

## Original Article

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

Kai Wang<sup>1</sup>, Dahui You<sup>1,5</sup>, Shrilatha Balakrishna<sup>5</sup>, Michael Ripple<sup>5</sup>, Terry Ahlert<sup>5</sup>, Baher Fahmy<sup>5</sup>, David Becnel<sup>1</sup>, Melissa Daly<sup>1</sup>, Wilma Subra<sup>2</sup>, James S. McElduff<sup>3</sup>, Larry G. Lomax<sup>4</sup>, Dana Troxclair<sup>6</sup>, and Stephania A. Cormier<sup>5</sup>

<sup>1</sup>Louisiana State University, Department of Biological Sciences, Baton Rouge, LA 70112, USA; <sup>2</sup>Subra Company, New Iberia, LA 70562, USA; <sup>3</sup>Altamont Environmental, Inc., Asheville, NC 28801, USA; <sup>4</sup>Louisiana State University School of Veterinary Medicine, Department of Veterinary Pathology, Baton Rouge, LA 70803, USA; <sup>5</sup>Louisiana State University Health Sciences Center, Department of Pharmacology & Experimental Therapeutics, 1901 Perdido Street, New Orleans, LA 70112, USA; <sup>6</sup>Louisiana State University Health Sciences Center, Department of Pathology, 1901 Perdido Street, New Orleans, LA 70112, USA

Received December 26, 2007; accepted February 16, 2008; available online February 28, 2008

**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  $\mu\text{m}$ . 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 PM<sub>10</sub> levels recorded in the New Orleans area post-Katrina were variable, ranging from 70  $\mu\text{g}/\text{m}^3$  in Gentilly to 688  $\mu\text{g}/\text{m}^3$  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 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/m<sup>3</sup>) post-Katrina; although PM 10 levels often peaked at >100 µg/m<sup>3</sup> with 688 µg/m<sup>3</sup> 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&q=>

<http://maps.google.com/maps/ms?hl=en&gl=us&ie=UTF8&om=1&msid=112347605037107176535.00000111e2bf9da31346e&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=112347605037107176535.0004447ccce3ef0db8a2d&ll=30.378086,89.268658&spn=0.003332,0.007167&z=18>). SS-13 was collected from a residential area just outside of the New Orleans metropolitan area (~47 miles from the city) and was selected for sampling due to over-wash that topped the Mississippi River levee at that location (approximately 40 feet south of the intersection of Cazenave Street and the toe of the Mississippi river levee; <http://maps.google.com/maps/ms?hl=en&gl=us&ie=UTF8&om=0&t=h&msa=0&msid=112347605037107176535.0004447cf4f355181b701&ll=29.65081,89.961821&spn=0.003357,0.007167&z=18>).

### 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,

**Table 1.** Metals analytical results

Sample	Collection date (mm/dd/yy)	Matrix	Metals						
			Arsenic mg/kg	Barium mg/kg	Cadmium mg/kg	Chromium mg/kg	Lead mg/kg	Selenium mg/kg	Mercury mg/kg
SS-1	09/30/05	Soil	1.6	15	ND	3.6	7.0	ND	0.015
SS-2	09/30/05	Soil	0.94	7.3	ND	4.5	3.6	ND	0.012
SS-3	09/30/05	Soil	7.5	14	ND	4.5	78	ND	0.052
SS-4	09/30/05	Soil	35	74	ND	27	140	ND	0.11
ADEM Draft Preliminary Screening Values			0.39	540		30	400		0.61
EPA Region IX Preliminary Remediation Goals			0.39	5,400	37	30	400	390	NE
SS-6	10/01/05	Soil	11	33	<0.10	21	33	<0.52	0.062
SS-6B	10/01/05	Soil	NA	NA	NA	NA	NA	NA	NA
SS-7	10/01/05	Soil	3.6	27	<0.15	4.2	25	<0.758	0.024
SS-8	10/01/05	Soil	10	35	<0.20	2117	27	<0.99	0.050
SS-9	10/01/05	Soil	11	26	<0.099	19	29	<0.50	0.016
SS-10	10/01/05	Soil	11	64	<0.10	15	22	<0.52	0.016
Tier 1 Target Remediation Goals - Restricted			3.82	14,300	1,020	381	1,700	1,020	61.3
Tier 1 Target Remediation Goals - Unrestricted			0.426	5,480	39.1	227	400	391	10
EPA Region IX Preliminary Remediation Goals			0.39	5,400	37	30	400	390	NE
SS-12	10/01/05	Soil	13	220	1.6	11	53	<1.0	0.054
SS-13	10/01/05	Soil	6.6	200	<0.097	9.4	17	<0.48	0.051
SS-C	10/01/05	Soil	0.9	11	<0.092	0.81	5.0	<0.46	0.016
LDEQ RECAP Standards for Soil			12	550	3.9	23	400	39	2.3
EPA Region VI Human Health Medium-Specific Screening Levels			0.39	5,500	39	30	400	390	NE

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.

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.

#### *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

**Table 2.** Semivolatile organic compounds (SVOCs) analytical results

Sample	Collection date (mm/dd/yy)	Matrix	SVOCs							
			Benzo(k)fluoranthene µg/kg	Benzo(b)fluoranthene µg/kg	Benzo(a)anthracene µg/kg	Benzo(e)pyrene µg/kg	Chrysene µg/kg	Fluoranthene µg/kg	Indeno(1,2,3-cd)pyrene µg/kg	Phenanthrene µg/kg
SS-1	09/30/05	Soil	<390	<390	ND	ND	ND	<390	ND	ND
SS-2	09/30/05	Soil	<350	<350	ND	ND	ND	<350	ND	ND
SS-3	09/30/05	Soil	450	420	ND	ND	ND	540	ND	ND
SS-4	09/30/05	Soil	<620	<620	ND	ND	ND	<620	ND	ND
ADEM Draft Preliminary Screening Values			6,200	620				230,000		
EPA Region IX Preliminary Remediation Goals			6,200	620				2,300,000		
SS-11	10/01/05	Soil	<430	<430	<430	<430	<430	<430	<430	<430
SS-12	10/01/05	Soil	1200	1200	900	1200	1500	2800	720	1400
SS-13	10/01/05	Soil	<340	<340	<340	<340	<340	<340	<340	<340
LDEQ RECAP Standards for Soil			6,200	620	620	330	62,000	220,000	620	2,100,000
EPA Region VI Human Health Medium-Specific Screening Levels			6,200	620	620	62	62,000	2,300,000	620	NE

Notes: 1) Table represents detected compounds only. 2) SVOCs Analysis by USEPA 8270. 3) ADEM Draft Preliminary Screening Values taken from "Alabama Risk-Based Corrective Action Guidance Manual", Table 2-2: "Draft Preliminary Screening Values" for Residential Soil, Alabama Department of Environmental Management, March 2005. 4) EPA Region VI recommended using EPA Region IX Preliminary Remediation Goals. USEPA. 2000. Supplemental Guidance to RABS: Region 4 Bullitins, Human Health Risk Assessment Bulletins. EPA Region 4, originally published November 1995, Website version last updated May 2000: <http://www.epa.gov/region4/waste/oftecsesr/healthbul.htm>. 5) EPA Region IX Preliminary Remediation Goals taken from "Region 9 PRG Table", Preliminary Remediation Goals (PRGs) for Residential Soil, October 2004. 6) 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. 7) 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. 8) Bold numbers indicate concentrations above applicable screening levels and/or remediation goals. ND: not detected.

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 pre-treated 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 performed 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 3.** Volatile organic compounds(VOCs) analytical results

Sample	Collection date (mm/dd/yy)	Matrix	VOCs
			Methylene Chloride µg/kg
SS-5	09/30/05	Soil	NA
SS-6	10/01/05	Soil	NA
SS-6B	10/01/05	Soil	8.3
SS-7	10/01/05	Soil	NA
SS-8	10/01/05	Soil	<10
SS-9	10/01/05	Soil	NA
SS-10	10/01/05	Soil	NA
Tier 1 Target Remediation Goals - Restricted			21,900
Tier 1 Target Remediation Goals - Unrestricted			14,300
EPA Region IX Preliminary Remediation Goals			9,100
			Naphthalene µg/kg
SS-11	10/01/05	Soil	NA
SS-12	10/01/05	Soil	12
SS-13	10/01/05	Soil	NA
SS-C	10/01/05	Soil	NA
LDEQ RECAP Standards for Soil			6,200
EPA Region VI Human Health Medium-Specific Screening Levels			120,000

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.

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% isoflurane 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*

**Table 4.** Microbial analytical results

Sample	Collection Date (mm/dd/yy)	Matrix	Microbial						
			Aerobic Plate count cfu/g	Total Coliforms cfu/g	E. Coli cfu/g	Staphylococcus a. cfu/g	Yeast count cfu/g	Mold count cfu/g	Salmonella MPN/g
SS-6	10/01/05	Soil	44,500,000	1,240,000	NG	10,000	22,000	7,000	NG
SS-6B	10/01/05	Soil	5,400,000	61,000	5,000	12,000	25,000	10,000	34
SS-7	10/01/05	Soil	123,000	9,000	NG	70	20,000	16,000	NG
SS-8	10/01/05	Soil	4,400,000	280,000	1,000	80,000	13,000	13,000	41
SS-9	10/01/05	Soil	6,000,000	350,000	NG	170,000	8,000	7,000	14
SS-11	10/01/05	Soil	21,400,000	7,800	NG	7,600	220,000	NG	13
SS-12	10/01/05	Soil	81,500,000	590,000	400	39,000	3,800	200	>1600

Notes: 1) Table represents detected microbes only. 2) cfu/g-colony forming units per gram. 3) MPN/g-most probable number per gram. 4) NG: no growth.

**Table 5.** Cytokine levels in BALF

Cytokines (pg/ml)	SAL	SS-C	SS-13	SS-12
IL-6	BD	1.91 ± 0.15	6.75 ± 2.50*	4.94 ± 1.82*
KC	45.87 ± 6.01	50.50 ± 7.40	63.20 ± 6.84	48.5 ± 7.05
TNF-α#	9.84 ± 1.41	ND	ND	139.48 ± 53.70*

Notes: BD: below detection limits; \*: p < 0.05; #: assayed by CBA kit; ND: not determined.

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.

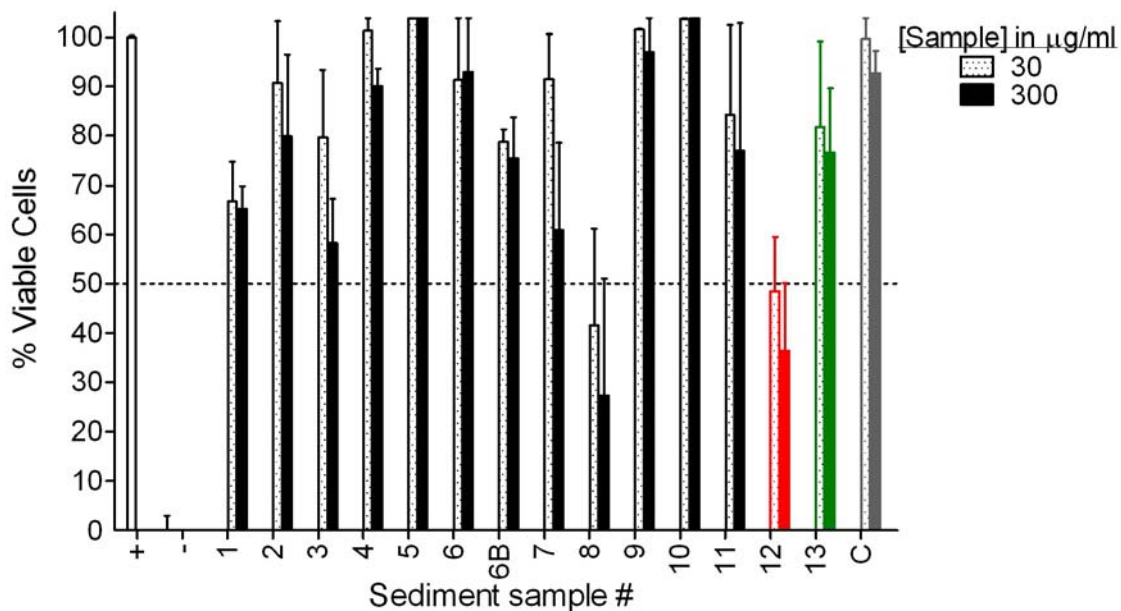
Cytokine levels were also measured from 50 μl of cell-free BALF using a high-throughput multiplex cytokine assay system (LincoPlex Mouse Cytokine Assay; Millipore) according to the manufacturer's instructions. Each sample was analyzed in duplicate on the Bio-Plex 200 system (BioRad). The sensitivity 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



**Figure 1.** Decreased viability of epithelial cells exposed to sediment samples. Human epithelial cells (HEp2) were exposed to various concentrations of sediment (30 or 300 µg/ml). After 24 h, cell viability was assessed. Data are shown as percentage of viable cells. The numbers in the abscissa refer to different sediment samples. For example, 12 represents sediment sample #12 (SS-12). All samples were assayed in duplicate and the experiment repeated two times.

**Glutathione estimation:** Measurement of GSH was performed according to the fluorimetric method of Hissin & Hilf [11]. Briefly, tissue was homogenized in 25% HPO<sub>3</sub> 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

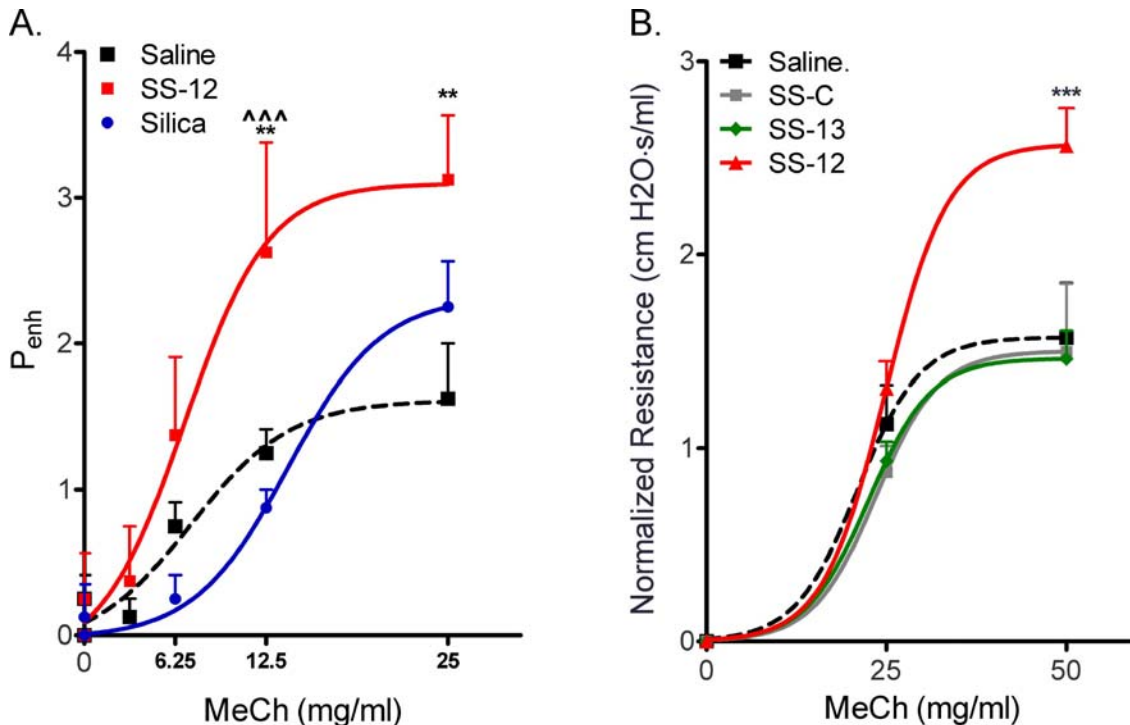
8600 (GE Healthcare). ImageQuant software (GE Healthcare; version 5.2) was used to quantify results.

#### 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*



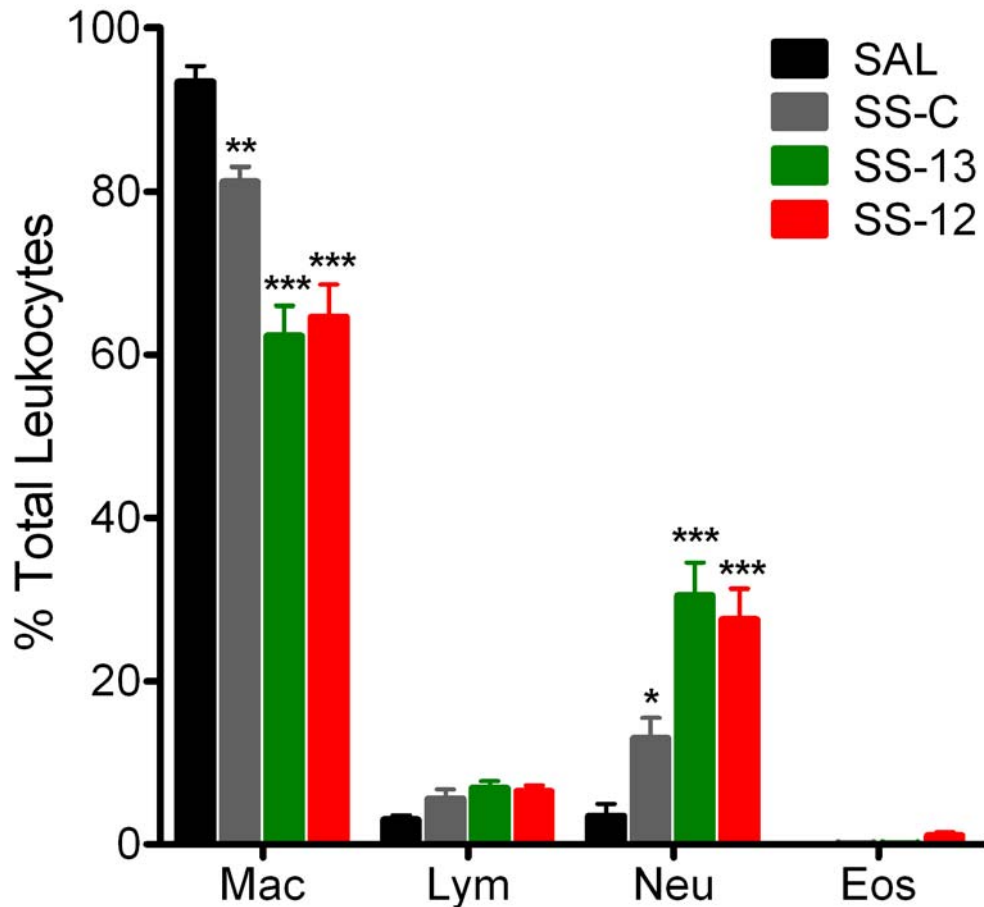
**Figure 2.** Significant changes in airway resistance is observed in mice exposed to Katrina sediment. A) Whole body plethysmography was performed to examine airway responsiveness (Penh). \*\* $p < 0.01$  vs. saline;  $^{***}p < 0.001$  vs. silica. In addition, measurements of specific airway mechanics in response to MeCh were performed using the forced oscillation technique (B). Mice exposed to silica (data not shown) were statistically no different than mice exposed to saline alone. Data are expressed as means  $\pm$  SE,  $n=5-6$ .  $^{***}p < 0.001$  vs. saline/SS-C/SS-13.

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  $\mu\text{g/ml}$ ). 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  $\mu\text{g/ml}$ ) and cell viability levels at less than 35% for the highest dose (300  $\mu\text{g/ml}$ ). 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.  $\sim 0.2$  mg). Although this seems like a relatively high dose, the dose was based on actual PM<sub>10</sub> 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  $\pm$  SE, n=6-8. \*\*\*\*p < 0.001 vs. saline. \*\*p < 0.01 vs. saline. \*p < 0.05 vs. saline.

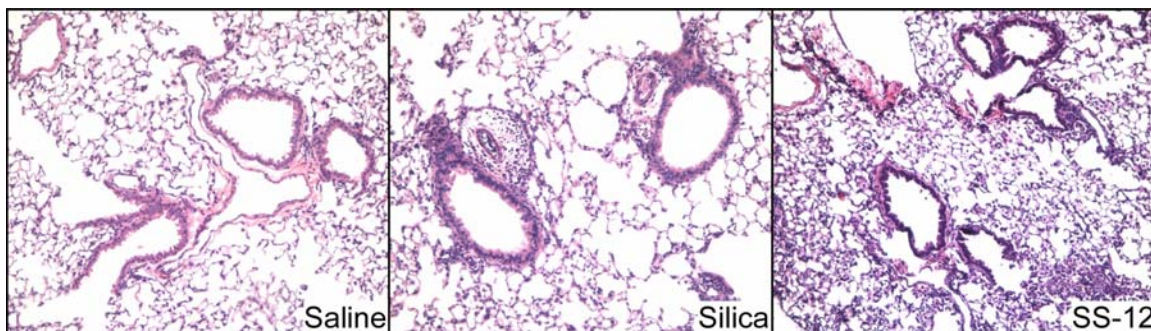
were exposed once per day for 3 days. Forty-eight hours after the final exposure, all assessments were made.

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  $2.625 \pm 0.754$  vs  $1.25 \pm 0.164$ ;  $p < 0.01$ ) and 25 mg/ml (Penh of  $3.125 \pm 0.441$  vs  $1.625 \pm 0.375$ ;  $p < 0.01$ ). 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  $2.625 \pm 0.681$  vs  $0.875 \pm 0.125$ ;  $p < 0.001$ ). 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 ( $2.56 \pm 0.194$ ) compared to saline ( $1.57 \pm 0.284$ ), SS-13 ( $1.46 \pm 0.145$ ), or SS-C ( $1.50 \pm 0.353$ ) at 50 mg/ml MeCh ( $p < 0.001$ ) (**Figure 2B**).

*Pulmonary dysfunction was accompanied by marked pulmonary inflammation*

To determine the amount of pulmonary



**Figure 4.** Histopathology associated with exposure to SS-12. Lung tissue sections were obtained from mice exposed to saline, silica, and SS-12. Mild inflammation was observed in the lungs of mice exposed to silica. Moderate inflammation, diffuse alveolitis, and mild type II pneumocyte hyperplasia were observed in the lungs of mice exposed to SS-12. Lung tissue sections were stained with H&E and representative photomicrographs taken at 100 $\times$ .

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 ( $4.3 \times 10^5$ ,  $4.2 \pm 0.71$ , or  $4.8 \pm 0.60$  respectively) was greater than in saline treated mice ( $2.9 \pm 0.31$ ) (**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- $\alpha$  and IL-6 were detected in the BALF recovered from mice exposed to SS-12*

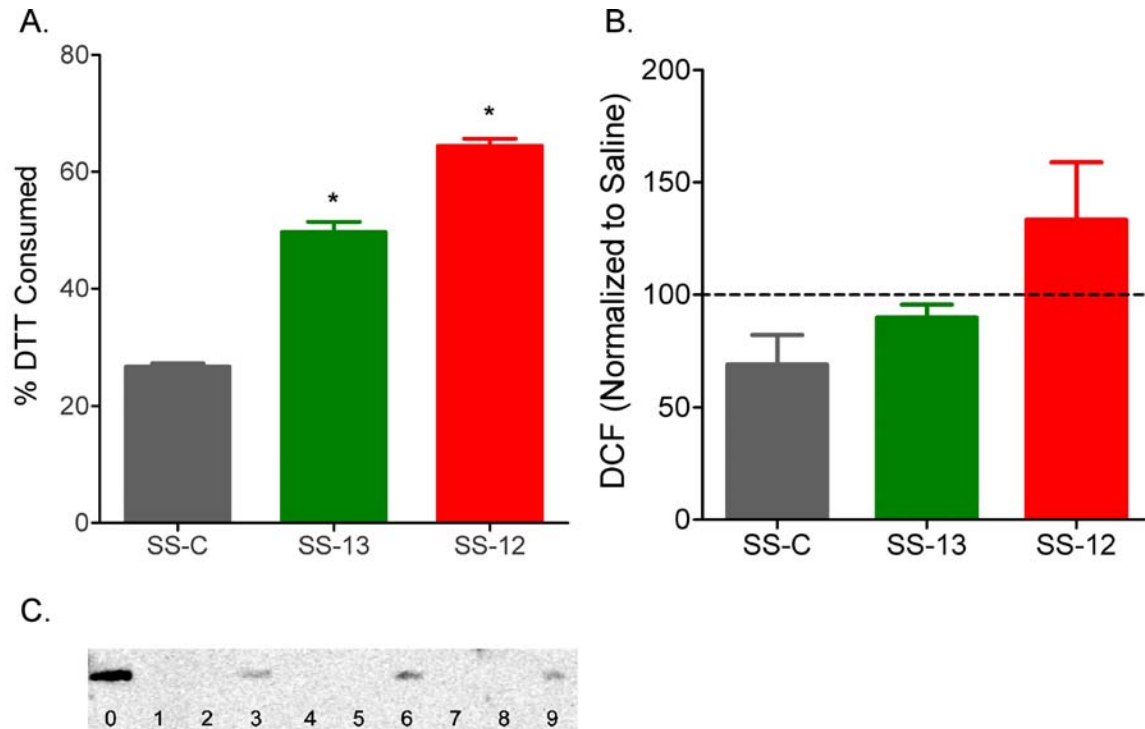
To determine how the inflammatory response to SS-12 was initiated, cytokine levels in the

BALF were measured (**Table 5**). TNF- $\alpha$  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- $\gamma$ , IL-1 $\beta$ , 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



**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  $\mu\text{g}$  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  $4.5 \pm 0.60 \mu\text{g}$  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  $\pm$  SEM ( $n = 3$ ).

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 ( $4.51 \pm 0.60 \mu\text{g}/\text{ml}$ ).

## 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

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 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)anthracene, 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  $\mu\text{g/ml}$ ). 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- $\alpha$  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- $\alpha$  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- $\alpha$  is typically generated by macrophages, neutrophils, and epithelial cells in the airway [31]. In SS-12 treated mice, an impressive increase of TNF- $\alpha$  in BALF was observed and suggests a role for TNF- $\alpha$  in SS-12 induced pulmonary inflammation and airways resistance. Although not statistically significant, a slight increase in TNF- $\alpha$  was also observed in mice exposed to silica particles

alone (data not shown). This was not entirely unexpected, since TNF- $\alpha$  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- $\alpha$ , 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- $\alpha$  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.

### Acknowledgements

The authors wish to thank Luis Marrero of the Louisiana State University Health Sciences Center – Morphology & Imaging Core Facility (Pathology) and Liren Ding for assistance in manuscript collation. This work was supported by NIH grant number P20 RR020159 from the LSU/Tulane COBRE-CEIDR Program of the National Center for Research Resources and NIH/NIEHS grant number 1 R01 ES015050-01 to SAC. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of NIH; similarly, the contents and opinions expressed in this manuscript do not represent the views of the Editorial Board of the IJCEM.

**Address correspondence to:** Stephania A. Cormier, PhD, Louisiana State University Health Sciences Center, Department of Pharmacology & Experimental Therapeutics, 1901 Perdido Street, MEB, P7-1, New Orleans, LA 70112, Fax: (504) 568-2361, Email: [scorm1@lsuhsc.edu](mailto:scorm1@lsuhsc.edu).

### References

- [1] National Resources Defense Council New Orleans Area Environmental Quality Test Results: Particulate Results by Neighborhood. <http://www.nrdc.org/health/effects/katrinadat/particulate2.asp>
- [2] Kumagai, Y.; Koide, S.; Taguchi, K.; Endo, A.; Nakai, Y.; Yoshikawa, T.; Shimojo, N., Oxidation of proximal protein sulfhydryls by phenanthraquinone, a component of diesel exhaust particles. *Chem Res Toxicol* 2002, 15, (4), 483-9.
- [3] de Haar, C.; Hassing, I.; Bol, M.; Bleumink, R.; Pieters, R., Ultrafine carbon black particles cause early airway inflammation and have adjuvant activity in a mouse allergic airway disease model. *Toxicol. Sci.* 2005, 87, (2), 409-18.
- [4] Eyles, J. E.; Spiers, I. D.; Williamson, E. D.; Alpar, H. O., Tissue distribution of radioactivity following intranasal administration of radioactive microspheres. *J. Pharm. Pharmacol.* 2001, 53, (5), 601-7.
- [5] Wang, B.; Feng, W. Y.; Wang, M.; Shi, J. W.; Zhang, F.; Ouyang, H.; Zhao, Y. L.; Chai, Z. F.; Huang, Y. Y.; Xie, Y. N.; Wang, H. F.; Wang, J., Transport of intranasally instilled fine Fe<sub>2</sub>O<sub>3</sub> particles into the brain: micro-distribution, chemical states, and histopathological observation. *Biol. Trace Elem. Res.* 2007, 118, (3), 233-43.
- [6] Barbarin, V.; Nihoul, A.; Misson, P.; Arras, M.; Delos, M.; Leclercq, I.; Lison, D.; Huaux, F., The role of pro- and anti-inflammatory responses in silica-induced lung fibrosis. *Respir. Res.* 2005, 6, 112.
- [7] Carter, J. M.; Driscoll, K. E., The role of inflammation, oxidative stress, and proliferation in silica-induced lung disease: a species comparison. *J. Environ. Pathol. Toxicol. Oncol.* 2001, 20 Suppl 1, 33-43.
- [8] Becnel, D.; You, D.; Erskin, J.; Dimina, D. M.; Cormier, S. A., A role for airway remodeling during respiratory syncytial virus infection. *Respir. Res.* 2005, 6, 122.
- [9] You, D.; Becnel, D.; Wang, K.; Ripple, M.; Daly, M.; Cormier, S. A., Exposure of neonates to respiratory syncytial virus is critical in determining subsequent airway response in adults. *Respir. Res.* 2006, 7, 107.
- [10] Driver, A. S.; Kodavanti, P. R.; Mundy, W. R., Age-related changes in reactive oxygen species production in rat brain homogenates. *Neurotoxicol. Teratol.* 2000, 22, (2), 175-81.
- [11] Hissin, P. J.; Hilf, R., A fluorometric method for determination of oxidized and reduced glutathione in tissues. *Anal Biochem* 1976, 74, (1), 214-26.
- [12] Matsubara, T.; Prough, R. A.; Burke, M. D.; Estabrook, R. W., The preparation of microsomal fractions of rodent respiratory tract and their characterization. *Cancer research* 1974, 34, (9), 2196-203.
- [13] CIIT Centers for Health Research; Dutch National Institute for Public Health and the Environment (RIVM) Multiple-path particle dosimetry (MPPD) model, 2.0; Research Triangle Park, 2006.
- [14] Slebos, D. J.; Ryter, S. W.; Choi, A. M., Heme oxygenase-1 and carbon monoxide in pulmonary medicine. *Respir. Res.* 2003, 4, 7.
- [15] Bernstein, J. A.; Alexis, N.; Barnes, C.; Bernstein, I. L.; Bernstein, J. A.; Nel, A.; Peden, D.; Diaz-Sanchez, D.; Tarlo, S. M.; Williams, P. B., Health effects of air pollution. *J Allergy Clin Immunol* 2004, 114, (5), 1116-23.
- [16] Presley, S. M.; Rainwater, T. R.; Austin, G. P.; Platt, S. G.; Zak, J. C.; Cobb, G. P.; Marsland, E. J.; Tian, K.; Zhang, B.; Anderson, T. A.; Cox, S. B.; Abel, M. T.; Leftwich, B. D.; Huddleston, J. R.; Jeter, R. M.; Kendall, R. J., Assessment of pathogens and toxicants in New Orleans, LA following Hurricane Katrina. *Environ.Sci.Technol.* 2006, 40, (2), 468-74.
- [17] Mielke, H. W.; Wang, G.; Gonzales, C. R.; Le, B.;

- Quach, V. N.; Mielke, P. W., PAH and metal mixtures in New Orleans soils and sediments. *Sci Total Environ* 2001, 281, (1-3), 217-27.
- [18] Fields, L.; Huang, A.; Solomon, G.; Rotkin-Ellman, M.; Simms, P. Katrina's Wake: Arsenic-Lead Schools and Playgrounds Put New Orleans Children at Risk; National Resources Defense Council: 2007.
- [19] Pardue, J. H.; Moe, W. M.; McInnis, D.; Thibodeaux, L. J.; Valsaraj, K. T.; Maciasz, E.; van Heerden, I.; Korevec, N.; Yuan, Q. Z., Chemical and microbiological parameters in New Orleans floodwater following Hurricane Katrina. *Environ.Sci.Technol.* 2005, 39, (22), 8591-9.
- [20] Seagrave, J.; McDonald, J. D.; Gigliotti, A. P.; Nikula, K. J.; Seilkop, S. K.; Gurevich, M.; Mauderly, J. L., Mutagenicity and in vivo toxicity of combined particulate and semivolatiles organic fractions of gasoline and diesel engine emissions. *Toxicol. Sci.* 2002, 70, (2), 212-26.
- [21] Kitchin, K. T., Recent advances in arsenic carcinogenesis: modes of action, animal model systems, and methylated arsenic metabolites. *Toxicol. Appl. Pharmacol.* 2001, 172, (3), 249-61.
- [22] Wijeweera, J. B.; Gandolfi, A. J.; Parrish, A.; Lantz, R. C., Sodium arsenite enhances AP-1 and NFkappaB DNA binding and induces stress protein expression in precision-cut rat lung slices. *Toxicol. Appl. Pharmacol.* 2001, 61, (2), 283-94.
- [23] Chen, Y. C.; Lin-Shiau, S. Y.; Lin, J. K., Involvement of reactive oxygen species and caspase 3 activation in arsenite-induced apoptosis. *J. Cell. Physiol.* 1998, 177, (2), 324-33.
- [24] Li, N.; Wang, M.; Oberley, T. D.; Sempf, J. M.; Nel, A. E., Comparison of the pro-oxidative and proinflammatory effects of organic diesel exhaust particle chemicals in bronchial epithelial cells and macrophages. *J. Immunol.* 2002, 169, (8), 4531-41.
- [25] Choi, A. M.; Alam, J., Heme oxygenase-1: function, regulation, and implication of a novel stress-inducible protein in oxidant-induced lung injury. *Am. J. Respir. Cell Mol. Biol.* 1996, 15, (1), 9-19.
- [26] Xiao, G. G.; Wang, M.; Li, N.; Loo, J. A.; Nel, A. E., Use of proteomics to demonstrate a hierarchical oxidative stress response to diesel exhaust particle chemicals in a macrophage cell line. *J. Biol. Chem.* 2003, 278, (50), 50781-90.
- [27] Otterbein, L. E.; Choi, A. M., Heme oxygenase: colors of defense against cellular stress. *Am J Physiol Lung Cell Mol Physiol.* 2000, 279, (6), L1029-37.
- [28] Ryter, S. W.; Otterbein, L. E.; Morse, D.; Choi, A. M., Heme oxygenase/carbon monoxide signaling pathways: regulation and functional significance. *Mol. Cell. Biochem.* 2002, 234-235, (1-2), 249-63.
- [29] Tapio, S.; Grosche, B., Arsenic in the aetiology of cancer. *Mutat. Res.* 2006, 612, (3), 215-46.
- [30] Pershagen, G.; Nordberg, G.; Bjorklund, N. E., Carcinomas of the Respiratory-Tract in Hamsters Given Arsenic Trioxide and or Benzo[a]Pyrene by the Pulmonary Route. *Environ. Res.* 1984, 34, (2), 227-241.
- [31] Bazzoni, F.; Beutler, B., The tumor necrosis factor ligand and receptor families. *N. Engl. J. Med.* 1996, 334, (26), 1717-25.
- [32] Tartaglia, L. A.; Weber, R. F.; Figari, I. S.; Reynolds, C.; Palladino, M. A., Jr.; Goeddel, D. V., The two different receptors for tumor necrosis factor mediate distinct cellular responses. *Proc. Natl. Acad. Sci. U. S. A.* 1991, 88, (20), 9292-6.
- [33] Wajant, H.; Henkler, F.; Scheurich, P., The TNF-receptor-associated factor family: scaffold molecules for cytokine receptors, kinases and their regulators. *Cell. Signal.* 2001, 13, (6), 389-400.
- [34] Piguet, P. F.; Vesin, C., Treatment by human recombinant soluble TNF receptor of pulmonary fibrosis induced by bleomycin or silica in mice. *Eur. Respir. J.* 1994, 7, (3), 515-8.