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Exposure to Deepwater Horizon Crude Oil Burnoff Particulate Matter Induces Pulmonary Inflammation and Alters Adaptive Immune Response

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Abstract

The "in situ burning" of trapped crude oil on the surface of Gulf waters during the 2010 Deepwater Horizon (DWH) oil spill released numerous pollutants, including combustion-generated particulate matter (PM). Limited information is available on the respiratory impact of inhaled *in situ* burned oil spill particulate matter (OSPM). Here we utilized PM collected from *in situ* burn plumes of the DWH oil spill to study the acute effects of exposure to OSPM on pulmonary health. OSPM caused dose- and time-dependent cytotoxicity and generated reactive oxygen species and superoxide radicals *in vitro*. Additionally, mice exposed to OSPM exhibited significant decreases in body weight gain, systemic oxidative stress in the form of increased serum 8-isoprostane (8-IP) levels, and airway inflammation in the form of increased macrophages and eosinophils in bronchoalveolar lavage fluid. Further, in a mouse model of allergic asthma, OSPM caused increased T helper 2 cells (Th2), peribronchiolar inflammation, and increased airway mucus production. These findings demonstrate that acute exposure to OSPM results in pulmonary

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ASSOCIATED CONTENT

Supporting Information

Methods for cytotoxicity assessment, ROS and superoxide radical assay, lung histology, and total IgE estimation in the lung homogenates. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b01439.

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inflammation and alteration of innate/adaptive immune responses in mice and highlight potential respiratory effects associated with cleaning up an oil spill.

Abstract

INTRODUCTION

The Deepwater Horizon explosion in the Gulf of Mexico in 2010 is considered as the largest accidental marine oil spill in global history. An estimated 4.9 million barrels of crude oil were released into the Gulf waters.¹ The released crude oil was contained from spreading in the Gulf waters by several methods, one of which was by means of surface booms and controlled burning of contained oil termed as “*in situ*” burning. Approximately 411 *in situ* burning operations were conducted to remove an estimated 5% of the total crude oil released.¹ Though *in situ* burning facilitates rapid removal of oil from the surface of water, it releases numerous pollutants including PM into the surrounding atmosphere. In this particular burn, the oil was corralled by booms being pulled in parallel from two boats. The collected surface oil was then ignited by igniter boats via an incendiary starter charge containing gelled diesel in a plastic container and equipped with a foam flotation and road flare (Gullett et al., unpublished observation). The heat stress experienced by the personnel limited them from wearing proper protective respiratory equipment during the burning activities and many days involved multiple simultaneous burns with as many as 16 *in situ* burns conducted simultaneously on a single day burning as much as 50,000–70,000 barrels of oil. The collected oil was burned for times varying from minutes to hours depending on the sea/wind conditions. The longest of the burns took 11 h. and 48 min to dissipate (On Scene Coordinator Report Deepwater Horizon Oil Spill, 2011).

During the initial period of cleanup, reports surfaced in which cleanup participants reported several lower respiratory tract problems such as wheezing, tightness in the chest, shortness of breath, itchy or runny nose and throat lasting at least 3 days.² Oil spill cleanup participants are often exposed to oil fumes, volatile organic compounds (VOCs), and/or combustion products from *in situ* burning of crude oil.³ Reports from previous oil-spill cleanup participants have indicated the persistence of respiratory symptoms such as bronchial hyperresponsiveness and increased levels of oxidative stress marker 8-isoprostane (8-IP) and vascular endothelial growth factor in exhaled breath of participants.^{4,5} However,

to our knowledge, no study has investigated whether exposure to PM from *in situ* burning of crude oil has a potential to cause or exacerbate respiratory health effects. Combustion derived PM such as diesel exhaust particles (DEPs) are known to promote a T helper 2 (Th2) biased immunologic responses, usually associated with allergic asthma. Indeed, in the presence of allergens, DEPs were shown to exacerbate Th2 biased immunologic responses.^{6,7} The residual carbonaceous components of DEPs are known to contribute to oxidative stress due to the redox potential of quinone radicals.⁸ The initial chemical characterization studies of the particulate emissions from *in situ* burns of BP crude oil showed the PAHs accounted for roughly 4.5 mg/kg of oil burned (Gullett et al., unpublished observations). The presence of PAHs suggests a similar ability to induce oxidative stress.

Epidemiological evidence of association between exposure to particulate air pollution and adverse respiratory health effects has been well documented.^{9–11} Our previous studies using animal models of asthma have shown that exposure to combustion derived PM affects pulmonary immunologic homeostasis and leads to adverse respiratory health effects such as asthma.^{12–14} Further, several studies have demonstrated the role of PM-induced oxidative stress in the exacerbation of asthma.¹⁵ Though there are numerous studies suggesting the risk of acute respiratory health effects due to exposure to particulate pollution,¹⁶ very few studies have specifically addressed the respiratory health effects due to exposure to *in situ* burned oil particulate matter (OSPM). Airborne pollutants such as residual oil fly ash are shown to enhance allergic pulmonary inflammation and exacerbate lung disease indicating air borne burnoff pollutants as important risk factors for asthma.¹⁷ Though limited information is available on the health effects of crude oil spills, epidemiological studies have shown increased prevalence of acute and prolonged respiratory health effects in oil spill cleanup workers and residents closer to the spill area.^{4,18} Epidemiological studies on respiratory health effects of previous crude oil spills have demonstrated increased prevalence of asthma in residents and children living near the spill area suggesting that exposure to crude oil spill is a risk factor for asthma.¹⁹

In the present study, we sought to determine the respiratory health effects associated with inhalation of PM generated as a result of controlled burning of spilled crude oil (OSPM). We tested whether acute exposure to OSPM induces oxidative stress and alters pulmonary immunologic responses to allergens. We determined the free radical content of OSPM particles and estimated their ability to induce oxidative stress *in vitro*. Additionally, we tested whether acute exposure to OSPM exacerbates pulmonary innate and adaptive immune responses in a mouse model of allergic asthma. Results from our studies demonstrated that exposure to OSPM results in systemic oxidative stress, pulmonary inflammation, and altered innate and adaptive pulmonary immune responses. Combining OSPM exposure with allergen challenge resulted in increased peribronchiolar inflammation and airway mucus production. This was accompanied by significant increase in Th2 responses, a hallmark of asthma. Taken together, these studies identify the respiratory health effects of acute exposure to OSPM in mouse models and suggests that exposure to OSPM may induce adverse respiratory health effects among cleanup participants, especially those with preexisting allergic asthma.

MATERIALS AND METHODS

Oil Sail Particulate Matter (OSPM)

The sample of OSPM was collected from black, particle-laden smoke plumes from incomplete combustion of crude oil. OSPM particles were collected by maneuvering an aerostat-lofted sampler into the *in situ* burn plumes of the DWH oil spill from a ship-mounted winch as described by Aurell et al.²⁰ PM filters were collected from a total of 27 oil burn plumes and the average concentration of PM content as measured by carbon balance method from a total of five composite over a period of 4 days was found to be 0.088 kg/kg carbon burned. PM contributed to 10% of total carbon emitted by mass and over 80% of the PM mass was elemental carbon.²¹

Cytotoxicity Assessment

OSPM particles were tested for cytotoxic effect using Alamar blue reagent (Life Technologies, Grand Island, NY). A detailed method is described in the Supporting Information.

Reactive Oxygen Species (ROS) and Superoxide Radicals Assay

OSPM particles were tested for generation of ROS and superoxide radicals using BEAS-2B cells following manufacturer's instructions. A detailed method is described in the Supporting Information.

Animals and Treatment

Female BALB/c mice (aged 6–8 weeks) were purchased from Harlan (Indianapolis IN). All animals were housed in ventilated cages and supplied with filtered air in a specific pathogen free environment. Mice had free access to food and water under controlled conditions with 12 h light/dark cycle, temperature, and humidity. All animal protocols were prepared in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by the LSU and UTHSC Institutional Animal Care and Use Committee. OSPM particles were suspended at a concentration of 1 mg/mL in irrigation saline (Vehicle) containing 0.02% Tween-80 and were subsequently subjected to probe sonication to disperse the particles from forming aggregates. Each mouse received 50 µg OSPM on protocol days 1, 3, and 5 via oropharyngeal aspiration (OA) as described previously.¹² In our studies, this has proven to be equivalent to an inhalation exposure of 230 µg/m³ for 30 min.¹⁴ We chose OA as the OSPM delivery method over inhalation route for administration of the particles due to limited availability of OSPM samples and to ensure accuracy of dosing. Vehicle and OVA group mice were exposed to the same volume of vehicle.

Serum 8-Isoprostane

Blood was collected 48 h after the last exposure of OSPM and was allowed to clot for 30 min. Serum was isolated by centrifugation and flash frozen in liquid nitrogen and stored at –80 °C until further analysis. Serum samples were saponified and subjected to solid phase extraction by using established methods as described earlier,²² and the level of 8-IP, a

marker of oxidative stress, was quantified by ELISA (Cayman Chemicals, Ann Arbor, MI) following the manufacturer's instructions.

Bronchoalveolar Lavage Fluid Cellularity

Bronchoalveolar lavage (BAL) was performed by flushing lungs with 1 mL of PBS containing 0.5% Bovine Serum Albumin (BSA). An aliquot of 200 μ L of isolated BAL fluid was used to count the total number of cells. A volume of BAL fluid equivalent to 20,000 cells was centrifuged onto glass slides using cytopsin. Subsequently, the slides were allowed to air-dry and stained using a Hema-3 staining kit (Fisher Scientific, Pittsburgh, PA) following manufacturer's instructions. Differential counts of cells were determined by an unbiased observer from a total of 20,000 cells per slide based on the morphology and the staining of the cells.

Mouse Model of Allergic Asthma

Mouse model of asthma was established by sensitizing and challenging the mice with chicken egg white ovalbumin (OVA). Briefly, mice were divided into Vehicle, OSPM, OVA, and OVA+OSPM groups ($n = 5-6$ per group). The OVA and OVA+OSPM group mice were sensitized with a mixture of 20 μ g of OVA (Sigma-Aldrich, St. Louis, MO) emulsified in Imject Alum (Pierce, Rockford, IL) via i.p. on protocol days 0 and 7. Subsequently, mice were challenged with a solution of 1% OVA in saline on protocol days 15, 16, and 17 and rechallenged on days 30, 31, and 32 by inhalation exposure for 20 min. OVA+OSPM group mice were exposed to 50 μ g of OSPM via OA route on protocol days 14, 16, and 18 (Figure 4A).

Assessment of Pulmonary T Cell Populations

Mice were euthanized, and blood was gently flushed from the lungs to remove excess red blood cells by retrograde vascular perfusion with isotonic saline. A single-cell suspension of lung cells was prepared using collagenase digestion and physical manipulation as described earlier.^{23,24} Briefly, perfused lungs were excised, cut into small pieces, and collected in chilled Hank's Balanced Salt solution (HBSS) containing 1 mg/mL collagenase and 150 ng/mL DNase I (Sigma-Aldrich, St. Louis, MO) and mechanically dissociated using Octodissociator (Miltenyi, Germany). Dissociated lungs were incubated at 37 °C with continuous shaking at 200 rpm for 30 min. Following incubation, lungs were dissociated a second time in the octodissociator, and single cell suspension was obtained by filtering the cell suspension through a 40 μ m cell strainer. The resulting cell suspension was treated with RBC lysis buffer to remove residual RBCs. A total of 1.2 million cells from each sample were stimulated with 5 ng/mL phorbol 12-myristate 13-acetate (PMA) and 500 ng/mL ionomycin (Sigma-Aldrich) in the presence of a protein transport inhibitor (GolgiPlug, BD Biosciences). Cells were subsequently fixed, permeabilized, and stained with eFluor450-CD3, PerCP-CD4, FITC-CD8, PE-IFN- γ and PE-Cy7-IL-4, APC-IL-17A, Fixable live/dead dye eFluor 780 (eBiosciences, San Diego, CA) was used to stain dead cells. After the staining procedure, cells were immediately analyzed by FACS Canto II (BD Biosciences, San Jose, CA). Dead cells were excluded from analysis. Flowcytometry data was analyzed using FlowJo software v.7.6.5 (Tree Star, OR).

Lung Histology

Our methods used for lung histopathology are described in the Supporting Information.

Statistical Analysis

All results were expressed as mean $\pm$ SEM and were analyzed using GraphPad Prism 6 software (GraphPad Software Inc., Version 6.03, CA). Two-way ANOVA with Bonferroni post-test, one way ANOVA with Dunnett's multiple comparisons test, unpaired *t* test, and multiple *t* tests with Holm-Sidak correction were used to determine statistical difference between groups. *p* values less than 0.05 were considered as statistically different.

RESULTS

Electron Paramagnetic Resonance Spectra of OSPM Particles

OSPM particles were analyzed for the presence of free radicals using EPR spectroscopy. A signal for the presence of paramagnetic center in the particles was observed with a H_{pp} of 5.669 G (Figure S1). The *g* value for the OSPM particles was found to be ~ 2.0034 . The *g* value indicated that OSPM predominantly consisted of phenoxyl type radicals. The radical concentration for the OSPM sample was 4.25×10^{16} radicals/g.

OSPM Caused Cytotoxic Effect in BEAS-2B Cells

The cytotoxic effect of OSPM particles was determined using BEAS-2B cells over a range of concentrations (0.97 to 31.24 $\mu\text{g}/\text{cm}^2$). The percentage viability of BEAS-2B cells decreased in a time and dose-dependent manner after treatment with OSPM particles (Figure 1). The lowest concentration at which cytotoxicity was observed was 7.81 $\mu\text{g}/\text{cm}^2$.

OSPM Induces Generation of ROS and Superoxide Radical Species

PM containing free radicals is known to generate reactive oxygen species *in vitro*²⁵ and *in vivo*.^{13,23} OSPM particles caused a significant dose-dependent increase in ROS at 20, 50, and 100 $\mu\text{g}/\text{cm}^2$ (Figure 2A). Significant generation of superoxide radicals was observed at 50 and 100 $\mu\text{g}/\text{cm}^2$ in BEAS-2B cells compared to untreated control (Figure 2B).

Effect of OSPM Exposure in Vivo

Exposure to PM is known to cause morbidity in the form of decrease in body weight gain.²³ Exposure to OSPM caused a significant decrease in body weight gain at 48 h after last exposure (Figure 3B). In addition, exposure to radical containing PM has been shown to induce systemic oxidative stress.^{13,24} To study whether exposure to OSPM induced systemic oxidative stress in mice, we studied indicators of oxidative stress such as 8-IP in the serum. Serum 8-IP levels were significantly increased at 48 h after the last OSPM exposure in mice compared to vehicle control (Vehicle: 106.9 ± 13.51 vs OSPM: 207.2 ± 38.93) (Figure 3C).

Effect of OSPM Exposure on Pulmonary Innate and Adaptive Immune Responses in a Mice Model of Asthma

To study whether exposure to OSPM exacerbated pulmonary inflammation, we quantified total and differential cell counts in bronchoalveolar lavage fluid (BALF). We found a

significant increase in the total number of cells in OSPM mice compared to vehicle control, which appeared to be due to significant increases in total number of macrophages and eosinophils (Figure 4B). Asthma is characterized by a Th2 biased immune response.²⁶ To understand whether exposure to OSPM has any effect on altering the asthma response, we studied the effect of OSPM on pulmonary T cell responses in a mice model of asthma at 48 h after the final exposure to OVA. A significant increase in adaptive T cell responses (Th1, Th2, and Th17) was observed in mice in the OVA and OVA+OSPM groups. However, exposure to OSPM caused a significant increase in the percentage of Th2 cells (OVA: 1.58 ± 0.24 vs OVA+OSPM: 2.57 ± 0.08) and a decreased percentage of Th1 cells (OVA: 4.68 ± 0.24 vs OVA+OSPM: 3.72 ± 0.05) (Figure 4C and S4) in OVA+OSPM exposed mice compared to OVA exposed mice. Although the data reproducibly suggested a decrease in Th17 responses in the OVA+OSPM mice, we were unable to demonstrate a statistical difference between these mice and OVA mice. Total IgE concentrations in the lungs of both OVA and OVA+OSPM groups were comparable. The concentration of IgE was significantly elevated in OVA+OSPM group compared to vehicle or OSPM exposed mice. Though the concentration of IgE was increased in OVA group mice, it is not statistically significant compared to the OSPM group (Figure S5).

OSPM Exacerbates Pulmonary Inflammation and Mucus Production in a Mouse Model of Asthma

To study whether exposure to OSPM caused any alteration in pulmonary inflammation and mucus production caused by OVA challenge, we studied pulmonary inflammation after exposure to OSPM in a mouse model of asthma. Lung histopathology showed a significant increase in inflammatory cell infiltration in the peribronchiolar region characterized by the presence of increased numbers of eosinophils (Figure 5A). Also, PAS staining demonstrated a significant increase in mucus production in the pulmonary bronchioles of OVA+OSPM mice as characterized by an increased percentage of PAS+ cells in OVA+OSPM mice compared to OVA mice (Figure 5B, 5C). Alveolar macrophages with engulfed OSPM were observed adjacent to the airways in both OSPM and OVA+OSPM group mice even at 20 days after exposure suggesting a delayed pulmonary clearance of these particles. Figure 5D shows a PAS stained photomicrograph of lung section containing macrophages laden with engulfed OSPM.

DISCUSSION

The association between exposures to elevated levels of PM from combustion processes and adverse respiratory health effects resulting in enhanced morbidity and mortality rates has been well documented,^{27–29} although the mechanisms responsible are not fully understood. Crude oil smoke is a mixture of volatile organic compounds (VOCs) and PM. Though the volatile constituents of crude oil are known to cause respiratory symptoms, the PM generated from burning crude oil may also elicit adverse respiratory events. The air quality monitoring data acquired by the EPA during the *in situ* burns demonstrated that in certain places PM₁₀ levels reached as high as 944 µg/m³ and the PM_{2.5} levels were as high as 213 µg/m³.³⁰ The EPA's health based National Ambient Air Quality (NAAQ) average annual

and daily level standards for PM_{2.5} are 12 µg/m³ and 35 µg/m³, respectively, and the daily standard for PM₁₀ is 150 µg/m³.³¹

The typical *in situ* burn operation of crude oil involves containment of the oil to achieve burnable thickness and is then ignited by means of a Heli-torch ignition system containing gelled gasoline.³² The smoke emitting from the crude oil combustion is a mixture of several gases and particulate matter. In addition, small amounts of toxic gases and polycyclic aromatic hydrocarbons are emitted from the burn.³³ Experimental results from Newfoundland off shore *in situ* burn studies showed that the concentration of particulates exceeded more than 150 µg/m³ at several places 10 miles down wind of the burn.³³ *In situ* burning of surface oil reduces coastal and marine environmental threat by rapid removal of surface oil. However, the largest disadvantage is the emissions from incomplete combustion of the oil and release of large volumes of combustion derived particulate matter, thus posing potential exposure conditions to the personnel involved in the cleanup activities and people in the vicinity. Though *in situ* burning of DWH crude oil on the surface of the seawater is different from oil well fires, it is expected that smoke from both the fires will both produce vaporization of VOCs - the primary risk is chemicals associated with the soot generated in the fire. Indeed, the soot was shown to contain incomplete combustion products including transition metals, salts, and organics and generate compounds like polychlorinated dibenzofurans (PCDDs/PCDFs)²⁰ and, as shown here, PM containing environmentally persistent free radicals (EPFRs), which are individually and collectively of potential health concern. In general, the median particulate size in the smoke of oil burns was estimated to be 0.5 µm.³⁴ Response personnel on the boats pulling the booms or on the igniter boats were not wearing respiratory protective devices; thus, exposure to OSPM represented a definite hazard to the respiratory system. Though studies showed that the concentration of PM₁₀ near burn sites would be below the permissive levels (8 h mean for PM₁₀ is 5 mg/m³),³⁴ the high permissive level does not preclude the fact that high levels were observed or that respiratory effects may still may be observed at lower levels (especially in susceptible populations).³⁴ There is little data concerning the effect of smoke from crude oil *in situ* burns on humans. Some of the previous epidemiological studies on Gulf war veterans exposed to oil well fires demonstrate an association between exposure to PM from crude oil well smoke and asthma³⁵ and a higher prevalence of reduced forced vital capacity.³⁶ Since PM from crude oil smoke is a complex mixture, it is difficult to attribute the observed respiratory health effects to specific constituents. However, PM is a major constituent of the smoke, and since PM is known to affect respiratory health, here we tested the effect of acute exposure to inhaled *in situ* burned oil PM (OSPM). The PM was collected from the smoke plumes during the cleanup of the oil on the Gulf surface resulting from the DWH.

Though epidemiological evidence of association between exposure to combustion derived PM and respiratory health effects exist, very few studies have actually addressed the mechanism responsible for such effects, and fewer studies have evaluated their effect in the exacerbation of asthma using animal models. A recent study has demonstrated that PM from residual oil fly ash exacerbates allergic airway inflammation using mice models.¹⁷ Our primary objective in this study was to understand the possible respiratory health consequences of acute exposure to OSPM from DWH oil spill using *in vitro* and *in vivo*

approaches. As expected, we observed the presence of EPFRs in OSPM and the EPR spectral signature indicated that they are predominantly phenoxyl type of radicals (Figure S1). Previously, phenoxyl-type radicals generated as a result of combustion processes were shown to be stabilized and resistant to oxidation and persist in the environment for long periods of time.^{37,38} Further, these EPFRs have the ability to produce ROS in biological systems due to their redox potential.³⁹ Our studies demonstrated that, *in vitro*, OSPM induced both reactive oxygen species and superoxide free radicals indicative of their ability to induce oxidative stress. Further, OSPM caused cytopathic effects on human bronchial epithelial cells in a dose dependent manner (Figure 1). Oxidative stress is known to play an important role in the pathogenesis of asthma⁴⁰ and augmentation of allergen induced lung inflammation.⁴¹ Moreover, exposure to ultrafine carbon particles is shown to exert an adjuvant effect on allergen induced airway inflammation.^{42,43} 8-IP, a stable byproduct of lipid peroxidation, is a known indicator of oxidative stress, and airborne PM has been shown to induce oxidative stress leading to lipid peroxidation and increased serum levels of 8-IP.⁴⁴ A significant increase in serum 8-IP levels was observed in mice exposed to OSPM at 48 h post-exposure, indicating a systemic oxidative stress response.

Despite our knowledge that particulate pollutants can interact with both innate and adaptive immune responses and exacerbate allergic airway disease, the mechanisms involved are not fully understood.⁴⁵ Using a mice model of allergic asthma, we further examined the role of these particles in altering pulmonary immunologic homeostasis and driving allergic airway inflammation and lung dysfunction. Asthma is typically characterized by an imbalance between Th1 and Th2 immune response,⁴⁶ and oxidative stress has been shown to play a role in favoring differentiation and polarization of CD4⁺ lymphocytes toward Th2 phenotype.⁴⁷ Also, our previous studies showed oxidative stress as a result of exposure to combustion derived PM was associated with increased maturation of dendritic cells and Th17 immune response. Our data demonstrated that prior exposure to OSPM enhanced adaptive, pulmonary T cell responses, specifically increasing the Th2 cells and decreasing Th1 cells in mice exposed to OSPM and challenged with allergen (OVA) compared to mice that were challenged with just OVA, thus leading to a Th2 biased immune response. However, no difference in Th17 responses was observed. These observations are consistent with other studies using DEP, where oxidative stress was found to favor a Th2 skewed immune response while suppressing the Th1 response.^{48–50} Consistent with these observations, OVA challenged mice exposed to OSPM exhibited significantly greater peribronchiolar inflammation compared to OVA exposed mice. Since inflammation was present in the BALF at 5 days after exposure to OSPM, it is possible that it resolved prior to T cell phenotype and histological analysis. Further, the presence of OSPM particles and the resulting oxidative stress due to OSPM exposure during OVA challenge might have favored the Th2 response observed. The increase in the airway mucus production in OVA+OSPM compared to OVA group mice is consistent with a Th2 biased response. Thus, our results indicate that OSPM synergizes with OVA and exacerbates OVA induced pulmonary pathophysiology.

In conclusion, our data suggests that exposure to OSPM causes systemic and local oxidative stress and pulmonary inflammation in the form of increased numbers of macrophages and

eosinophils in the lungs of mice exposed to OSPM. Also, in a mouse model of asthma, OSPM exacerbated allergic asthma response by significantly increasing the Th2 biased immune response to OVA as evidenced by increased peribronchiolar inflammation and airway mucus production in the lungs. These responses may be attributed partially to oxidative stress induced by these particles. It is unclear if the respiratory health of cleanup participants was affected; however, our results provide valuable information to understand the possible health effects observed as a result of acute exposure to OSPM, especially in those with preexisting pulmonary conditions.

LIMITATIONS

Due to limited sample, our study had limitations including the fact that we performed only acute exposures at one dose mimicking high PM_{2.5} levels recorded by the EPA and our route of exposure bypassed the nasal passages. We are also aware that potential human health risks would be best determined by determining the amount of actual inhaled OSPM, the duration of exposure, and repeating these studies with those parameters in mind. Moreover, further studies are required to understand the long-term respiratory health effects of exposure to OSPM.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Dose and time dependent decrease in percent viability of BEAS-2B cells following exposure to OSPM. BEAS-2B cells were exposed to OSPM particles (7.81, 15.62, and 31.24 $\mu\text{g}/\text{cm}^2$), and cell viability was assessed 30 and 60 min post-exposure. Data are expressed as means $\pm$ SEM of percentage viability. * $p < 0.05$ vs media exposed control; two-way ANOVA with Bonferroni post-test.

Figure 2.

OSPM generates ROS (A) and superoxide free radicals (B) in vitro. Cells were treated with either vehicle (medium) or OSPM at concentrations 10, 20, 50, and 100 $\mu\text{g}/\text{cm}^2$ for 1 h prior to the addition of ROS and superoxide radical detection reagent. Data are expressed as means $\pm$ SEM of fluorescence intensity ($n = 6$). * $p < 0.05$ compared to vehicle control. One way ANOVA with Dunnett's multiple comparisons test.

Figure 3.

OSPM decreases body weight gain and induces oxidative stress *in vivo*. (A) Schematic of exposure protocol. (B) Body weight gain analysis at 7 days post-exposure. (C) Exposure to OSPM induces systemic oxidative stress. 8-IP levels were measured in the serum of OSPM and vehicle exposed mice at 6 days after initial exposure. Data plotted as mean $\pm$ standard error of mean (SEM). * $p < 0.05$ vs vehicle exposed control; unpaired t test for both (B) and (C).

Figure 4.

OSPM enhances pulmonary inflammation in a mouse model of asthma. (A) Schematic of exposure protocol to OSPM and induction of mouse model of asthma. (B) Total cell numbers in BAL fluid and differential counts for macrophages (Mac), lymphocytes (Lym), neutrophils (Neu), and eosinophils (Eos) in vehicle and OSPM exposed mice at 5 days after exposure. (C) Pulmonary adaptive T cell responses as determined on protocol day 34 and quantification using flow cytometry. Data are expressed as percentage of CD4⁺ cells. * $p < 0.05$ indicates significant difference between vehicle control and treatment groups, and # $p < 0.05$ indicates significant difference between OVA and OVA+OSPM groups. Multiple comparisons were performed by multiple t tests with Holm-Sidak correction.

Figure 5.

OSPM enhances pulmonary inflammation and airway mucus production in a mouse model of asthma. (A) Representative photomicrographs of histological sections of mice lungs stained with hematoxylin and eosin. Black arrow heads point to peribronchiolar inflammation. Scale bar = 100 μ m. (B) Representative photomicrographs of mouse lung histological sections stained with Periodic Acid-Schiff (PAS) for mucus. Black arrow heads point to mucus producing goblet cells (stained bright magenta color) in the airways. (C) Quantified, percent mucus-positive cells to total epithelial cells in the airways. * $p < 0.05$,

indicates significant difference compared to OVA exposed mice. (D) Photomicrograph of mouse lung exposed to OSPM and challenged with OVA. White arrow heads point to alveolar macrophages with engulfed OSPM particles. Scale bar = 50 μm .