KEYWORDS: Neurotoxicology; Inhalation Toxicology; In Vivo Models; Black Carbon; BBB

ABSTRACT: Background and Purpose: Black carbon, a major contributor to global warming, is emitted into the atmosphere through the combustion of fossil fuels. Upon inhalation, it poses significant health risks, inducing toxic effects in various human organs, including the respiratory system and the brain. While epidemiological studies have well-documented the relationship between black carbon exposure and its adverse effects on human respiratory and neurological systems, preclinical studies on its neurotoxic effects remain scarce. Comprehensive research is urgently needed to elucidate the toxic mechanisms of black carbon on the brain. In this study, we investigated the neurotoxic effects of black carbon following inhalation exposure in mice, with a focus on its impact on cerebral regions. **Methods:** Black carbon was generated by combusting propane gas and subsequently dispersed in an aqueous solution to prepare a black carbon suspension. The suspension was sprayed and dried to produce black carbon aerosols. Mice were exposed to these aerosols in a nose-only inhalation toxicity chamber at a concentration of 9.01 mg/m^3 for 6 hours per day over a period of two weeks. Brain tissues were collected post-exposure and subjected to immunohistochemical staining with NeuN, MAP2, NFL, Iba1, GFAP, AQP4, CD31, IgG, and Fibrinogen. These markers were used to evaluate changes in neuronal cells, microglial cells, astrocytes, cerebral vasculature, and blood-brain barrier integrity. **Results:** In the cerebral regions, a reduction in NeuN expression indicated neuronal loss. A decrease in MAP2 was observed, suggesting damage to neuronal dendrites. However, NFL levels remained unchanged, implying that axonal structures were not affected by black carbon exposure. Immunostaining for Iba1 revealed an increase in activated microglial cells, while no activation of astrocytes was detected. Around cerebral blood vessels, a significant reduction in AQP4, a key component of the glymphatic system, was observed. Despite this, CD31 and IgG staining did not show signs of vascular hemorrhage. However, the reduced fluorescence intensity of IgG staining suggests a potential decrease in cerebral blood flow. **Conclusions:** Two weeks of inhalation exposure to black carbon induced pathological changes in cerebral blood vessels and neuronal cells. These alterations are likely to affect various human activities, including cognitive functions. Further studies, such as behavioral assessments and mechanistic investigations, are required to comprehensively understand the impacts of black carbon on brain health.

ABSTRACT NUMBER: 5008 **Poster Board Number:** LB109

TITLE: Characterization of engineered stone dust reactive oxygen species generation and cytotoxicity *in vitro*

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KEYWORDS: Particulates; Safety Evaluation; Respiratory Toxicology; Reactive Oxygen Species; Silica

ABSTRACT: Background and Purpose: Engineered stone (ES), a composite material composed primarily of crushed quartz and a polymer binder, is widely used for countertops and flooring due to its durability and aesthetic appeal. However, the fabrication processes of cutting, grinding, and polishing generate respirable engineered stone dust (ESD), posing a significant occupational health hazard. ESD contains high concentrations of respirable crystalline silica (CS), a well-established causative agent of silicosis, lung cancer, and other respiratory diseases. Furthermore, these processes release volatile organic compounds (VOCs), potentially exacerbating toxicity. Recent global outbreaks of accelerated silicosis among ES workers, often affecting young individuals with relatively short exposure durations,

underscore the urgent need for comprehensive research and effective workplace safety regulations. The underlying mechanisms driving this accelerated form of silicosis remain incompletely understood. This study investigated ESD-induced cellular toxicity and the generation of reactive oxygen species (ROS) in vitro, comparing the effects of freshly generated and aged ESD particles. **Methods:** Particles were generated using a custom-built, automated system mimicking countertop fabrication. A circular saw with a stone-cutting disc cut ES slabs within a sealed stainless-steel chamber with HEPA-filtered air intake. Aerosols exited at 30 L/min, passing through a cyclone (5µm cut size) to select respirable dust, and collected on Teflon filters. Collected particles were stored under nitrogen gas at -80°C. A subset was aged in air at room temperature for two weeks. Three ES materials (A, B, C), natural granite, and Min-u-sil-5 (MS5, a CS positive control) were used. CS content varied: 60% (ES A), 20% (ES B), 0% (ES C), 30% (Granite), and 99.5% (MS5). Electron paramagnetic resonance (EPR) spin-trapping assessed free radical generation. Particles were exposed to hydrogen peroxide (H2O2) to evaluate hydroxyl radical (.OH) production via a Fenton-like reaction. RAW 264.7 macrophages were cultured and exposed to 10µg/well of fresh or aged ESD, or MS5, for 24 hours. Cell viability, apoptosis (Caspase 3/7), necrosis (Propidium Iodide), and ROS production (CellRox Deep Red) were measured using high-content imaging. The antioxidant N-acetyl cysteine (NAC) was used to assess the contribution of ROS to cytotoxicity. **Results:** Fresh materials exhibited significantly higher EPR peak intensities (indicating greater radical generation) than aged counterparts. All materials generated more radicals than background and MS5. In the presence of cells, fresh materials still showed higher EPR intensities, but aging significantly reduced this effect. Live cell counts were significantly reduced in wells treated with fresh ES materials and granite compared to controls, while aged materials and MS5 showed no significant difference. The percentage of apoptotic cells increased for all materials compared to controls, with no significant differences between fresh and aged materials. Necrotic cells increased in samples treated with fresh granite, fresh and aged ES A, and fresh ES B, with fresh materials showing significantly higher levels than aged counterparts. Oxidative stress increased in cells exposed to fresh and aged ES A and C, and aged granite. Despite decreased cell counts, total cell area increased in wells containing fresh and aged granite, fresh and aged ES A, fresh ES C, and MS5. Adding NAC did not attenuate the cytotoxic effects of ES exposure. **Conclusions:** Freshly-generated ES dusts exhibit different radical generating capacity compared to their aged counterparts. This capacity was only partially silica-content dependent. Cell viability, apoptosis, necrosis, and total cell area were all altered differently by aged vs fresh ES materials. The addition of antioxidant to the culture media did not attenuate any cytotoxic effects of ES exposure, indicating that ROS may not be the main factor contributing to the observed effects. Granite and ES A exposure tended to elicit the largest changes in toxicity and ROS production, while ES B produced fewer changes. ES C, which contains no measurable CS, was still capable of inducing ROS generation and cytotoxicity. These findings suggest that material silica content is an important, but not the sole factor contributing to cytotoxicity. Additionally, particle aging plays a significant role in ESD toxicity and should be a consideration in any future studies with these materials.

ABSTRACT NUMBER: 5009 **Poster Board Number:** LB110

TITLE: A study of ambient air monitoring of particulates and crystalline silica near aggregate production operations (APOs) in central Texas

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KEYWORDS: Crystalline Silica, particulate matter

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