Original Article

Sediment from Hurricane Katrina: Potential to Produce Pulmonary Dysfunction in Mice

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Abstract: On August 29, 2005, Hurricane Katrina made landfall along the Gulf Coast as a Category 3 hurricane. The associated storm surge and heavy rainfall resulted in major flooding throughout the New Orleans area. As the flood waters receded, thick sediment was left covering the ground and coating the interior of homes. This sediment was dispersed into the air and inhaled as dust by returning residents and workers. Our objective in this study was to evaluate the potential pulmonary effects associated with the respirable particulate matter (PM) derived from Hurricane Katrina (HK-PM) in mice. Samples of PM were collected from several locations along the Gulf Coast on September 30 and October 2, 2005 and had a mean aerodynamic diameter ranging from 3-5 µm. Chemical analysis and cytotoxicity assays were performed for all HK-PM samples. A few samples with varying levels of cytotoxicity were chosen for an acute inhalation exposure study. Airborne PM10 levels recorded in the New Orleans area post-Katrina were variable, ranging from 70 µg/m3 in Gentilly to 688 µg/m3 in Lakeview (residential areas). Mice exposed to one of these samples developed significant pulmonary inflammation and airways resistance and hyperresponsiveness to methacholine challenge. These studies demonstrate that dispersion of certain Katrina sediment samples through either natural (e.g., wind) or mechanical (e.g., vehicles) processes promotes airflow obstruction in mice.

Key Words: Hurricane Katrina, pulmonary dysfunction, respiratory toxicology

Introduction

Hurricane Katrina was one of the strongest hurricanes seen in the United States in the past 100 years. Striking the gulf coastal regions of Louisiana on August 29, 2005, Hurricane Katrina resulted in one of the worst natural disasters in United States history. The city of New Orleans, Louisiana was further damaged by severe flooding when major levees broke and released water from Lake Pontchartrain. The infrastructure of the city including electrical transmission, water, and sewage services were destroyed. The residents were evacuated to neighboring states and cities. Although two and a half years have passed since Hurricane Katrina, the potential public health risks caused by the biological and chemical contaminants dispersed in the floodwaters remain unresolved.

Flood waters left from the breached levees/canals deposited a layer of sediment, sometimes half-a-foot in depth, onto the ground and inside many homes. On September 30 and October 2, 2005, Subra Company and Altamont Environmental Inc. collected samples of sediment at 18 locations along the coast of the Gulf of Mexico. The
sampling was conducted as part of an effort to assess potential biological and chemical contamination resulting from the hurricane. Chemical analysis revealed that some of these sediments contained heavy metals such as arsenic and several kinds of semi-volatile organic compounds (SVOCs) at levels exceeding the Environment Protection Agency (US EPA) and Louisiana Department of Environmental Quality (DEQ) standards (Tables 1-4). The minimum average PM 10 data recorded in the New Orleans area (i.e., 35 μg/m3) post-Katrina; although PM 10 levels often peaked at >100 μg/m3 with 688 μg/m3 being recorded in the Lakeview area of New Orleans (a major site of debris cleanup) [1]. Dispersion of dried sediment either through natural (e.g., wind), mechanical (e.g., vehicles), or thermal (e.g., combustion of sediment covered debris) processes is occurring and may elicit harmful effects on the pulmonary health of residents and workers. To date, however, the effect of aerosolized dust created from these sediments on pulmonary function remains unknown.

The purpose of this study was to perform a preliminary assessment of the short-term impact of exposure to inhaled sediment on pulmonary function. We found that exposure to sediment sample-12 (SS-12) resulted in increased pulmonary inflammation, airway resistance, and distinct histopathologies in mice. These events correlated with an increase in various markers of oxidative stress and suggest that oxidative stress may, in part, be responsible for these observed effects in mice.

Materials and Methods

Materials

Sediment samples (SS) were collected by Altamont Environmental, Inc. and Subra Company on September 30 and October 2, 2005 from various sites along the coast of the Gulf of Mexico including schools, residential, and commercial areas. These samples were then supplied to our laboratory and stored at -80C until use. SS-12 was collected from a residential area in the New Orleans metropolitan area (near the center of St. Ferdinand Street, 100 feet south of Higgins Boulevard and approximately 15 feet east of the fence enclosing the Agriculture Street NPL; http://maps.google.com/maps/ms?hl=en&gl=us&ie=UTF8&om=0&t=h&msa=0&msid=11234760537107176535.000447ccc3ef0d8a2d&ll=30.378086,89.268658&spn=0.003332,0.007167&z=18). SS-C was collected from the grounds of the DeLisle Elementary School, MS (approximately 50 feet south of a portable classroom and 200 feet west of West Wittman Road; http://maps.google.com/maps/ms?hl=en&gl=us&ie=UTF8&om=0&t=h&msa=0&msid=11234760537107176535.000447ccc3ef0d8a2d&ll=29.989044,90.039182&spn=0.013548,0.028667&om=1&msa=0). SS-C was collected from the grounds of the DeLisle Elementary School, MS (approximately 50 feet south of a portable classroom and 200 feet west of West Wittman Road; http://maps.google.com/maps/ms?hl=en&gl=us&ie=UTF8&om=0&t=h&msa=0&msid=11234760537107176535.000447ccc3ef0d8a2d&ll=29.989044,90.039182&spn=0.013548,0.028667&om=1&msa=0).

Chemical & biological characterization of sediment samples

The chemical and microbial composition of the sediment samples was analyzed by Pace Analytical Services in Asheville, North Carolina using the following methods; Volatile Organic Compounds (VOCs) by US EPA Method 8260; SVOCs by US EPA Method 8270; the eight Resource, Conservation, And Recovery Act metals by various US EPA Methods in the 6000/7000 series; dioxins/furans by US EPA 8290; and microbiology by standard methods.

SS-12, -13, and -C were tested for endotoxin using the Limulus Amebocyte Lysate PYROGENT Plus assay (Cambrex) as per the manufacturer’s instructions. Reagent blanks and a 6-point standard curve using control standard endotoxin were assayed in the same manner as the samples. Lysate sensitivity was calculated at 0.09 endotoxin units (EU) / ml. Endotoxin content of SS-12, -13, and -C were all below the level of sensitivity for the assay at the doses used. More than 82.8% of the particles had a particle size of 1-3 µm, with a size distribution of 0.3-10 µm (AeroTrak 8220, TSI).

Redox-active compounds catalyze the reduction of oxygen to superoxide by DTT,
which is oxidized to its disulfide [2]. The remaining thiol is allowed to react with DTNB, generating the mixed disulfide and 5-mercapto-2-nitrobenzoic acid. The amount of 5-mercapto-2-nitrobenzoic acid formed is then determined by its absorption at 412 nm. The sediment sample-dependent DTT consumption is measured under conditions such that the rate is linear, i.e., when less than 20% is depleted. Catalytic activity is expressed as the rate of DTT consumption less that observed in the absence of sediment sample and plotted as % DTT consumed.

Table 1. Metals analytical results

<table>
<thead>
<tr>
<th>Sample</th>
<th>Collection date (mm/dd/yy)</th>
<th>Matrix</th>
<th>Arsenic mg/kg</th>
<th>Barium mg/kg</th>
<th>Cadmium mg/kg</th>
<th>Chromium mg/kg</th>
<th>Lead mg/kg</th>
<th>Selenium mg/kg</th>
<th>Mercury mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS-1</td>
<td>09/30/05 Soil</td>
<td></td>
<td>1.6</td>
<td>15</td>
<td>ND</td>
<td>3.6</td>
<td>7.0</td>
<td>ND</td>
<td>0.015</td>
</tr>
<tr>
<td>SS-2</td>
<td>09/30/05 Soil</td>
<td></td>
<td>0.94</td>
<td>7.3</td>
<td>ND</td>
<td>4.5</td>
<td>3.6</td>
<td>ND</td>
<td>0.012</td>
</tr>
<tr>
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<td>09/30/05 Soil</td>
<td></td>
<td>7.5</td>
<td>14</td>
<td>ND</td>
<td>4.5</td>
<td>78</td>
<td>ND</td>
<td>0.052</td>
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<tr>
<td>SS-4</td>
<td>09/30/05 Soil</td>
<td></td>
<td>35</td>
<td>74</td>
<td>ND</td>
<td>27</td>
<td>140</td>
<td>ND</td>
<td>0.11</td>
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<tr>
<td>ADEM Draft Preliminary Screening Values</td>
<td></td>
<td></td>
<td>0.39</td>
<td>540</td>
<td>30</td>
<td>400</td>
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<td></td>
<td>0.39</td>
<td>5,400</td>
<td>37</td>
<td>30</td>
<td>400</td>
<td>390</td>
<td>NE</td>
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<tr>
<td>SS-6</td>
<td>10/01/05 Soil</td>
<td></td>
<td>11</td>
<td>33</td>
<td>&lt;0.10</td>
<td>21</td>
<td>33</td>
<td>&lt;0.52</td>
<td>0.062</td>
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<tr>
<td>SS-6B</td>
<td>10/01/05 Soil</td>
<td></td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>SS-7</td>
<td>10/01/05 Soil</td>
<td></td>
<td>3.6</td>
<td>27</td>
<td>&lt;0.15</td>
<td>4.2</td>
<td>25</td>
<td>&lt;0.758</td>
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<tr>
<td>SS-8</td>
<td>10/01/05 Soil</td>
<td></td>
<td>10</td>
<td>35</td>
<td>&lt;0.20</td>
<td>2117</td>
<td>27</td>
<td>&lt;0.99</td>
<td>0.050</td>
</tr>
<tr>
<td>SS-9</td>
<td>10/01/05 Soil</td>
<td></td>
<td>11</td>
<td>26</td>
<td>&lt;0.099</td>
<td>19</td>
<td>29</td>
<td>&lt;0.50</td>
<td>0.016</td>
</tr>
<tr>
<td>SS-10</td>
<td>10/01/05 Soil</td>
<td></td>
<td>11</td>
<td>64</td>
<td>&lt;0.10</td>
<td>15</td>
<td>22</td>
<td>&lt;0.52</td>
<td>0.016</td>
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<tr>
<td>Tier 1 Target Remediation Goals - Restricted</td>
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<td></td>
<td>3.82</td>
<td>14,300</td>
<td>1,020</td>
<td>381</td>
<td>1,700</td>
<td>1,020</td>
<td>61.3</td>
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<td>Tier 1 Target Remediation Goals - Unrestricted</td>
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<td>0.426</td>
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<td>227</td>
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<td>391</td>
<td>10</td>
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<td>0.39</td>
<td>5,400</td>
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<td>400</td>
<td>390</td>
<td>NE</td>
</tr>
<tr>
<td>SS-12</td>
<td>10/01/05 Soil</td>
<td></td>
<td>13</td>
<td>220</td>
<td>1.6</td>
<td>11</td>
<td>53</td>
<td>&lt;1.0</td>
<td>0.054</td>
</tr>
<tr>
<td>SS-13</td>
<td>10/01/05 Soil</td>
<td></td>
<td>6.6</td>
<td>200</td>
<td>&lt;0.097</td>
<td>9.4</td>
<td>17</td>
<td>&lt;0.48</td>
<td>0.051</td>
</tr>
<tr>
<td>SS-C</td>
<td>10/01/05 Soil</td>
<td></td>
<td>0.9</td>
<td>11</td>
<td>&lt;0.092</td>
<td>0.81</td>
<td>5.0</td>
<td>&lt;0.46</td>
<td>0.016</td>
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<tr>
<td>LDEQ RECAP Standards for Soil</td>
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<td></td>
<td>12</td>
<td>550</td>
<td>3.9</td>
<td>23</td>
<td>400</td>
<td>39</td>
<td>2.3</td>
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<tr>
<td>EPA Region VI Human Health Medium-Specific Screening Levels</td>
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<td></td>
<td>0.39</td>
<td>5,500</td>
<td>39</td>
<td>30</td>
<td>400</td>
<td>390</td>
<td>NE</td>
</tr>
</tbody>
</table>

Notes: 1) Metals analysis by USEPA method 6010 with the exception of Mercury by USEPA methods 7471. 2) LDEQ RECAP Standards for Soil taken from “Table 2: Management Option 1, Standards for Soil, Non-Industrial Soil” Louisiana Department of Environmental Quality, Risks Evaluation/Corrective Action Program, October 20, 2003. 3) EPA Region VI Human Health Medium-Specific Screening Levels taken from “Region 6 Human Health Medium-Specific Screening Levels 2004-2005 Table, Residential Soil” 12/21/2004. 4) Hexavalent Chromium used for Soil Standards and Screening Level. 5) Bold numbers indicate concentrations above applicable screening levels and/or remediation goals. 6) NA: not analyzed. 7) NE: chemical Not Established in applicable screening levels and/or remediation goals. ND: not detected.

Cellular cytotoxicity

Human epithelial cells (HEp2; ATCC # CCL-23) were plated at a concentration of 5000 cells per well on Costar 96-well plates and allowed to attach overnight. Sediment samples were
suspended in tissue culture media at 30 and 300 μg/ml, added to the cells, and allowed to incubate for 24 h. All samples were pretreated with primocin (Invivogen, San Diego, CA), an antimicrobial reagent active against both Gram-positive and Gram-negative bacteria, mycoplasma and fungi; and the cytotoxicity assay preformed in the presence of this antimicrobial reagent using the CyQuant Kit (Invitrogen) according to manufacturer’s directions. All samples were compared to tissue culture media (positive control; 100% viable) and 0.1% saponin (negative control; 0% viable). Exposures were for 24 hours, and the resulting sample data was graphed as a percentage of viable cells.

Mice

Male BALB/c mice (8-12 weeks of age) were purchased from the Division of Laboratory Animal Medicine (School of Veterinary Medicine, LSU). All mice were maintained in ventilated micro-isolator cages housed in a specific, pathogen-free animal facility. Animal protocols were prepared in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at Louisiana State University.

Table 2. Semivolatile organic compounds (SVOCs) analytical results

<table>
<thead>
<tr>
<th>Sample</th>
<th>Collection date (mm/dd/yy)</th>
<th>Matrix</th>
<th>Benz[ka]fluoranthene μg/kg</th>
<th>Benz[eb]fluoranthene μg/kg</th>
<th>Benz[a]anthracene μg/kg</th>
<th>Benz[pyrene] μg/kg</th>
<th>Chrysene μg/kg</th>
<th>Fluoranthene μg/kg</th>
<th>Indeno[1,2,3-cd]pyrene μg/kg</th>
<th>Phenanthrene μg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS-1</td>
<td>09/30/05 Soil</td>
<td>&lt;390</td>
<td>&lt;390</td>
<td>ND</td>
<td>ND</td>
<td>&lt;390</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SS-2</td>
<td>09/30/05 Soil</td>
<td>&lt;350</td>
<td>&lt;350</td>
<td>ND</td>
<td>ND</td>
<td>&lt;350</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SS-3</td>
<td>09/30/05 Soil</td>
<td>450</td>
<td>420</td>
<td>ND</td>
<td>ND</td>
<td>540</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SS-4</td>
<td>09/30/05 Soil</td>
<td>&lt;620</td>
<td>&lt;620</td>
<td>ND</td>
<td>ND</td>
<td>&lt;620</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>EPA Region IX Preliminary Remediation Goals</td>
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<tr>
<td>SS-T1</td>
<td>10/01/05 Soil</td>
<td>&lt;430</td>
<td>&lt;430</td>
<td>&lt;430</td>
<td>&lt;430</td>
<td>&lt;430</td>
<td>&lt;430</td>
<td>&lt;430</td>
<td>&lt;430</td>
<td>&lt;430</td>
</tr>
<tr>
<td>SS-T2</td>
<td>10/01/05 Soil</td>
<td>1200</td>
<td>1200</td>
<td>900</td>
<td>1200</td>
<td>1500</td>
<td>2800</td>
<td>720</td>
<td>1400</td>
<td></td>
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<tr>
<td>LDEQ RECAP Standards for Soil</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPA Region VI Human Health Medium-Specific Screening Levels</td>
<td>6,200</td>
<td>620</td>
<td>620</td>
<td>620</td>
<td>620</td>
<td>620</td>
<td>620</td>
<td>620</td>
<td>620</td>
<td>620</td>
</tr>
</tbody>
</table>

Mice were divided into three exposure groups: SS-12, SS-13, SS-C, silica (chemically inert control for particles), and saline (vehicle control) and anesthetized using 5% isofluorane in oxygen. Once anesthetized, mice inhaled intranasally (i.n.) a single 50 μl aliquot of SS or silica (0.2 mg) or vehicle alone for three consecutive days. The dose chosen (0.2 mg/mouse i.n.) was well within the range of doses (0.2 – 1 mg/mouse delivered i.n.) used in similar exposure studies [3-7]. All samples were pre-treated with primocin (Invivogen, San Diego, CA), an antimicrobial reagent active against both Gram-positive and Gram-negative bacteria, mycoplasma and fungi, prior to use. All assessments were performed 48 hours following the final exposure.

Pulmonary function

Whole body plethysmography (Buxco Electronics and EMKA Technologies) was used to examine airway responsiveness to methacholine (Sigma; 0, 6.25, 12.5, and 25 mg/ml in isotonic saline) as previously described [8]. Measurements of airway resistance, elastance, and compliance using a computer-controlled ventilator (flexiVent; SciReq) were also recorded [9]. Just prior to data collection, the volume history of the respiratory system was standardized by inflating the lungs to total lung capacity. The resulting input impedance was further analyzed using the Single Compartment Model.

Bronchoalveolar lavage fluid (BALF) cellularity

BALF was harvested in 1 ml of PBS containing 2% heat-inactivated FBS. BALF was diluted and used to determine total number of leukocytes. Cytospin slide preparations were stained with the Diff-Quick kit (IMEB) and used for differential cell counts. All counts were performed by two unbiased observers using standard morphological criteria to classify individual leukocyte populations. Four mice from each group were used for these analyses, and 200-300 cells were counted per mouse.

Cytokines level in BALF

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Table 3. Volatile organic compounds (VOCs) analytical results

<table>
<thead>
<tr>
<th>Sample</th>
<th>Collection date (mm/dd/yy)</th>
<th>Matrix</th>
<th>VOCs</th>
<th>Methylen Chloride (μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS-5</td>
<td>09/30/05</td>
<td>Soil</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>SS-6</td>
<td>10/01/05</td>
<td>Soil</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>SS-6B</td>
<td>10/01/05</td>
<td>Soil</td>
<td>8.3</td>
<td></td>
</tr>
<tr>
<td>SS-7</td>
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<td>Soil</td>
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</tr>
<tr>
<td>SS-8</td>
<td>10/01/05</td>
<td>Soil</td>
<td>&lt;10</td>
<td></td>
</tr>
<tr>
<td>SS-9</td>
<td>10/01/05</td>
<td>Soil</td>
<td>NA</td>
<td></td>
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<td>SS-12</td>
<td>10/01/05</td>
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<td>SS-13</td>
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<td>SS-C</td>
<td>10/01/05</td>
<td>Soil</td>
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Notes: 1) Table represents detected compounds only. 2) VOCs Analysis by USEPA 8260. 3) LDEQ RECAP Standards for Soil taken from “Table 2: Management Option 1, Standards for Soil, Non-Industrial Soil” Louisiana Department of Environmental Quality, Risks Evaluation/Corrective Action Program, October 20, 2003. 4) EPA Region VI Human Health Medium-Specific Screening Levels taken from “Region 6 Human Health Medium-Specific Screening Levels 2004-2005 Table, Residential Soil” 12/21/2004. 5) NA: not analyzed.
IL-2, IL-4, IL-5, IFN-γ, and TNF-α level in the BALF were examined using the Mouse Th1/Th2 Cytokine Cytometric Bead Array Kit (BD Biosciences) as per the manufacturer’s instructions. Data were acquired with a BD FACScan™ and analyses performed using the BD Cytometric Bead Array Software to generate standard curves for each cytokine and to determine sample cytokine levels. The sensitivity for each cytokine is as follows: 5.0 pg/ml for IL-2, IL-4 and IL-5; 2.5 pg/ml for IFN-γ, and 6.3 pg/ml for TNF-α.

Cytokine levels were also measured from 50 µl of cell-free BALF using a high-throughput multiplex cytokine assay system (LincOplex Mouse Cytokine Assay; Millipore) according to the manufacturer’s instructions. Each sample was analyzed in duplicate on the Bio-Plex 200 system (BioRad). The sensitivity of the assay (i.e. minimum detectable concentrations) ranged from 0.7 to 10.3 pg/ml (depending on the analyte). The concentrations of analytes in these assays were quantified using a standard curve and a 5-parameter logistic regression was performed to derive an equation that was then used to predict the concentration of the unknown samples. The following cytokines were assayed: KC, IL-1β, IL-15, IL-6, and IL-10. The data presented here excluded any number below the range of sensitivity for the particular analyte.

**Pulmonary histopathology**

Lungs were inflated and preserved with 1 ml of Histochoice tissue fixative MB (Amresco). Tissues were then embedded in paraffin, cut in 4 µm frontal sections, and stained with H&E. Lung sections were stained with gram stain and safranin O to test for gram (+) and (-) bacteria. To ensure accuracy of staining and interpretation of results, reference slides with gram (+) and (-) bacteria were also stained. Specific histopathological diagnoses were performed by pathologists (L.G.L. and D.T.).

**In Vivo indicators of oxidative stress**

**Dichlorofluorescein Assay:** Reactive oxygen species generation in lung homogenates was assayed using dihydrodichlorofluorescein diacetate, a non-polar compound that, after conversion to a polar derivative by intracellular esterases, rapidly reacts with ROS to form the highly fluorescent compound dichlorofluorescein [10]. Briefly, the reaction mixture containing Locke’s buffer (pH 7.4), 50µL homogenate and 10µL of DCFH-DA (10µM) was incubated for 15 min at room temperature to allow the DCFH-DA to be incorporated into any membrane-bound vesicles and the diacetate group cleaved by esterases. After 30 min of further incubation, the conversion of DCFH-DA to the fluorescent
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Glutathione estimation: Measurement of GSH was performed according to the fluorimetric method of Hissin & Hilf [11]. Briefly, tissue was homogenized in 25% HPO3 and centrifuged at 10,000 rpm for 15 min. An aliquot of the supernatant was added to the reaction mixture containing phosphate buffer, (0.1M, pH 8.0, 5 mM EDTA) and 100μL of O-phthalaldehyde (50 μg/mL) and incubated at room temperature for 15 min. The fluorescence was measured at an excitation wavelength 350 nm and emission 420 nm.

Heme Oxygenase-1 (HO-1) Expression: To detect HO-1 protein in lung tissue, lungs were homogenized and microsomes prepared as previously described [12]. Equal amounts of protein (25 μg) for each sample and 50 ng recombinant HO-1 (positive control) were separated by SDS polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane using a semi-dry transfer apparatus. Antibodies against HO-1 (Stressgen Biotechnologies) were diluted 1:2000 and hybridized to the membranes for 8600 (GE Healthcare). ImageQuant software (GE Healthcare; version 5.2) was used to quantify results.

Statistical analysis

Data are presented as mean ± SE obtained from experiments with n = 8 for whole body plethysmography analysis of airway responsiveness, n = 5-6 for airway resistance, and n = 4-5 for pulmonary histology. Two-way ANOVA was used to evaluate the differences of airway responsiveness, airway resistance, BALF cellularity, and cytokine levels between groups (Prism). One-way ANOVA was used to evaluate the differences of DTT consumption and DCF fluorescence. Bonferroni post-tests were performed to test for significance between the groups. Differences between means were considered significant when p < 0.05.

Results

Exposure to sediment samples reduced the viability of HEp2 cells
Sediment samples (13 in total) were obtained from various sites including educational, residential, and commercial areas and supplied to our laboratory. HEp-2 cells were exposed to various doses of sediment suspended in media (30-300 μg/ml). As shown in Figure 1, the majority of samples showed dose-response cytotoxicity. The three most toxic samples (1, 8, and 12) had cell viability levels at approximately 60% at the lowest dose (30 μg/ml) and cell viability levels at less than 35% for the highest dose (300 μg/ml). SS-12 was selected for the following studies based on the sampling location (a residential area in New Orleans), the in vitro cytotoxicity data, and the low level of endotoxin.

Pulmonary function decreased in the mice exposed to SS-12

To evaluate the pulmonary response to inhaled sediment, mice were exposed to sediment, silica, or vehicle (saline + primocin). Silica was chosen as the non-specific, standard particle control, since all sediment samples collected contained either chemical and/or biological contaminants. Additional sediment samples from nearby areas were also included as controls. One sample (SS-13) contained moderate levels of arsenic but lacked SVOCs and VOCs and microbials. The other sample (SS-C) lacked arsenic, SVOCs, VOCs and microbials. Mice inhaled sediment/silica suspended in saline at the dose of 8 mg/kg body weight mouse per day (i.e. ~0.2 mg). Although this seems like a relatively high dose, the dose was based on actual PM10 levels recorded in the New Orleans area post-Katrina [1], MPPD modeling to account for an equivalent mass deposition in alveoli between humans and mice [13], and similar exposure studies reported in the literature (range: 0.2 – 1 mg/mouse delivered i.n.) [3-5]. To understand the effects of acute exposure to inhalation of these samples, mice...
Figure 3. Exposure of mice to SS-12 increased leukocyte numbers in the BALF. Significant increases in BALF cellularity was observed only when mice were exposed to SS-12. The increase in total BALF cellularity correlated with elevated levels of neutrophils. Data are expressed as means ± SE, n=6-8. **p < 0.01 vs. saline. ***p < 0.001 vs. saline.

Pulmonary dysfunction was accompanied by marked pulmonary inflammation

To determine if SS-12 hindered pulmonary function, whole body plethysmography was performed to examine airway responses to methacholine (MeCh) (Figure 2A). Compared with the animals exposed to saline, mice exposed to SS-12 developed significant airway responses at the MeCh dose of 12.5 mg/ml (Penh of 2.625 ± 0.754 vs 1.25 ± 0.164; p<0.01) and 25 mg/ml (Penh of 3.125 ± 0.441 vs 1.625 ± 0.375; p<0.01). Significantly increased airway reactivity in SS-12 treated mice was also observed in comparison with the mice exposed to silica at the 12.5 mg/ml dose (Penh of 2.625 ± 0.681 vs 0.875 ± 0.125; p<0.001). These data were confirmed with a more rigorous and invasive technique using forced oscillations. As with the plethysmography data, lung resistance in response to MeCh was significantly greater in SS-12 exposed mice (2.56 ± 0.194) compared to saline (1.57 ± 0.284), SS-13 (1.46 ± 0.145), or SS-C (1.50 ± 0.353) at 50 mg/ml MeCh (p<0.001) (Figure 2B).

To determine the amount of pulmonary
inflammation occurring in response to sediment exposure, the lungs were lavaged and the fluid (i.e., bronchoalveolar lavage fluid; BALF) was recovered. Total cells of BALF were counted and differentiated using morphological criteria. The total number of leukocytes recovered from the BALF of SS-12, SS-C, and SS-13 treated mice (4.3 x 10^5, 4.2 ± 0.71, or 4.8 ± 0.60 respectively) was greater than in saline treated mice (2.9 ± 0.31) (Figure 3). Elevated levels of neutrophils correlated with the increase in total BALF cellularity in the SS-12 and were statistically greater than saline (p < 0.001). In addition, macrophages were significantly lower in the SS-12 treated mice compared to saline (p < 0.001). No significant differences in lymphocyte or eosinophil numbers were observed among any of the groups.

As shown in Figure 4, the mice exposure to SS-12 developed both peribronchial and perivascular inflammation. In addition, exposure to SS-12 resulted in a diffuse alveolitis and mild type II pneumocyte hyperplasia. Only mild pulmonary inflammation was observed in the lungs of mice exposed to silica alone. Tissue sections from controls and SS-12 were negative for gram (+) and (-) bacteria (data not shown).

Elevated concentrations of TNF-α and IL-6 were detected in the BALF recovered from mice exposed to SS-12

To determine how the inflammatory response to SS-12 was initiated, cytokine levels in the BALF were measured (Table 5). TNF-α and IL-6 were found to be significantly elevated in the BALF from mice exposed to SS-12 compared to mice exposed to either vehicle alone or silica. There were no differences in the concentrations of IL-2, IL-4, IL-5, IFN-γ, IL-1β, IL-15, or IL-10 between groups.

SS-12 induces oxidative stress in the lungs of exposed mice

Histopathological examination showed significant changes to lung structure and was consistent with the observed, diminished lung function in response to MeCh challenge. Arsenic and SVOCs, present at levels above EPA standards in SS-12 (Table 2), have been shown to elicit oxidative stress. The oxidative ability of the HK-PM was assessed in vitro with the DTT assay (Figure 5A). This assay demonstrated that SS-13 and SS-12 had significantly higher redox potential than SS-C with SS-12 consuming 65% of the DTT in the reaction.

The amount of non-specific generation of reactive oxygen species in the lung tissue of mice exposed to HK-PM was assessed by DCF staining (Figure 5B) and use of a fluorescent microplate reader. Lung homogenates from SS-12 exposed mice demonstrated significant increases in DCF fluorescence compared to saline controls (p < 0.05); while lung homogenates from SS-C or SS-13 exhibited a significant decrease or no change in DCF staining (respectively). A major contributor to the redox state of the lungs are GSSG and
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GSH. The GSSG/2GSH ratios were elevated in lungs of all HK-PM treated mice compared to lungs from saline treated control mice (data not shown).

Heme-oxygenase-1 (HO-1), the rate-limiting enzyme in the production of the potent antioxidant bilirubin [14]. Recent data have shown that HO-1 is protective against PM induced oxidative stress and is induced early in the hierarchic oxidative stress model [15]. Since our exposures were acute, we assayed for HO-1 as an indicator of the oxidative effects of HK-PM on the lungs. We found that mice exposed to SS-12 exhibited de novo expression of HO-1 in lung tissue homogenates (Figure 5C). HO-1 was detected only in lung microsomes isolated from SS-12 treated mice (4.51 ± 0.60 μg/ml).

Discussion

A previous study assessing the environmental impact of Hurricane Katrina in New Orleans reported that a “powdery dust was aerosolized or resuspended via vehicular traffic disrupting sediments on previously flooded roadways and other hard surfaces” [16]. The authors further reported the presence of numerous microbial and environmental contaminants present in sediment samples collected from the New Orleans area and suggested an “inhalational hazard” to residents and workers returning to the New Orleans area. While a much earlier

Figure 5. The oxidative ability of SS-12 is elevated and SS-12 induces oxidative stress in the lungs of exposed mice. A) The DTT consumption of HK-PM after a thirty minute incubation at room temperature. Oxidative ability was measured in quadruplicate by use of the DTT assay. DTT consumption was normalized to vehicle control and expressed as the percent maximum consumption. B) The amount of ROS in HK-PM exposed lungs was assessed by DCF staining. The increase in DCF fluorescence was then calculated by normalizing the mean fluorescence intensity of the lung homogenates from HK-PM treated mice to that of saline treated mice. C) Lung microsomes were prepared and 25 μg of microsomal protein was used for western blot analysis of HO-1. Recombinant HO-1 (50 ng) was used as a positive control (Lane 0). Quantitative analysis of the western blot revealed that lung microsomes from mice exposed to SS-12 contained 4.5 ±0.60 μg HO-1 per ml of sample. Three separate samples (corresponding to three separate mice) were obtained for each exposure group: lanes 1, 4, and 7 are saline; lanes 2, 5, and 8 are silica; and lanes 3, 6, and 9 are SS-12. Values are shown as the mean ± SEM (n = 3).
study of soils and sediments from various urban sites around the New Orleans area clearly demonstrated that polycyclic aromatic hydrocarbons were a part of the soil mixture in 2001 [17], a recent report suggests that this contamination (at least with arsenic) appears to have developed in post-flood spots after Hurricane Katrina (i.e. Lakeview, Gentilly, Lower Ninth) [18]. Since we have no way of truly knowing what the soils in the New Orleans area contained immediately prior to the flooding, the present study makes no attempt to directly link the contaminants to the flooding.

In this study, we report on our efforts to collect environmental samples and to conduct a preliminary assessment of the short-term and potentially long-term impact to public respiratory health in the New Orleans area due to Hurricane Katrina. Within a week of the hurricane, several investigations were conducted on soil and sediment samples to determine what, if any, biological and/or chemical contaminants were present. Interestingly, some reported only moderately elevated levels of metals [19]; while others reported concentrations of aldrin, arsenic, lead, and seven SVOCs in sediments/soils exceeding EPA thresholds [16]. Our chemical analysis, performed independent of the bio- and physio-logical analyses, showed that SS-12 contained significant levels of arsenic (approximately 30 times higher than USEPA screening levels) (Table 1). In addition, SS-12 contained high concentrations of SVOCs, including Benzo(b)fluoranthene, Benzo(a) anthraecne, Benzo(a)pyrene, and Indeno (1,2,3-cd)pyrene (Table 2).

In vitro, SS-12 elicited marked cytotoxicity reducing the viability of HEp2 cells to less than 50% at the lowest exposure dose (30 μg/ml). We further demonstrated that acute exposure to SS-12 resulted in diminished pulmonary function. In particular SS-12 induced an abnormal increase in airflow limitation in response to MeCh provocation (i.e., increase airway resistance). Elevations in pulmonary resistance were accompanied by a moderate increase in the inflammatory response including the total number of leukocytes present in the BALF. Significant increases in the number of neutrophils seemed to account for the elevation in the total number of leukocytes observed. The analysis of BALF cellularity showed an increase in neutrophils in SS-12 treated mice, while histopathology demonstrated that exposure to SS-12 caused significant pulmonary inflammation with monocyte/macrophage infiltration of the alveolar ducts/alveoli and mild type II pneumocyte hyperplasia. Moreover, exposure to SS-12 resulted in a significant increase of TNF-α and IL-6 in the BALF. The lack of microbial presence in the lungs of SS-12 treated mice suggested that viable microbes were most likely not responsible for these observed responses. Mice treated with SS-13 also developed significant neutrophilic inflammation and elevated IL-6 levels in their BALF. While IL-6 levels correlated well with neutrophilia in the BALF, airway resistance was most strongly correlated with levels of arsenic (common to both SS-12 and SS-13).

Both arsenic and SVOCs are associated with many adverse human health effects including cardiovascular, lung, hepatic, urinary, and renal diseases [20, 21]. While acute exposure to arsenic has been shown to induce bronchiolitis, SVOCs emitted from vehicles and instilled via the trachea have also been shown to elicit many of the same effects that were observed with short-term exposure to SS-12 including cytotoxicity, inflammation, and parenchymal changes in the lung [20]. In fact, pneumocyte hyperplasia was the principal histopathological effect induced by exposure to SVOCs from gasoline and diesel engine exhaust. Although no correlations between SVOC content and the amount of parenchymal changes observed were presented by Seagrave and colleagues, their data compliment our data and suggest that exposure to samples containing greater than 50% SVOC results in significantly elevated levels of total BALF cell counts due to elevations mainly in neutrophils. Thus, it is possible that the cellular cytotoxicity and the acute lung damage observed in response to inhalation of SS-12 are due to the chemical constituents of this sample.

Arsenic and SVOCs have also been shown to elicit oxidative stress [22]. In particular, arsenic has been shown to directly attack -SH groups of proteins and induce the generation of reactive oxygen species (ROS) in both cells and tissues [23]. HO-1 is the rate limiting enzyme in the production of the bilirubin, an important anti-oxidant [14]. In bronchial epithelial cells, expression of HO-1 has been shown to be a sensitive marker for oxidative
stress [24, 25] and is often induced at very low levels of oxidative stress [26]. Studies have suggested that HO-1 acts as an inducible defense, against oxidative stress in models of inflammation with the products of the HO-1 reaction potentially participating in cellular defense [27, 28]. The induction of HO-1 in lung tissue isolated from mice exposed to SS-12 suggests an escalating pulmonary response to oxidative stress. SS-12 showed significant oxidative ability in vitro and its ability to induce oxidative stress was confirmed by DCF staining of lung homogenates. The correlation between HO-1 expression, DCF staining, GSH/GSSG ratio, and redox activity of SS-12 as measured by DTT consumption provides evidence for the role of oxidative stress in HK-PM induced pulmonary dysfunction and toxicity.

Interestingly, epidemiological studies have shown that chronic exposure to arsenic-contaminated dust is associated with increased risks of lung cancer [29]. Although it has been difficult to confirm the carcinogenicity of arsenic in animal models (probably due to the long latency period), intratracheal instillations of arsenic trioxide have been shown to induce pulmonary adenomas and papillomas [30]. Although the mode of action of arsenic carcinogenicity has not been established, intracellular production of ROS may play a role in mediating DNA damage and initiating carcinogenic processes. Currently, chronic exposure studies using SS-12 are being developed to address the potential for SS-12 to induce pulmonary carcinogenesis and understand the role of ROS and oxidative stress in these processes.

TNF-α is known to play a critical role in the pathogenesis of airway inflammation and airway responsiveness in a number of pulmonary diseases often mediating cell differentiation, activation, apoptosis and release of pro-inflammatory mediators by specific binding to TNFR1 and TNFR2 [31-33]. TNF-α is typically generated by macrophages, neutrophils, and epithelial cells in the airway [31]. In SS-12 treated mice, an impressive increase of TNF-α in BALF was observed and suggests a role for TNF-α in SS-12 induced pulmonary inflammation and airways resistance. Although not statistically significant, a slight increase in TNF-α was also observed in mice exposed to silica particles alone (data not shown). This was not entirely unexpected, since TNF-α has been shown to be involved in the pathogenesis of silica induced lung disease [34]. However, the inflammatory, pulmonary function, cytokine, and HO-1 data suggest that something inherent to the samples, and not just particulates such as silica, were responsible for the enhanced pulmonary pathophysiology observed in these mice. Although outside of the scope of the current studies, our data and that of others suggest that treatment with antioxidants may reduce pulmonary disease associated with SS-12 exposure.

The present study demonstrated that inhalation exposure to SS-12 leads to acute lung injury in mice, which is characterized by increased pulmonary inflammation and decreased lung function. The pathophysiological processes in the lung correlated to increases in TNF-α, IL-6, and biomarkers of oxidative stress. These results suggest that oxidative stress may, in part, be responsible for these observed respiratory effects. Studies are ongoing to determine the long-term effects of acute and chronic exposure to HK-PM and the precise role of oxidative stress and TNF-α in these events in animal models.

Limitations

There are several limitations of this study. First, all in vivo experiments used intranasal aspiration to deliver sediment samples to the respiratory tract rather than the more physiologically relevant inhalation technique. Intranasal aspiration has many advantages over inhalation techniques as described in the methods section. In addition, at the time of exposures the potential contaminants were unknown and this exposure methodology posed the least risk to personnel. It has been successfully used in similar studies in this field [3-7]. In our laboratory, this technique routinely delivers >90% of the administered sample directly to the lung. Second, these studies only evaluated the short-term respiratory effects of inhaled sediment following acute exposure protocols. The effects of chronic exposure are unknown. Third, each sediment sample differed in content making it extremely difficult to determine the precise cause for the observed respiratory effects. These experiments were designed to determine the pulmonary effects...
associated with exposure to HK-PM and as such did not attempt to reduce the complexity of the samples to their individual constituents. Finally, it may be possible that samples with similar chemical/microbial properties exist in other metropolitan regions; therefore one must use caution in attributing their de novo appearance solely to Hurricane Katrina related flooding.

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