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Novel Approaches to Air Quality Monitoring by Measuring Biological Responses to
Environmental Aerosols

A Thesis submitted in partial satisfaction
of the requirements for the degree of

Master of Science

in

Biomedical Sciences

by

Malia Le Shapiro

June 2024

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Committee Chairperson

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ABSTRACT OF THE THESIS

Novel Approaches to Air Quality Monitoring by Measuring Biological Responses to
Environmental Aerosols

By

Malia Le Shapiro

Master of Science, Graduate Program in Biomedical Sciences
University of California, Riverside, June 2024
Dr. David Lo Chairperson

Air quality is measured by various factors: ground-level ozone, particle pollution (particulate matter such as PM2.5 and PM10), carbon monoxide, sulfur dioxide, and nitrogen dioxide. PM2.5 and PM10 refer to a particulate matter with a diameter equal to or less than 2.5 micrometers or 10 micrometers, respectively. Exposure to PM2.5's extremely fine particles allows for them to travel through the respiratory tract and reach the lungs, which can then be absorbed into the bloodstream via the alveolar sacs. Long-term exposure to PM2.5 has been linked to increased respiratory and cardiovascular hospital emissions. These factors play a large role in air quality, however defining a new category of biological pollutants that have health impacts on the community will be key in moving towards creating healthier communities.

The Salton Sea is the largest inland lake in terms of area located in California. This 376 square mile wide sea is located at the border of Riverside and Imperial counties. The cities surrounding the Salton Sea are plagued with unusually high asthma rates, ranging

from 20-22%, significantly higher than the California state average of 14.5% (Farzan et al., 2019). Recognizing the urgent need for better air quality monitoring in these areas, it is essential to explore innovative methods to identify and address the environmental factors contributing to these health issues.

Air quality monitors are typically outfitted with sensors that are designed to detect specific pollutants, using either scanners to measure particulate matter density or satellite imaging to measure energy reflected or emitted by the earth. Currently, there is no measure of natural biological aerosol pollutants, such as endotoxin, in current air quality monitoring protocols. Proposing a new way of monitoring, based on mouse exposure studies with aerosolized dust particles, will enable air quality monitoring that provides identification of inflammatory stimuli that contribute to the incidence of diseases like asthma.

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Impact of Biological Aerosolized Hazards - Natural Biological Hazards

The United States Environmental Protection Agency (EPA) categorizes biological pollutants as bacteria, mold, mildew, viruses, animal dander and cat saliva, house dust, mites, cockroaches, and pollen. Many of these pollutants come from everyday household items and activities, such as purchasing indoor and outdoor plants, having people over, and owning pets. Currently, biological pollutants are not monitored as part of the international air quality guidelines and database. Many of these biological contaminants can cause allergic reactions and long-term clinical diseases with repeated exposure, therefore it is important to shift air quality monitoring guidelines to incorporate these everyday pollutants.

Most commonly, asthma-like symptoms as well as asthma can be developed after long-term exposure to these biological pollutants. Asthma is described as an airway restriction caused by an increase in hyperreactivity due to airway inflammation in response to allergens. This is accompanied by increased immunoglobulin E (IgE) production, Th-2 cytokine production, eosinophil recruitment, airway remodeling, and airway hyperresponsiveness [39]. In sensitive individuals, continued exposure to allergens can cause symptoms such as wheezing, shortness of breath, chest tightness, and coughing; all symptoms are accompanied with widespread but variable airflow obstruction that is at least partly reversible either spontaneously or with treatment [33]. According to the Centers for Disease Control, asthma typically affects 1 in 10 children and typically worsens with triggers such as cold air, pollen, etc.

To start analyzing biological pollutants and agents as sources of air quality pollution, we need to classify known biological inflammatory components. *Alternaria alternata (Alternaria)* is one of the most common fungi associated with developing asthma and/or asthma-like symptoms. This chronic, adaptive inflammatory allergen is typically found in and outside of households and thrives in warmer, dry environments [40]. Studies show a positive correlation between increasing *Alternaria* spore concentrations and the presence of symptomatic asthma [17]. *Alternaria* exposure seems to be associated with the development of co-sensitization to other allergens and possibly exacerbate symptoms of asthma [40].

Lipopolysaccharide (LPS) *and Lipoteichoic acid* (LTA) are well established acute, innate inflammatory agents used to explore innate immune responses in mammals. LPS is a major component of the cell wall in gram-negative bacteria. LTA, alternatively, is a major component of the cell wall in Gram-positive bacteria. Although both of these agents are not inherently toxic in environmental settings, when inhaled and recognized by the immune system both of these are potent endotoxins that trigger strong inflammatory reactions in the body. In mammals, it triggers the activation of toll-like receptor 4 (TLR4) by LPS and activation of toll like receptor 2 (TLR2) by LTA, which both then cause a downstream release of proinflammatory cytokines such as Tumor Necrosis Factor Alpha (TNF-α), Interleukin 1 (IL-1), and Interleukin 6 (IL-6) [41].

Exposure to *Alternaria,* LPS, and LTA have well-categorized and reproducible inflammatory responses in scientific literature. Therefore, at the most basic level of categorization, these three immune response profiles are essential. When gathering

immune responses from different environmental dust collection sites, these three agents will be used as a baseline for comparison.

Impact of Biological Aerosolized Hazards - Man-Made Biological Hazards

Man-made technological advancements have always been the priority of society, starting with handmade fires to modern day coal-powered plants that provide energy to entire communities. While advancements in society are consistently made to improve our lives, these improvements, unfortunately, have consequences for the environment.

An ongoing battle in air quality improvement has been the pollution and emissions created by gas and diesel cars. Unlike countries that were built around walkability and accessible public transportation within a majority of their cities, such as Japan and Korea, the United States (US) relies heavily on personal use vehicles to get around the country, states, cities, and sometimes even neighborhoods depending on their structure. With the average income of a citizen living in the US sitting at $59,384, and the average hybrid and/or electric vehicle costing around $39,040 and $48,430, respectively, there is a huge lack of accessibility for the everyday community member to purchase a vehicle that can help improve air quality conditions in their neighborhood [46].

The health effects of diesel and gas emissions from transportation vehicles are well-documented and studied. Diesel exhaust contains a mixture of particulate matter (PM) and gasses, such as nitrogen oxides, which are both well-known airway inflammatory agents. Exposure to both of these pollutants can lead to increased susceptibility to lung infections and exacerbation of asthma [42] [43]. Similarly, emissions from gas vehicles release volatile organic compounds (VOCs) and carbon

monoxide, both of which impair lung function and can exacerbate asthma [44]. In addition to impairing healthy lung function, both types of emissions contribute to the formation of ground-level ozone and fine particulate matter [45].

In an effort to move towards clean energy, there is heavy emphasis on purchasing electric vehicles as a band aid for the harm that diesel and gas vehicles have caused to our changing ozone. Unfortunately, no matter how clean an energy is, there are always negative consequences to man-made biological hazards. While electric cars lack vehicle emissions, they have larger batteries which are more difficult to recycle after the car has reached its limit. Additionally, they are usually heavier than an everyday gas vehicle which causes more wear and tear on tires, constituting the need to be replaced more frequently. Furthermore, creating the energy to supply an electric vehicle is costly, indicating the need to factor in a cost-benefit analysis [47][48]. Researchers have concluded that, although there are still environmental risks from electric vehicles, overall electric vehicles have a smaller environmental risk than traditional gas and diesel vehicles. [47][48]

While technological advancements have significantly improved our quality of life, they have also introduced various environmental challenges. The reliance on gas and diesel vehicles in the United States has contributed to significant air pollution, resulting in numerous health issues that necessitate further research. Although the transition to electric vehicles presents a promising solution to reduce these emissions, it is not without its own environmental impacts. Therefore, it is essential to continue exploring and mitigating the effects of both chemical and biological pollutants to achieve

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comprehensive air quality improvement. This includes understanding the role of

biological endotoxins, like LPS and LTA, which can further exacerbate respiratory

conditions.

Current air monitoring protocol

The Air Quality Index (AQI) is the predominant monitoring scale for assessing air quality, utilizing a numerical range of 0-500. Its classifications are as follows: 0 to 100 for good to moderate conditions, 100 to 200 indicating unhealthy conditions for the public, especially affecting sensitive groups, and 200 to 500 representing significantly hazardous levels [24]. The United States EPA established this scale in reference to the five major pollutants monitored by the Clean Air Act: ground-level ozone, particle pollution (particulate matter, i.e. P.M. 2.5 and P.M. 10), carbon monoxide, sulfur dioxide, and nitrogen dioxide [19][20]. Each AQI established is based on a national air quality standard established and supported by scientific research [19][20].

In 1970, the Clean Air Act (CAA) was established to create guidelines for air pollution standards in order to protect public and environmental health. The CAA required the EPA to regulate emissions of air pollutants from a designated list of toxic substances, including benzene and volatile organic compounds (VOCs). Since passing and implementing the Clean Air Act in 1970, air pollution in the United States has decreased by over 78%, while Gross Domestic Product increased by more than 272% according to the EPA's annual "Our Nation's Air 2021" report [21]. The CAA was the first set of federal legislation that emphasized the importance of clean air for pulmonary health.

Traditional air quality monitoring standards are monitored and established by reliable instruments approved by the EPA. These conventional instruments are intricate, using infrared absorption spectroscopy or automatic concentration correction for pressure

and temperature changes, allowing for accurate real-time data collection. However, the complexity of these instruments comes with several limitations [49]. These include but are not limited to an inability to move instruments from their set monitoring center due to their heavy weight, high power consumption, and overall high cost of operation. [23] Altogether, the complexity of air quality monitoring instruments is essential in ensuring accurate, real-time air quality monitoring and adherence to standards.

Furthermore, failing to meet air quality standards in the United States has wide-ranging consequences, from public and community health impacts to economic and legal regulatory consequences. At the economic and legal regulatory level, areas that fail to meet the air quality standards can be deemed nonattainment areas by the EPA [28]. This classification implies additional restrictions and monitoring until the area meets air quality standards, along with the possibility of substantial fines. Failing to meet standards also increases economic costs through increases in hospital visits and hospitalizations while simultaneously causing a loss in productivity from those who are unable to work due to their symptoms. At the community health level, short-term exposure to poor air quality is linked to high rates of hospitalization and developing chronic obstructive pulmonary disease (COPD), coughing, shortness of breath, asthma, and respiratory disease[26]. Long-term effects of exposure to poor air quality are associated with the development of chronic asthma, pulmonary insufficiency, cardiovascular diseases, and cardiovascular mortality [25]. One review highlighted several studies identifying an association between communities with lower socioeconomic status (SES) and exposure to higher concentrations of air pollutants, outlined as the five major pollutants defined

and monitored by the CAA [27]. Many low-SES communities lack resources specifically access to healthcare services, to help mitigate the higher risks associated with air pollutants. The gap between healthcare outcomes, SES, and air pollution necessitates new approaches to air quality monitoring and community-based monitoring becoming a priority in order to shift healthcare outcomes.

As air quality monitoring becomes a higher priority for community members, several ambient air quality monitoring systems such as PurpleAir and TemTop have become available for at home monitoring. PurpleAir monitors rely solely on laser particle counters to measure the amount of PM1.0 , PM2.5 and PM10, within the air. Unfortunately, these low-cost monitoring systems cannot achieve the same accuracy and reliability that conventional monitoring center instruments are capable of. While these low-cost systems may not match the precision of conventional instruments, they offer a valuable alternative by providing accessible and comprehensible data to the public.

One advantage of real time home air quality monitoring systems is the ease with which the information can be understood and utilized by community members seeking to improve their health. While the information provided by in-home sensors are not necessarily the entire picture, these monitoring systems do provide a greater understanding of air quality throughout regions that lack a larger well-established monitoring system. One example of translatable monitoring systems is the Imperial County Community Air Monitoring Network (the Network). Their goals and objectives are to identify community concerns, establish research questions and protocol as well as community-engagement structure, monitor selection and installation, collect and analyze

data, and implement public health actions, with the focus on improving public health outcomes [3] [7]. Through their process of setting up monitors, community members and officials were all taught the basics of air quality and how the monitors work. These educational opportunities were essential for understanding how the air quality monitoring system will help the community improve their health outcomes, such as identifying when it is safer to go outside, when to shelter in place, especially for those who suffer from asthma, and even to help advocate for policy changes that actively reduce PM sources. Overall, this monitoring system is a smaller, less accurate scale of current government monitoring systems, but still provides a good structural example of how engaging community members is essential to creating translatable data that can be used to create movement towards healthy communities and policies. However, even with improved structural community monitoring, our current air quality monitoring systems are not equipped to measure environmental aerosols that also have health effects, such as LPS.

The Salton Sea Background

The Salton Sea located near southern Riverside and northern Imperial Counties in Southern California is the largest inland lake, area wise, located in California. This 376 square mile wide sea is the product of a canal break in 1905, allowing flooding from the Colorado River. Two years later the break was fixed, but continuous flooding from the Colorado river over this time filled the sink basin, creating what is now known as the Salton Sea. Many predicted that the sea would dry in the following decade post-break, however agricultural runoff flowed and by 1925, water levels had stabilized at 250 feet (76.2 m) below sea level (Blaney 1955). Since then, the Salton Sea has been maintained primarily by agricultural runoff from farming from the major agricultural centers of California, Eastern Coachella, and Imperial Valley (38).

During its prime, the Salton Sea was an incredible site of biodiversity, supporting myriads of fish, avians, and plants. In 1950, the valued game fish species Sargo (*Anisotremus davidsoni*), Gulf croaker (*Bairdiella icistia)*, and orangemouth corvina (*Cynoscion xanthulus)* were introduced into the sea, catalyzing the beginning of a thriving sport fishery spot. The original salinity, almost identical to saltwater concentration of 35 parts per million (PPM), supported marine fish well, and later on, other species were introduced, such as Tilapia (*Oreochromis mossambicus* x *O. urolepis hornurum)*. Additionally, Desert pupfish (*Cyrinodon macularius)* and Sailfin molly *(Poecilia latipinna)* were found in streams and canals that bordered the sea, eventually making their way into the sea itself to add to its thriving biodiversity. As the sea began to slowly dry due to reduced agricultural runoff and increasingly hotter, drier summers,

salinity levels began to increase, eventually killing off a majority of the once thriving

marine fish that inhabited the sea, leaving Tilapia at the top of the food chain [1].

Furthermore, the Salton Sea was an essential part of migratory birds flying

through the Pacific Flyway, becoming either a stopover spot or home during the winter

for nesting birds. Throughout its lifetime, there have been upwards of 400 species of

birds recorded at the Sea. The most popular species include Eared grebes, wintering gulls,

and egret herons. The diverse climate supported many species - subtropical birds used the

barnacle-covered sandpits of the sea and shorebirds bred and feasted along the vast

shoreline. There have been few Sea-wide bird surveys completed, putting exact bird

count and data at a disadvantage. The latest bird count, completed in 2022, showed 147

species still thrive at the Sea today. As climate change and the newest water transfer

policies began to shrink the Sea, salinity levels continued to rise to 60 PPM, saltier than

the Pacific Ocean. This is the tip of the threshold of tolerance for Tilapia to comfortably

breed, and unfortunately this once thriving species has now declined and died off,

resulting in an absence of piscivorous birds. With salinity continuing to increase,

halophilic invertebrates such as benthic algae and water boatmen will continue to support

shore bird species and filter feeding ducks.

Once a flourishing haven for ecological biodiversity, the Salton Sea is now

struggling to maintain its previous water levels due to consequences of reduced

agricultural runoff and intensified, hotter, drier summers over the years. The alarming

increase in salinity within the Salton Sea is creating an inhospitable environment for

many species, leaving the once-thriving populations of fish, birds, and wildlife with an

environment that can no longer support their existence. The repercussions extend beyond the lake's shores, impacting the populations residing in the surrounding Salton Sea cities. The drying sea exacerbates existing environmental challenges, posing a threat to the well-being of the local communities.

Emergence of Exposure Air Monitoring Protocols

Within the last several years, the communities on and around the Salton Sea have become a priority due to the rapid shrinking of the sea which has exposed dust and playa. This exposed dust and playa have been a major factor in increasing dust storms, which have been linked to cardiovascular mortality, increased asthma hospitalizations, and the development of Chronic Obstructive Pulmonary Disorder (COPD). [36] The prevalence of childhood asthma is high among children living around the Salton Sea, at a rate of 20% - 22.4%. This range is significantly higher than the California state average of 14.5% [32]. The communities surrounding the Salton Sea face many socioeconomic disparities, such as linguistic isolation, lack of education, and lack of funding and resources for health professionals. All of these barriers likely exacerbate the ongoing health disparities experienced by these communities of predominantly low-income, rural, Latino groups. In a 2022 survey done with 158 community members of Coachella Valley, only 11% of surveyors reported an official diagnosis of asthma from a physician, however, 20% of surveyors reported asthma-like symptoms with no diagnosis of asthma. Additionally, it was found that those who were diagnosed with asthma had poorly controlled symptoms. [35]

In response to the increased incidence of asthma in the region, several studies and programs have been launched to better understand and address the needs of these communities. Studies conducted in our laboratory have shown that dust from sites collected around the Salton Sea triggered lung neutrophil inflammation, an acute inflammatory profile that resembled the response to innate immune ligands LTA and LPS, but isolated from immune allergic response [38]. Other epidemiological studies have shown correlation between PM exposure and biological responses of innate immunity inflammation, oxidative stress, apoptosis and autophagy, and an imbalance of T-cells, all consistent with pathological changes in allergic respiratory diseases [37].

In a recent unpublished study done in our laboratory, the David Lo Laboratory, on different dust collection sites surrounding the Salton Sea we found that a large component of asthma symptoms could be linked to high concentration levels of LPS in the dust samples. Inhalation of LPS has been shown to cause neutrophil-dependent emphysema-like changes in lung structure, including apoptosis of lung parenchyma, and chronic long-term exposure has been associated with development of COPD. This discovery emphasized the importance of exploring not only specific particle size and type when determining air quality, but also the potential of organic biological inflammatory factors affecting pulmonary health.

Impact of Having A Biological Air Quality Monitoring Protocol

As society strives to improve air quality through community monitoring efforts [3], there remains a critical need to assess and diagnose additional components of air quality. Current monitoring methods predominantly focus on chemical pollutants, such as PM, nitrogen oxides (NOx), and sulfur dioxide (SO2). However, these methods overlook biological components that have significant impacts on respiratory health. Currently, there is no consistent method for monitoring the biological components that impact respiratory health, such as bacteria, fungi, and endotoxins.

To address this gap, we have developed a method to collect dust samples from regular community sites and categorize the immune response elicited. By comparing these reactions to known biological inflammatory agents, such as LPS and LTA, we can identify and categorize the components of dust that cause inflammatory responses in the area. This approach enhances our understanding of the biological factors affecting air quality and their impact on respiratory health but also empowers community members and officials with information and data to help tailor air quality guidelines and laws to reduce the impact of these inflammatory components. For example, if a community was found to have higher levels of fungal spores that correlated with increased respiratory issues, targeted measures could be implemented to mitigate exposure.

Furthermore, as more data is collected and analyzed, a comprehensive database can be established. This database would serve as a valuable reference and resource for national monitoring programs, enabling a standardized approach to assess biological air quality components across different regions. This resource would facilitate better

collaboration between different communities and researchers, ultimately leading to more effective strategies for improving air quality and public health. By bridging the gap in current monitoring practices, we are providing tools for policymakers and communities to improve public health as well as gaining a deeper understanding of current understudied factors that affect air quality.

Creating a Simplified Exposure Chamber Model

When performing exposures using a chamber exposure method, the focus is to replicate the natural mode of inhalation. To do this efficiently and effectively, we first tried to use the original large-scale chambers used in previous studies [38]. We then determined that smaller chambers would be a more effective route for the simple exposures desired.

After working with the UCR C-CERT engineering team, a 6x6x8 inch chamber with a four-inch spacer was built to imitate the use of an inhaler (Figure 1). We also determined that using an Auglam nebulizer for a standardized five-minute exposure would help facilitate more direct inhalation because particles are distributed within the size range of one to five microns, making them ideal for deep inhalation similar to typical environmental dust that causes respiratory issues.

Figure 1

Miniature Chamber for Single Aerosol Exposure
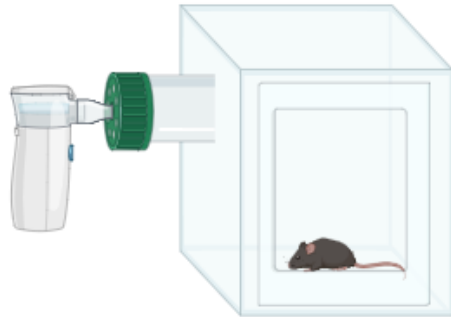
Note. This figure shows the design idea for the miniature single aerosol exposure chamber before its creation. The design avoids the animal needing to be anesthetized, in contrast to intranasal or intratracheal exposure protocols.

Methods

Animal Model and Exposure:

All animal studies were done following the UCR institutional IACUC and NIH guidelines. Both male and female C57BL6 mice (8-9 weeks) were purchased from Jackson Laboratory, Sacramento, and acclimated for one week in the University of California, Riverside SPF vivarium. After acclimation, mice were placed in either an environmental exposure chamber or in a smaller single aerosolized exposure chamber. Mice in the environmental exposure chambers were housed in group cages, with three to four mice per cage, and allowed food and water ad libitum. Mice placed in the smaller single chamber were individually exposed before being placed in an overnight home for 24 hours before animal processing began. Exposures in the environmental exposure chambers lasted for 48-hours and a 12-hour day/night cycle was provided whereas the single mouse chambers lasted for 5 minutes before being moved to the overnight cages.

Simplified Environmental Exposure Chamber

To investigate the potential of a smaller, simplified model of exposure using environmental samples, we exposed mice in a large exposure chamber for 48 hours and compared this with a variety of exposures in the miniature chamber. To establish a protocol for the simplified environmental exposure chamber, LPS was used as an exposure control model because of previously observed and characterized responses to LPS from both a 48 hour and 7-day time period [38]. Our first experiment was a singular dose of LPS at a concentration of 75 mg/mL in comparison to two doses of LPS at a concentration of 75 mg/mL, 6 hours apart. Mice were exposed for the amount of time the

nebulizer took to disperse the liquid, which varied from 2-5 minutes. Because of the variability of time in dispersal, we chose to use a set time point of 5 minutes total for mice to be set in the chamber, regardless of nebulizer dispersal. We chose 5 minutes because the particle size created by the nebulizer stayed floating in the air for around 5 minutes before dropping off as validated by a general scanning mobility particle sizer (SMPS). In this exposure we discovered that the two low dose exposure was more reliable in producing an inflammatory response, however it was still not significant enough to produce reliable data. In our final exposure, we did two high concentration doses of LPS, 375 mg/mL, 6 hours apart. We also did two doses of Ag dust as well as control PBS for comparison.

Animal Processing:

After either exposure, mice were removed from either of the exposure chambers for further processing. Mice were then anesthetized using isoflurane and euthanized by cervical dislocation. Bronchoalveolar lavage fluid (BALF) was collected by flushing the lungs three times with 0.8 mL of PBS. After flushing, the lungs were dissected out for lung digestion or RNA extraction. The right lobe was taken, and flash frozen in liquid nitrogen and kept at -80 °C until RNA extraction was completed. The left lobe was minced into small sections (~1-2mm) and digested using 0.5 mg/mL collagenase D (Roche Diagnostics, Mannheim, Germany) and 50 U/mL DNase I (Sigma Aldrich, St. Louis, USA) in RPMI 1640 (Gibco, Grand Island, USA) secured with 10 % heat-inactivated FBS (Gibco, Grand Island, USA) preheated to 37 °C. The mixture then incubated for 30 minutes at 150 rpm in 37 °C, then the lung was agitated using an 18-

gauge needle and incubated for another 15 min under the same conditions. After digestion was complete, the lung was pushed through a 100 μm cell strainer (Corning, Corning, USA). The cell strainer was then washed with RPMI 1640 with 10 % heat inactivated FBS before centrifugation and resuspension for use in flow cytometry. Alternatively, the lungs were dissected out for lung digestion or RNA extraction, however the left lobe was agitated and digested using the GentleMacs protocol 37C_m_LDK_1 and then tissues were processed the same post cell strainer as the above protocol. All samples were then resuspended and fixed using 1% paraformaldehyde until the next day when processing the samples on the NovoCyte Flow Cytometer.

Flow Cytometry:

For flow cytometry, both BALF and left lobes were collected to examine the cellular recruitment of immune cells. Cells were first stained with 1:100 dilution of Zombie Yellow dye and then dead cells were excluded from further analysis. Cell populations were determined by using a variety of surface markers as follows: neutrophils were identified by CD45+Cd11b+Ly6G+SiglecF-CD11c-, eosinophils were identified by CD45+CD11b+SiglecF+CD11c-, T-cells were identified by CD45+CD3+-SiglecF−CD11c−, and B-cells were identified by CD45+CD19+SiglecF+CD11c−. Samples were run on a NovoCyte Quanteon and gating and analysis were performed using FlowJo.

Statistical Analysis

Statistical analysis was done using GraphPad Prism 9 (GraphPad, San Diego, USA). T-tests and Mann-Whitney U tests were done to calculate p-value.

Dust Collectors:

Dust collectors were standardized for dust collection. Sterilized round, Bundt pans (Nordic Ware, Minneapolis, MN, USA) coated with Teflon (25.4 cm in diameter), lined with Kevlar mesh (Industrial Netting, Maple Grove, MN, USA) were filled with sterilized marbles, then a set of two plastic pieces were secured over the top of the Bundt pan in an X-shape. These two plastic pieces were covered in a clear sticky substance to act as an anti-bird repellent. Each Bundt pan was placed around 5-6 feet off of the ground, at minimum, and monitored for weather changes, such as rainfall or snow.

Dust collectors were placed at 33° 56 '33.4"N 117° 18' 15.2"W, a site labeled as Highway Dust, from August 1st, 2023 to October 11th, 2023. Collectors were also placed at 33° 10 '07.9"N 115° 51' 21.8"W, a site labeled Ag Dust, in 2021. Dust collectors were then placed into sterilized Whirl-pak bags and transported to the laboratory at UC Riverside for further processing.

Dust Collections:

Bundt pans were first uncovered and then 750 mL of MilliQ water was added to the pan from a 1L bottle. Pans were swirled around and gently poured into a sterilized tray with the mesh filter. After all marbles were in the tray, 250 mL of MilliQ water was added to the remaining empty Bundt pan. All leftover dust and debris were collected and poured into the tray. The mesh lining and marbles were washed and removed from the tray. Water was then swirled around and poured into a Buchner funnel back into the original 1L bottle that held the MilliQ water. After all water was poured through, the dust

suspension was placed in the -20*C freezer for storage. Suspensions were thawed and

then filtered to get rid of larger debris. Filtered dust was refrozen to be lyophilized.

Results

Comparison of Highway Dust Exposure vs. Control

To assess the biological responses to the highway dust exposure, we did aqueous extractions of collected highway dust and exposed mice to these extracts for 48-hours in our large chambers. Mice exposed to highway dust showed no significant increase in immune cellular recruitment in the lung tissue (Figure 2a,b). In contrast, there was a statistically significant increase in neutrophil, eosinophil, and alveolar macrophage recruitment in collected BALF (Figure 3a,b). This type of inflammatory cellular recruitment is consistent with active tissue inflammation and cellular responses to innate immune triggers, such as LPS and LTA.

Limulus Assay to Assess LPS Content

In order to confirm the hypothesis that Salton Sea dust caused pulmonary inflammation due to LPS content and exposure, a Limulus Amebocyte Lysate-Biological Assay was performed on several Salton Sea collection sites, including Ag Dust as well as the highway dust samples collected. This assay confirmed the presence of LPS in both Ag Dust and Highway dust at 1518.19 EU/mL and 31.08 EU/mL, respectively (Figure 4).

Comparison of LPS, Ag Dust, and PBS Exposure in Simplified Chambers

To assess the validity and consistency of the simplified exposure chambers, we made aqueous extracts from LPS, Ag Dust, and used PBS as a control. We exposed 2 mice per experimental group. Experimental group one was a single dose of LPS and group two was two doses of LPS, 6 hours apart. We then repeated that exposure, but with the three other experimental groups, each with two mice per group. Overall, in the tissue

there was no significant inflammatory cellular recruitment in single dose LPS, double

dose LPS, high dose LPS and Ag dust when compared to the PBS control group, however

there was a statistically significant increase in recruitment of neutrophils in the BALF

following high dose LPS exposure (Figure 5a,b).

Discussion

Overall, our results indicate that environmental samples and the described simplified protocol did not produce an immune response. As a result of the Limulus Assay measuring a high LPS concentration in the Ag dust, we hypothesized that a two-dose exposure of Ag dust, similar to the two-dose high LPS concentration exposure, would elicit an immune response, however the lack of significant immune response necessitates a modification of protocol and retesting of this exposure. Conversely, using environmental samples in the large chamber was sufficient in producing an immune response in the mice.

The miniature chamber was found to be sufficient in eliciting an immune response to high concentration laboratory LPS but needs modification and further studies in order to use environmental samples. The miniature chamber's design is unique as it enables simpler exposures and eliminates the need for anesthetization of mice, unlike intranasal exposures, while better simulating the body's natural inhalation of particles. These chamber exposures provide new ways to explore modes of delivery and exposure in pulmonary inflammation.

Conclusion

As it becomes a more pressing concern, new methods towards improving air quality monitoring are quickly becoming a priority. Addressing the impact of both natural and man-made biological aerosolized hazards and prioritizing improving current air monitoring protocols is essential for improving air quality and public health. Natural biological pollutants, such as endotoxins, along with man-made emissions from diesel and gas vehicles, contribute significantly to development of respiratory issues, such as asthma. Even with an increase in traditional monitoring systems and contribution from community members using at home monitoring systems, there are still limitations with immobility of traditional instruments and lack of precision in the instruments, respectively. Community-based initiatives, such as the Imperial County Community Air Monitoring Network, highlight the importance of engaging and educating community members to enhance public health outcomes. It remains essential to incorporate a more comprehensive monitoring system that focuses on biological pollutants that we encounter every day.

The miniature chamber was successful in eliciting immune responses when working with lab grade, high concentration LPS doses, however exposed mice did not produce an immune response when working with environmental samples. This lack of response could be due to the inability to know the environmental sample concentration, a factor that is normally mitigated by the use of the SMPS in large chamber exposures. Due to the smaller sample size, exposures in the simplified chamber should be repeated with modifications to the time of exposure, concentration of exposure, and possibly even dust

collection process to properly elicit an immune response from environmental samples. Additionally, multiple day exposures with environmental samples would be key to continually emulating natural inhalation and exposure. Incorporating guidelines and focusing on monitoring biological pollutants in air quality will shift health outcomes in areas, such as the Salton Sea, that suffer from high rates of asthma and pulmonary issues. By shifting our focus, we can ensure a cleaner, healthier air quality environment for future generations

Future Direction

Future directions for this project include initiating collecting environmental dust samples from different sample sites in order to continue quantifying inflammatory profile responses to the different biological endotoxins that affect air quality. While the simplified protocol proposed for this thesis was unsuccessful with the use of the given environmental samples, with more experimental analysis and adjustments to the protocol, evidence from the simplified LPS exposures show that this newer individualized chamber protocol could be used with environmental samples. Another future direction is completing RNA sequencing via Nanostring Technology in order to visualize the gene expression patterns that occur post-exposure. This would help us identify the genes that are upregulated or down regulated in response to the environmental exposure. This will help us understand the underlying biological mechanisms and pathways that are activated or suppressed in response to these biological endotoxins.

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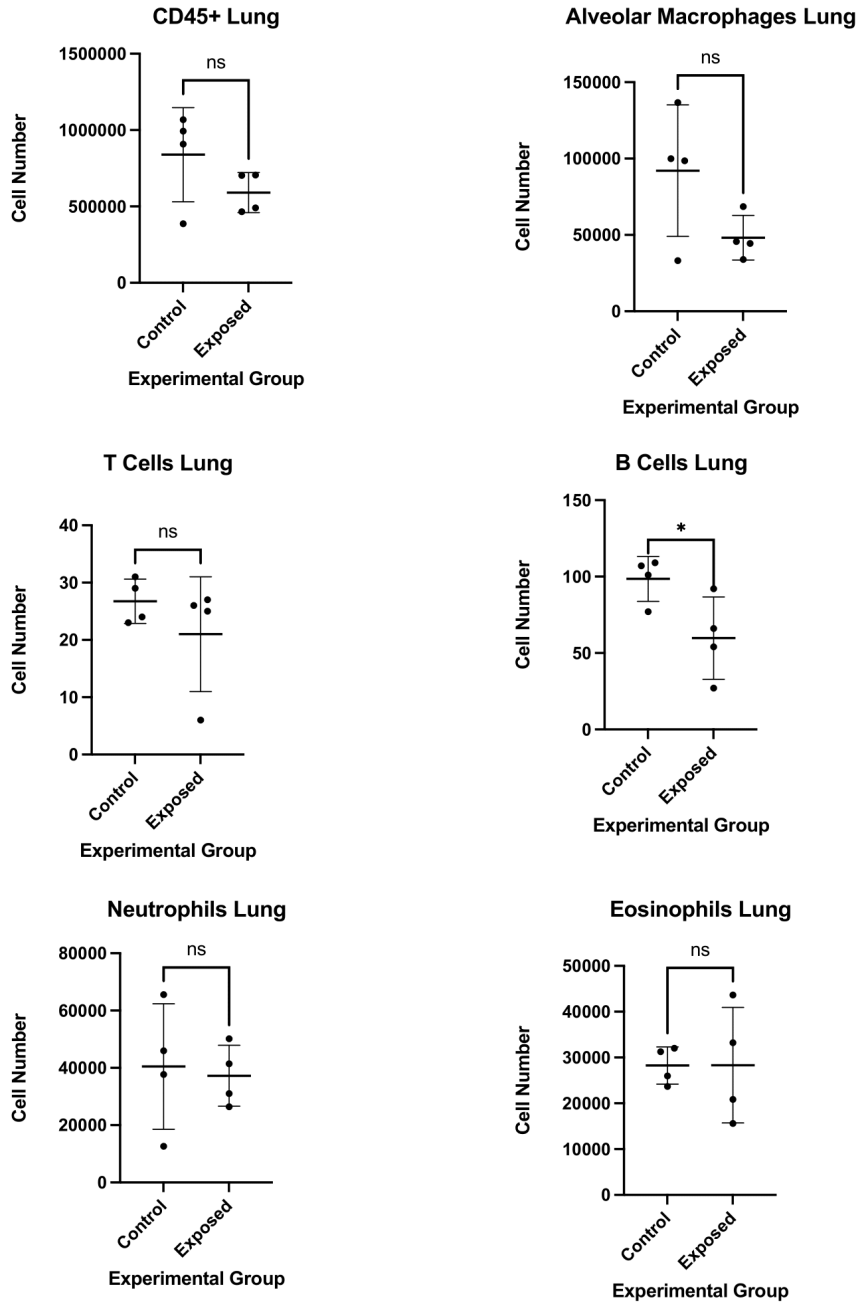
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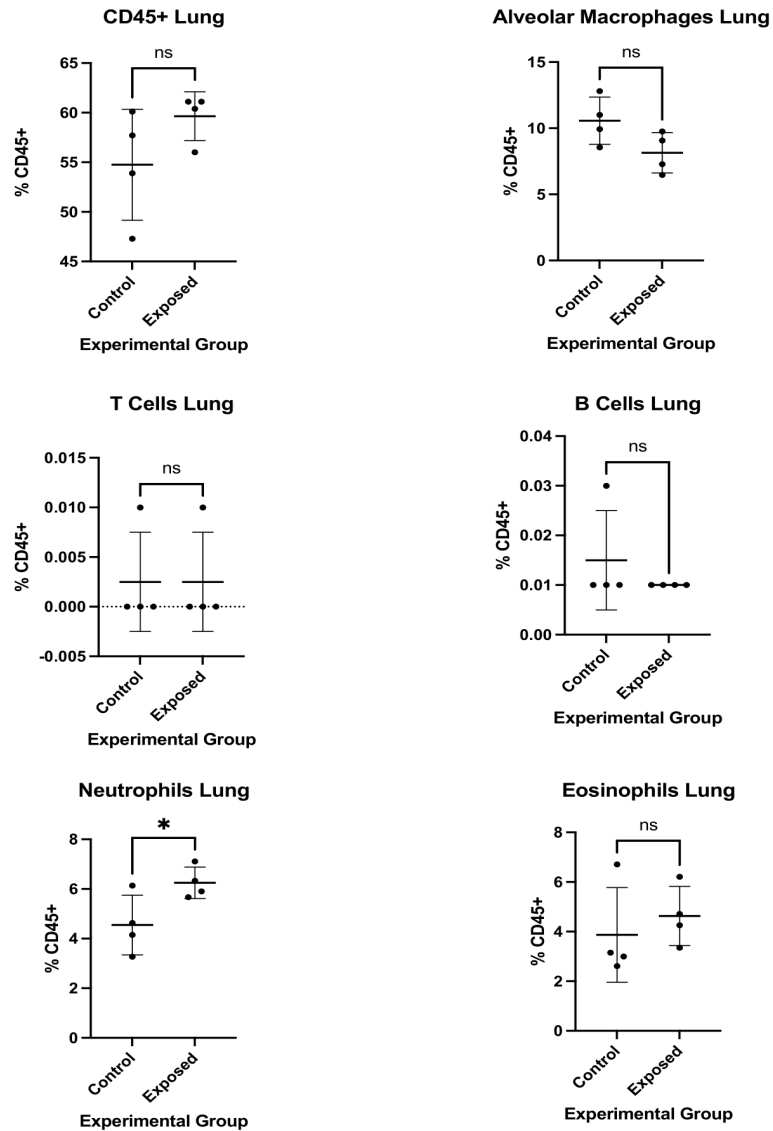
Figure 2

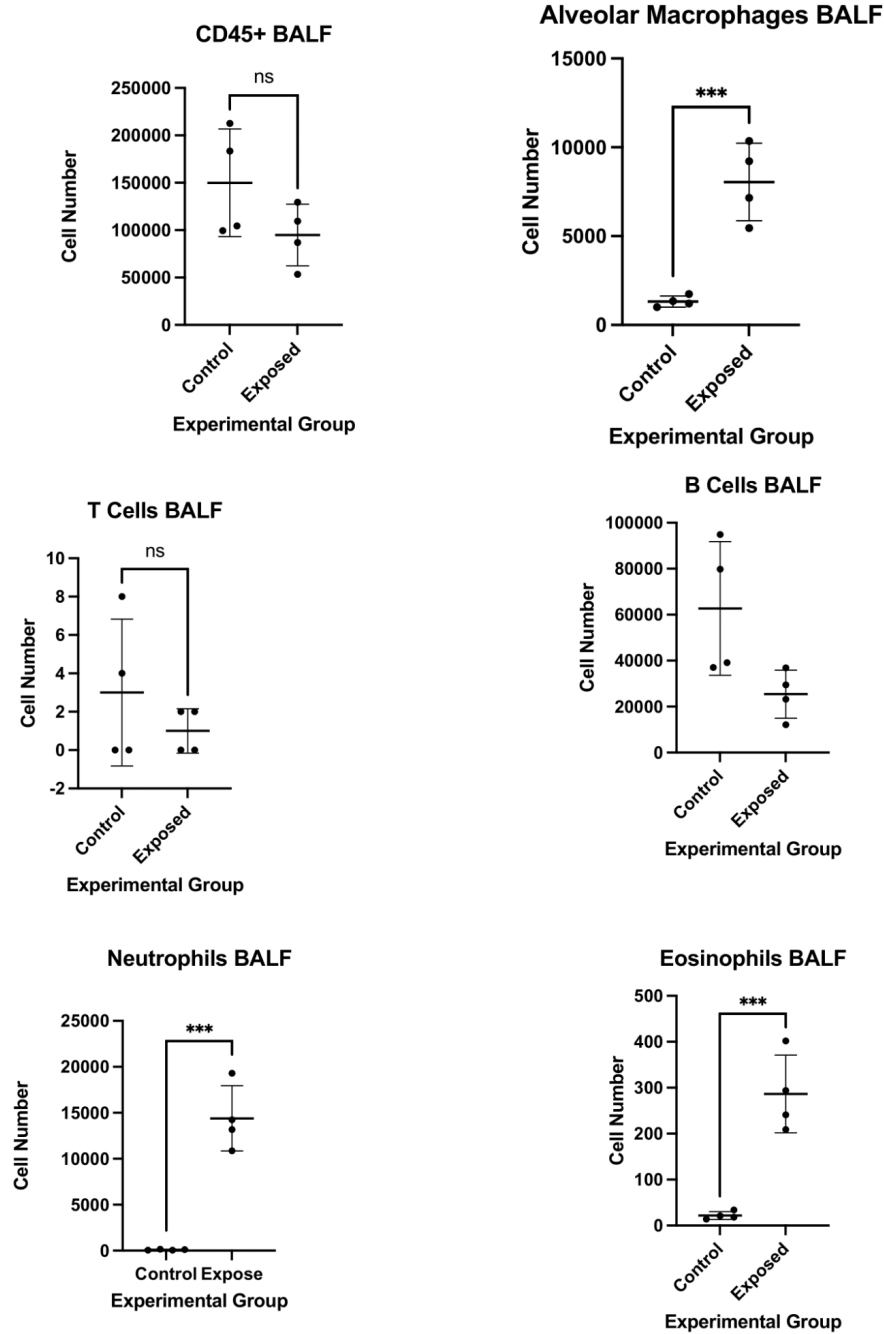Lung Cell Population Counts and Percentages - Highway Dust 48 Hour Exposure

A.

B.

Note: Inflammatory cell recruitment due to Highway Dust. Mice were exposed to filtered control air and filtered, aerosolized Highway Dust for 48 hours. Tissue was digested for flow cytometry. (A) Lung cell counts. (B) Lung cell percentages. Digested lung was stained and analyzed via flow cytometry. Cell populations are represented as the percentage of CD45+ cells. Representative dot plots for the control air and highway dust are shown. Eosinophils are stained with CD45+CD11B+SiglecF+Cd11c- (n=4 per experimental group). Neutrophils are stained with CD45+CD11b+Ly6G+SiglecF-CD11c- (n=4 per experimental group). B cells are stained with CD45+CD19+SiglecF+CD11c- (n=4 per experimental group). T cells are stained with CD45+CD3+SiglecF-CD11c- (n=4 per experimental group). * = p < 0.05.
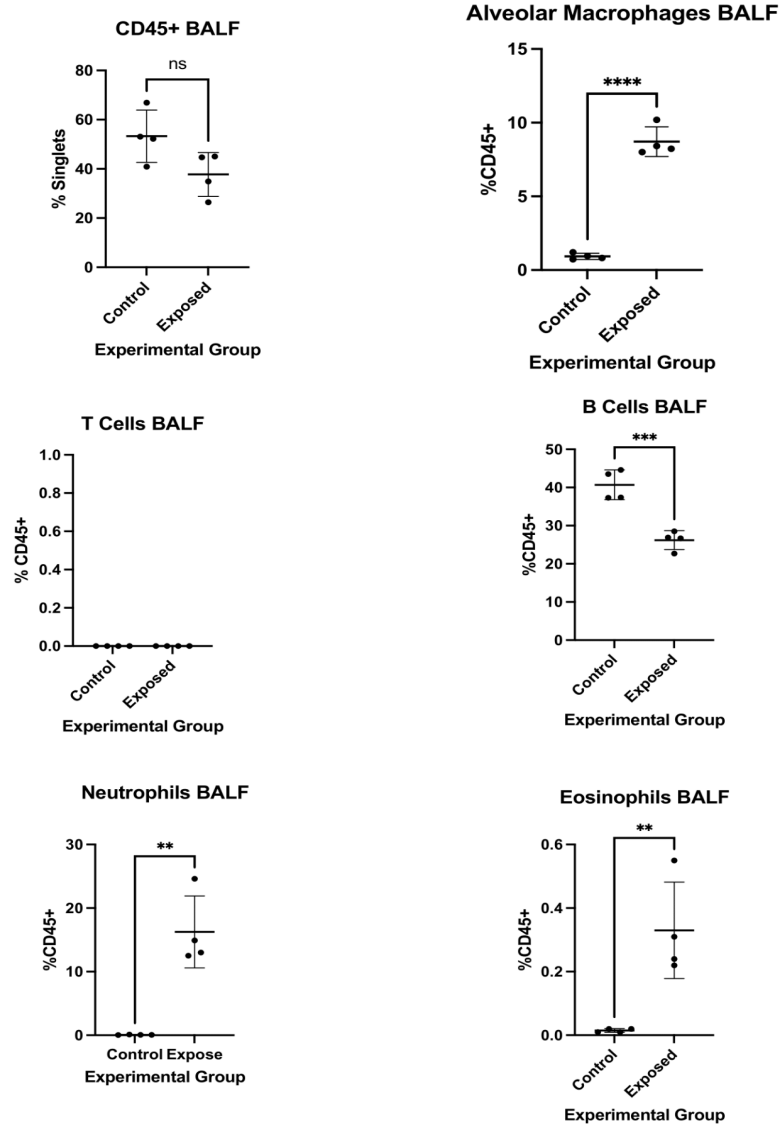
Figure 3

BALF Cell Population Counts and Percentages - Highway Dust 48 Hour Exposure

A.

B.

CD45+ BALF

Alveolar Macrophages BALF

T Cells BALF

B Cells BALF

Neutrophils BALF

Eosinophils BALF

Note: Inflammatory cell recruitment due to Highway Dust. Mice were exposed to filtered control air and filtered, aerosolized Highway Dust for 48 hours. BALF was processed for flow cytometry. (A) BALF cell counts. (B) BALF cell percentages. BALF was stained and analyzed via flow cytometry. Cell populations are represented as the percentage of CD45+ cells. Representative dot plots for the control air and highway dust are shown. Eosinophils are stained with CD45+CD11B+SiglecF+Cd11c- (n=4 per experimental group). Neutrophils are stained with CD45+CD11b+Ly6G+SiglecF-CD11c- (n=4 per experimental group). B cells are stained with CD45+CD19+SiglecF+CD11c- (n=4 per experimental group). T cells are stained with CD45+CD3+SiglecF-CD11c- (n=4 per experimental group). * = p < 0.05.

Figure 4
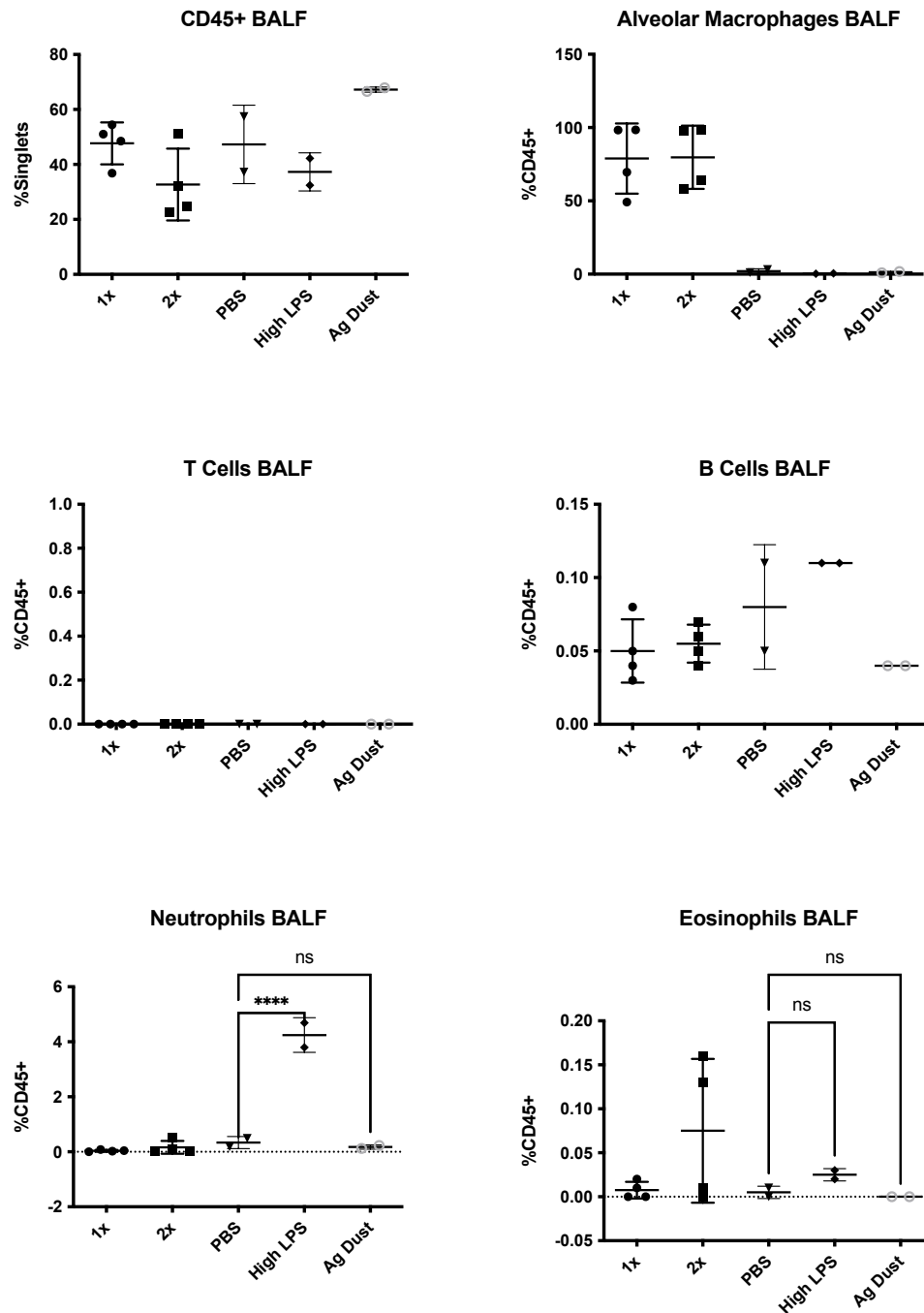
Limulus Amebocyte Lysate-Biological Assay

Site	LPS Concentrations (EU/mL)	LPS Concentrations/months of collection
AG Dust	1518.190547	253.0317578
West Corvina Beach Playa	22.815534	
Wister Dust	8737	2912.333333
North Shore Sea Water	0.810791237	
Salton Sea Water	0.997525499	
Salton City Filtrate	9.07	
Malia's House Dust	31.08	15.54
Wister Water	338.578896	

Note: This assay was performed to assess the potential presence or concentration of LPS within several dust and water collection sites around the Salton Sea as well as a highway dust environmental sample, used as a control site for future environmental samples.
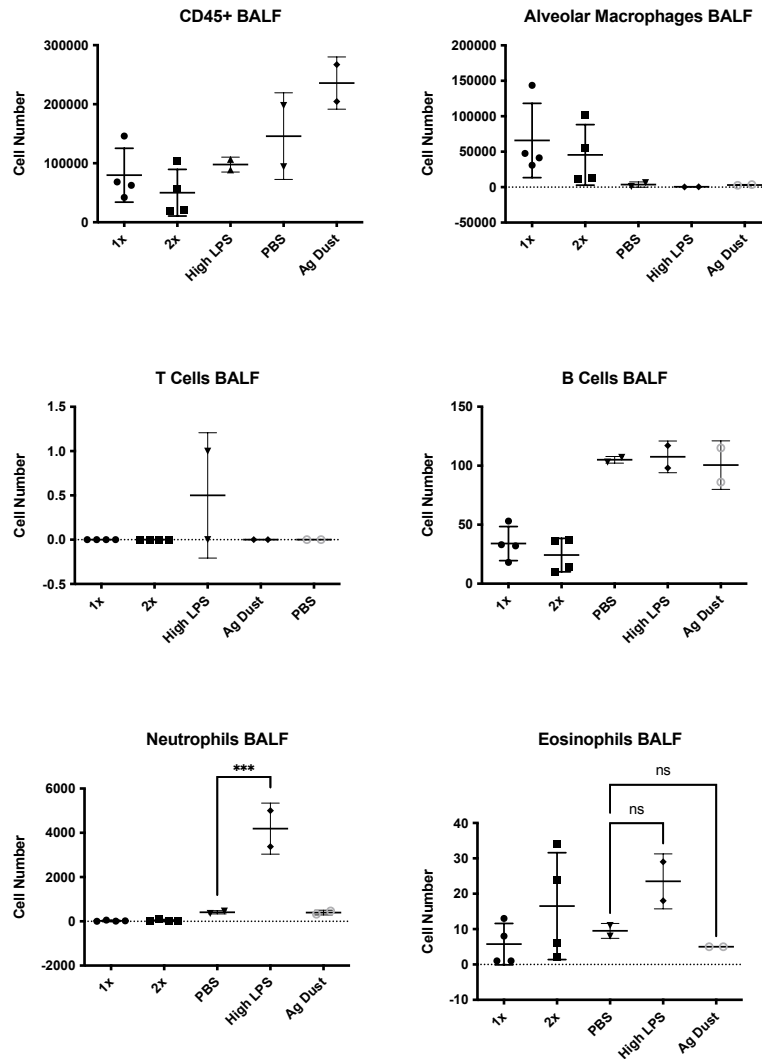
Figure 5

Single Low Dose, Double Low Dose, Double High Dose, Ag Dust, and PBS Exposure BALF Cell Count and Percentages

A. BALF Cell Percentages

B. BALF Cell Count

CD45+ BALF

Alveolar Macrophages BALF

T Cells BALF

B Cells BALF

Neutrophils BALF

Eosinophils BALF

Note: Inflammatory cell recruitment due to Single low dose LPS, double low dose LPS, double high dose LPS, Ag Dust, and PBS exposure BALF Cell Count and Percentages. Mice were exposed to PBS and each experimental group. BALF was processed for flow cytometry. (A) BALF cell percentages. (B) BALF cell counts. BALF was stained and analyzed via flow cytometry. Cell populations are represented as the percentage of CD45+ cells. Representative dot plots for each individual exposure are shown. Eosinophils are stained with CD45+CD11B+SiglecF+Cd11c- (n=4 per experimental group). Neutrophils are stained with CD45+CD11b+Ly6G+SiglecF-CD11c- (n=4 per experimental group). B cells are stained with CD45+CD19+SiglecF+CD11c- (n=4 per experimental group). T cells are stained with CD45+CD3+SiglecF-CD11c- (n=4 per experimental group). * = p < 0.05.