters (Sterlitech Corporation, VA) for SEM (S-4800, Hitachi, Tokyo, Japan). **Results:** Particle sizer assessments revealed polydisperse PSDs (i.e., GSD>1.5) within the ranges of PM 2.5 (<2.5 µm), PM 1 (<1 µm) and ultrafine particles (UFPs: <0.1 µm). Smaller sized particles resulted from combusting wood alone (45-276 nm) or with OSB (45-247 nm). The addition of 3% FB slightly increased the particle MMAD of the particles (42-303 nm). Pellet type with 83% wood + 17% VF and the Mix of all 3 synthetics considerably increased the median diameter 53-440 nm and 61-388 nm, respectively. TEM and SEM assessments revealed a variety of non-spherical shapes including chains, clumps, and spikes between 0.4-1 µm. Unique morphological features of particles emitted from wood alone and OSB (consisting mainly of wood and glue products) indicate particle conjugation, agglomeration, and aggregations versus the other synthetic materials studied (VF, FB, and MIX). Greater particle agglomerate density, elongated chains and clumps tend to deposit in the upper respiratory tract, while smaller particles penetrate deeper into respiratory regions. Further, clearance of larger agglomerates compared to smaller particles locally and systemically may contribute to biological impacts. These morphological features may explain wider PSDs evaluated by particle sizers since optical sensors could not differentiate particle morphology and metal constituents that are apparent in high-resolution TEM and SEM images. EDS did not detect any evidence of heavy metal components. Based on this evidence, TEM and SEM can provide high-resolution morphological features which are essential for fully understanding particle characterizations and structural configurations. **Conclusions:** Novel mixtures emitted in WUI fires requires in-depth assessments as PSD measurements alone may not be sufficient to provide complete particle characterizations. Along with size, electron microscopy also provides particle morphology and metal constituents. Further, it is less influenced by the sampling and algorithmic nuances of particle sizer instruments. To understand the dynamics of pulmonary depositions for local or systemic outcomes from WUI fire emissions, interpretation of PSDs is necessary. To achieve this, optical sensor-based PSDs should be complemented by electron microscopy. Inhalation of diverse non-spherical particles may result in differences in respiratory tract deposition as well as clearance. Therefore, in addition to particle sizers, native physical properties of particles should be assessed using electron microscopy. TEM and SEM images provide confirmatory evidence of size, diverse morphological features, densities, and the metal constituents of particles produced in WUI fire emissions. In future studies, the electron microscopy results of this study will be used to categorize scale from least to most complex based on the presence of particle morphological feature(s) such as sphericity in 3D, circularity in 2D, frequency of heterogeneous shapes, surface textures, agglomerate density, length of elongated chains etc. This scaled assessment may be valuable to establish on objective directory/library and advance particle characterizations by cross comparing emissions from diverse material combustion involved in WUI fires. Qualitative assessment of metal constituent of particles by SEM + EDS will bring focus to quantitative analysis using induction coupled plasma mass spectrometry techniques. Fuel accelerants will be added during material combustion to mimic emissions from military burn pits and other open air combustion processes.

PS 4186 Inflammatory impacts of tire-related particles: do all roads lead to Rome?

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Background and Purpose: Current air pollution regulations focus entirely on exhaust emissions. However, inhalation of tire wear particles (TWP), produced when tires are worn down in contact with the road surface, could pose an emerging threat to human health. The environmental burden of TWP is expected to increase in coming years as more consumers transition to heavier electric vehicles that produce greater TWP emissions compared to conventional vehicles. TWP are composed of a unique chemical cocktail, including antioxidants like N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine, or 6PPD, which can be converted to toxic quinones under environmental conditions, impacting the toxicity of these particles with time. TWP have demonstrated toxicity to several species of freshwater fish, yet understanding of their impact on human health is still rudimentary. This study investigates the inflammatory effects of tire wear particle exposure in C57BL/6 wild-type mice and NLRX1-knockout mice. NLRX1 is a modulator of inflammation, often contributing to the control of inflammation initiation and preventing an overactive immune response. We predicted that exposure to TWP will elicit a pulmonary inflammatory response, with a more pronounced response occurring in the NLRX1-KO mice. **Methods:** TWP from commercial passenger vehicle tires were prepared by either grinding or cryomilling. Physical characteristics and chemical composition were assessed via scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDS), and high-performance liquid chromatography (HPLC). We additionally evaluated the potential of particles to generate oxidants/free radicals using electron paramagnetic resonance (EPR). 9-12-week-old male and female C57BL/6 wild type and NLRX1 knockout mice were used for this study. Mice were exposed via intratracheal instillation to 1 mg/Kg body weight TWP suspension. 24 hours after treatment, mice were euthanized and bronchoalveolar lavage (BAL) was performed on the left lung while the right lung was collected. Total cell counts and differential cell counting were performed on the bronchoalveolar lavage fluid. RNA was extracted from the right lung for real-time PCR analysis of inflammatory cytokine expression, including the cytokines KC, TNF-α, IL-6, IL-10, and STAT3. **Results:** SEM of TWP demonstrated a wide range of sizes (100 nm - 80 micron) and EDS demonstrated a complex elemental composition including Fe, Cu, Zn, S, Si, O, C and Cr. We validated the method for the extraction of 6PPD and 6PPD-Q from TWP using HPLC coupled with mass spectrometry. EPR analyses demonstrated significantly elevated oxidant levels in the BAL fluid. Moreover, BAL fluid from mice exposed to TWP exhibited an increase in the total number of cells, macrophages, and neutrophils. NLRX1-KO mice demonstrated significantly greater neutrophil recruitment in lavage compared to WT mice. Increased KC, TNF-α, and IL-6 gene and protein expression was observed after TWP exposure. **Conclusions:** In sum, we demonstrate that TWP have a complex composition and elicit a pulmonary inflammatory response depending on their composition and genotype of the mice. Further studies are underway to determine the mechanistic pathways implicated in the inflammatory response and to clarify the relative contribution of different components of TWP in their pulmonary toxicity.

PS 4187 Sustained, age-dependent neurometabolic changes following wood smoke exposure in mice

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Background and Purpose: Owing to the increasing severity of wildfires in the US, millions of people are routinely exposed to particulate matter from these events that may be harmful to the lungs and the brain. The severity of the neuroinflammatory response that was previously found to be associated with WFS exposure in young mice was dynamic and resolved approximately 4 weeks after exposures ended. Subsequent studies showed that this sustained effect could be worse in aged mouse models. The purpose of the study was to better understand the relationship between aging and neuroinflammation resolution. **Methods:** 15-, 18-, and 21-month-old (mo) mice were exposed to either filtered air (FA) or simulated wildfire smoke (WFS; 500 µg/m^3, 4h/d every other day for 14 days) and sacrificed 30-days, 3-months, or 5-months post-exposure. Untargeted metabolomics and targeted metabolomics (NAD$^+$ pathway) were performed on left brain hemispheres. Pathway analyses were performed following identifications of significantly altered metabolites. **Results:** Clear metabolomic differences were found in the brains of mice exposed to FA and WFS at age of 15-mo and sacrificed 1-month post-exposure (e.g., L-threonine, asparagine); age of 18-mo and sacrificed 1-month post-exposure (e.g., L-threonine, asparagine); and age of 18-mo and sacrificed 5-months post-exposure (e.g., palmitoylcarnitine). Aging itself affected metabolomics at 15-, 18-, and 21-mo mice exposed to FA and sacrificed 30-days post-exposure. Phe/Tyr/Trp biosynthesis was significantly downregulated while purine metabolism was significantly upregulated in both the WFS-exposed mice and the advanced aged mice. Pathway analyses further revealed that significantly altered metabolites shared between the WFS and aging had significant overlap in purine metabolism and Phe/Tyr/Trp biosynthesis. NAD$^+$

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