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ABSTRACT
First Responders (FR) present at Ground Zero during the first 72 hr after the WTC collapse have suffered significant damage to their respiratory health, including chronic disorders like granulomatous pulmonary disease and persistent airway hyper-reactivity (AHR; feature of chronic asthma). Early animal studies performed to understand the etiology of some ailments primarily used only fine WTC dust fractions (≤ 2.5 μm), dusts collected after 9/13/01, or regimens that did not reflect exposure scenarios most FR underwent. Because significant coarse (> 10 μm) WTC dust particle deposition was found in the lungs of FR, and the alkalinity of WTC particles increased with size, we hypothesized that: (1) an increased presence of coarse alkaline particles in their lungs contributed to the high incidence\severity of pulmonary diseases; and, (2) a mechanism underlying these disorders was that (A) alkalinity of the large particles damaged lung epithelium so airway remodeling was triggered and particle clearance reduced, and (B) metals associated with retained WTC particles exerted a variety of toxicities including promoting airway remodeling. To verify the hypotheses, we proposed - using an animal model, relevant WTC dusts and exposure scenarios, inter-related Specific Aims to demonstrate the incidence\severity of lung diseases was related to: (1) presence of significant amounts of large WTC particles in the lungs; (2) specific presence of alkaline portions of WTC dusts; (3) altered dust retention; and/or, (4) dust-induced airway epithelium damage\subsequent release of factors to promote remodeling. The earliest period of the grant focused on development\validation of a novel WTC dust exposure system to deliver particles to rats in a manner that mimicked what FR underwent (i.e., extensive mouth breathing/entrainment of large diameter particles). Thereafter, exposures (on 2 consecutive days, 2 hr/d, via intratracheal inhalation) to WTC dusts (collected 9/12-13/01) were performed and biologic endpoints examined over a 1-yr post-exposure period. Other biomaterials from the rats were archived for use by other investigators to assess effects of the dusts on non-pulmonary systems. Results showed the exposures (at highest proposed dose) resulted in significant changes in: lung weights due to dust deposition; methacholine responsivity (AHR index); viability of lavaged (BAL) cells and BAL total protein levels reflecting WTC dust toxicities *in situ*; ciliated cell levels in upper airways (re: impacted on retention of WTC dust/other co-inhaled particles); goblet/mucin-bearing cell levels; and, expression (within 24 hr) of key genes associated with inflammation, oxidative stress, cell cycle, and immune system activation in the lungs. The studies also indicated damage to ciliated cells likely led to the observed prolonged retention (to 1 yr) of WTC dust deposited by the exposure. The studies also permitted a determination that Ti and Al were good “markers” of WTC dust exposure. Results regarding shifts in responsivity to antigen challenge were inconclusive; further studies are needed. We expect data obtained here will provide valuable information about relationships between physicochemical properties of building collapse-/demolition-associated dusts and toxicologic effects in the lungs, and permit Investigators in Worker Safety\Emergency Planning and in Respiratory Medicine to develop prospective action plans and focused treatments to protect respiratory health of firefighters/rescuers called to any future building collapses.

Section 1

Background: In this application, we hypothesized that in First Responders who were at Ground Zero during the first 72 hr after the WTC collapse: (1) an increased presence of large (>10 μm) alkaline particles in the lungs contributed to a high incidence\severity of pulmonary diseases; and, (2) a mechanism underlying these disorders was that the alkalinity of the large particles damaged lung epithelium so that airway remodeling was triggered and particle clearance was reduced, and also that metals associated with retained WTC particles exerted a variety of toxicities including promotion of airway remodeling. To verify these hypotheses, we proposed - using an animal model, relevant WTC dusts, and relevant exposure scenarios, four inter-related Specific Aims: (1) increases in amounts of large (>2.5 **μ**m) WTC particles in the lungs contribute to increases in AHR and SLGPD incidence\severity; (2) presence of alkaline portions of WTC dusts was critical to observed AHR and SLGPD incidence\severity; (3) AHR incidence\severity after WTC dust exposure depended on initial airway epithelium damage and subsequent release of factors to promote remodeling; and, (4) SLGPD and AHR incidence and/or severity after WTC dust exposure was related to altered dust retention.

Significant (Key) Findings. In these studies, rats were exposed to WTC dusts (collected 9/12-13/01) either once or on consecutive days (each exposure, 2 hr/d via intratracheal inhalation) and several biologic endpoints that would address the goals outlined in the Specific Aims then examined for (in some cases) up to a 1-yr post-exposure. For many endpoints, analyses from exposures of rats to the highest (99 mg WTC_{53}/m^3) and lowest (33 mg WTC_{53}/m^3) proposed doses are complete; a few results from the lower dose exposure are still pending (indicated as such below). Results of these remaining analyses will be reported in publications generated after the grant has ended. The major findings from the studies (and where indicated, with which dust dose level) were that one or two WTC dust exposures resulted in significant:

- changes (starting within 1 hr) in lung weights (absolute/relative) that appeared to persist [at both dust levels];
- changes in methacholine responsivity (index for AHR) [seen at high dust level; not seen at low level];
- changes in goblet/mucin-bearing cell levels (markers for damage to airway epithelium) [seen at low dust level; high dust level tissues not yet examined];
- retention of WTC dusts (up to 1 yr) that were inhaled in a single set of exposures
- changes (time-dependent) in lavaged (BAL) cell viability, lavage total protein levels, and presence of select key cytokine/modeling enzymes (indicating progressive WTC dust-induced toxicities *in situ*) [at high dust level; not consistent with low dust level];
- changes in ciliated cell levels in upper airways that persisted/seemed to worsen with time post-exposure (reflective of potential impact on subsequent retention of WTC dust/other co-inhaled Ground Zero particles) [seen at low dust level; high dust level not examined];
- changes (≈24 hr) in expression of genes associated with inflammation, oxidative stress, immune system activation, and cell cycle in the lungs [seen at high dust level; low dust level and persistence outcomes pending];
- retention of the dusts in the lungs (using Al, Ti markers to verify) [seen at low dust level; high dust level not examined].

The studies also permitted a determination to be made that Ti and Al (but not Sb) were good "markers" of WTC dust exposure [at high and low dust levels]. Results regarding potential dust-induced shifts in host responsivity to antigen challenge in the lung were inconclusive [only low dust level tested]; further studies are still needed to fully resolve this issue.

Translation of Findings. At this time, we do not feel the findings are fully complete (i.e., some data still pending) to allow us to be able to draw firm conclusions as to how the outcomes could be specifically applied to prevent workplace diseases and injuries among those who comprise First Responder populations (i.e., firefighters, police, rescue personnel). We still believe the data

obtained so far (and to come) will provide valuable information about relationships between physicochemical properties of building collapse-/demolition-associated dusts and toxicologic effects in the lungs that will permit Investigators in Worker Safety\Emergency Planning and in Respiratory Medicine to develop, respectively, prospective action plans and focused treatments to protect respiratory health of firefighters/rescuers called to any future building collapses. We also feel the data provides a firm basis for future studies to examine the impact of the actual co-pollutant exposures that occurred at Ground Zero (i.e., dust and diesel exhaust fumes, dust and metal cutting fumes); grant applications addressing such exposures have been/will be submitted to CDC/NIOSH and other government funding agencies so we can ultimately attain an even more complete understanding of the bases for the increased incidence/severity of chronic pulmonary (and, increasingly, cardiovascular) disorders among the First Responders.

Outcomes/ Impact. In the context of "How did this project lead to improvements in occupational safety and health?" at this point we can only address potential outcomes, i.e., findings, results, or recommendations that could impact workplace risk if used. Specifically, in the context of dusts from building collapses/demolition, it would seem imperative steps be taken to minimize the potential for entrainment of such dusts via mouth breathing. Clearly, Personal Protective Equipment (PPE) already exist for use by those who might be face these types of exposures; however, it is clear these PPE may need to be modified to minimize the discomfort that could cause individuals to remove the PPE during heavy labor under conditions of warm humid weather/ongoing on-site fires. With respect to "How can the findings of this study guide future investigations and research?", we feel the data obtained with the dust alone exposures now permits us to move on to investigations of contributions to chronic pulmonary (and, increasingly, cardiovascular) disorders among the First Responders from other major types of co-pollutant exposures that occurred at Ground Zero, specifically those from WTC dusts and diesel exhaust fumes, and from WTC dusts and metal cutting fumes.

Section 2 **Scientific Report**

Background for the project: At the time of the original grant submission, it had been ≈7+ yr since the disaster in Lower Manhattan and it was increasingly evident that chronic respiratory problems were becoming apparent primarily among firefighters and rescue personnel who were at Ground Zero for repeated/prolonged periods in the first 72 hr after the buildings collapsed on 9/11 (i.e., "First Responders"). While 'WTC cough' was one of the first respiratory anomalies reported in First Responders, increases in more chronic disorders like sarcoid-like granulomatous pulmonary disease (SLGPD) and persistent airway hyper-reactivity (AHR; defining feature of asthma) were documented. Attempts to better understand the etiologies of these health problems had initially focused on effects from the dusts derived from the buildings (i.e., WTC dusts). However, those studies that employed animal models mainly utilized: dust size fractions (i.e., fine) most likely to be entrained and deposited in the lungs; WTC dusts that physico-chemically differed from those the First Responders encountered during 9/11-9/13; or, regimens that did not truly reflect the exposure scenarios the majority of First Responders underwent (i.e., high dust levels, non-use of respirators, extensive mouth breathing). Because studies had shown: there was significant coarse WTC dust particle deposition in First Responders' lungs; and, that the alkalinity of WTC particles rose as their aerodynamic diameters (d_a) increased, we hypothesized that an increased presence of these large (>2.5 μm d_a) alkaline particles in the lungs contributed to the high incidence\increased severity of SLGPD and persistent AHR in First Responders. We also hypothesized that a mechanism underlying these chronic disorders was that **(A)** alkalinity of the large particles initially damaged the lung epithelium so that airway remodeling was triggered and particle clearance was reduced, and **(B)** metals associated with subsequently-retained WTC dust particles could then exerte a variety of toxicities including, possible granuloma induction and promotion of airway remodeling, for extended periods.

To test the hypotheses, studies were proposed that would employ rats exposed for consecutive days (4 hr/d, via mouth breathing [**intratracheal inhalation**]) to WTC dusts collected on 9/12-13/01. Since rats are routinely used in studies of toxicant-induced persistent lung diseases, they were selected as models for First Responders who remained on, stayed near, or returned many times to the Main Pile at Ground Zero during 9/11-13/01 and thus were repeatedly exposed to these "unique" early dusts. All exposures would use dust concentrations extrapolated from those present at the Main Pile during those first 3 days. Unfortunately, because large debris (e.g., glass\metal shards, stones, carpet fragments) that would never be entrained into the lungs was also present in collected samples, parent WTC dust *per se* cannot be used; the material used herein would be WTC dust sieved to yield all particles of diameters ≤ 53 μm (i.e., WTC_{53-}). Four specific aims were proposed for the studies:

1. *To demonstrate that increases in amounts of large (>2.5 μm d_a) WTC particles present in the lungs could contribute to increases in AHR and SLGPD incidence\severity;*
2. *To establish that a presence in the lungs of the alkaline portions of the WTC dusts was critical to AHR and SLGPD incidence\severity*;
3. *To determine whether AHR incidence\severity after WTC dust exposure depended on initial airway epithelium damage and subsequent release of factors that promote remodeling; and,*
4. *To confirm that SLGPD (and AHR) incidence\severity after WTC dust exposure was related to alterations in WTC dust retention.*

It was anticipated successful performance of these unique comprehensive studies using relevant WTC dusts and exposure scenarios would: (1) permit clarification of what role WTC dusts inhaled during the first 72 hr at Ground Zero may have had in changes in First Responder respiratory health; (2) provide important clues to the etiology of some reported chronic lung disorders; and, (3) for first time, allow effects from exposures to WTC dusts (or respective coarse\supercoarse fractions) on the initiation\progression of two disorders, AHR and SLGPD, to be monitored after exposure. The data obtained would also provide valuable information concerning relationships between physic-chemical properties of building collapse-/demolition-associated dusts and toxicologic effects (acute and chronic) in the lungs.

Methodology

General Overview: The earliest portion of the grant-work focused on development\validation of a novel WTC dust exposure system to deliver the dust particles to rats in a manner that mimicked what First Responders underwent (i.e., extensive mouth breathing/entrainment of large diameter particles) in the first days after the buildings collapsed. Originally, exposures were to occur on 3 consecutive days (2 hr/d, via intratracheal inhalation) to WTC dusts that were collected 9/12-13/01; however, issues of dust lethality (at highest [first] dose tested) precluded 3 days of exposures (see below). Thus, for remainder of the grant period, rat exposures to the WTC dusts took place on 2 consecutive days (2 hr/d, via intratracheal inhalation) unless elsewise indicated. Rats were exposed to 99 mg WTC_{53}-/m^3 (or in latter part of grant period, 33 mg WTC_{53}-/m^3) atmospheres each day. Each dust dose was selected - after accounting for species-specific breathing/lung parameters and utilizing appropriate modeling software - to correspond to ≈750 and 250 mg WTC dust/m^3 atmospheres that were likely encountered by the First Responders (and entrained during extensive mouth breathing while performing heavy labor at the site) at Ground Zero during the first 72 hr after the buildings collapsed. Biologic endpoints in the rats were then examined over a period of up to 1-yr post-exposure (detailed below).

Results (by year[s] of project) and Discussion

Yr 1-2

Exposure System Creation/Validation

Efforts over the first year of the project focused on obtaining all materials essential to construct the intratracheal inhalation (ITIH) system to be used for WTC dust exposures. The two major constituents, a Fishing Line system (miniaturized from prototype) and an 8-port ITIH exposure conduit, were successfully constructed. During assembly of the ITIH exposure/in-line anesthesia system, design obstacles became evident. Among the most critical of these included how to: (1) modulate the amount of flow used to generate WTC dust particles (i.e., potentially for 12-15 lpm) from that to which rats could be optimally exposed (i.e., 3 lpm, maximum); (2) monitor particle concentrations in-line without causing potentially-risky spikes in flow delivered to the anesthetized rats; (3) introduce isoflurane anesthetic (ISO) into the particle stream and minimize risk of inadvertent exposure of Investigators; and, (4) assure particles delivered via the system reflected those to which First Responders were exposed to at Ground Zero (i.e., size fraction distributions [≤ 2.5 µm, 2.5-10 µm, 10-53 µM] were not inherently skewed by delivery system). Standardized protocols were developed for all exposures, including use of defined air-flows/line speeds to generate desired exposure levels (i.e., 33-99 mg dust/m^3) and intubation techniques to prepare rats for each exposure, These protocols addressed all the above-noted 'obstacles'.

Initial Findings

Having defined the system conditions/settings, WTC dust exposures were performed at the highest (99 mg dust/m^3) proposed concentration. Originally planned as 2-hr exposures on 3 consecutive days, it became evident rats could not withstand this dust level; thus, some rats were only exposed once and a subset on 2 days - no rats reached the original 3-d goal. Two sets of controls were used; rats given ISO only by nose or via ITIH. Longitudinal studies/analyses of any changes in: airway hyper-responsivity (AHR; Aim 1); airway epithelium status (damage)\release of factors to promote remodeling (Aim 3) were then performed on the rats. Another set of rats underwent a 2 hr exposure at 99 mg dust/m^3 to confirm the WTC dusts were being delivered into lungs and to verify utility of three proposed metals (e.g., Sb, Al, Ti) as 'markers' of dust burden - a critical endpoint to ultimately confirm that AHR\SLGPD incidence and/or severity post-exposure is related to any potential change in WTC dust retention (Aim 4).

The major findings of these initial studies were that

- The novel exposure system produced atmospheres to model those experienced by First Responders;
- Lung weights showed a significant change in exposed rats due to dust deposition;

- Measures of soluble ion levels in lungs indicated that Ti, Al, Sb were likely good markers of WTC dust exposure;
- Prolonged (≥ 2 hr) anesthesia by ISO (2.5% in O_2) did not effect airway response to methacholine (Mch);
- A single 2-hr WTC dust exposure at 99 mg dust/m^3 caused significant changes in Mch responsivity (index for AHR); however, Mch responsivity was not apparently altered after two sequential daily 2-hr dust exposures to the dust - possibly due to damage to cells/associated [muscarinic] receptors in the airways; and,
- Time-dependent decreases in the viability of lavaged (BAL) cells and increases in BAL total protein levels indicated significant WTC dust-induced toxicities had occurred and were - as hypothesized - progressive.

Significance of the Initial Studies' Findings

The main significance of these initial efforts were: optimization/standardization of the WTC dust generation/exposure system; succesful perfomance of exposure(s) to highest proposed dust level (99 mg WTC_{53}-/m^3); determination of immediate/acute mortality in dust-exposed rats; preliminary validation of the three hypothesized select (metal) markers of WTC dust exposure *in situ*; and, establishing that even a single high level exposure to intact WTC dusts caused significant changes in airway (hyper)responsivity (in re: AHR, Aims 1-4)

Progress Not Made during Initial Studies

A major problem (as related to achieving certain goals outlined in Specific Aims) was an inability to gain access to a micro-computed tomography (micro-CT) system as was originally expected. This was due to problems associated with renovations at the laboratory of the investigators who were to perform the off-site experiments with the exposed rats. Thus, endpoints associated with measures of SLGPD were not achieved (or likely to be so unless renovations to the facilities housing the system were completed in a timely manner).

Yr 2-3

Continuation of Highest Dust Level Exposures /Initiation of Lower Dust Level Exposures

WTC dust exposures were done at the highest (99 mg dust/m^3) proposed level; two controls were again used. All rats underwent a single 2-hr exposure (to mimic exposure that may have occurred in original plume cloud[s]) and then longitudinal studies/analyses of changes in airway hyper-responsivity (AHR; Aim 1) and analyses of intra-pulmonary release of factors to promote lung remodeling (Aim 3) were undertaken. Other rats underwent a single exposure to confirm WTC dusts were delivered to the lungs and to verify the potential utility of three metals (e.g., Sb, Al, Ti) as dust burden markers - this was critical to confirm that lung disease incidence/severity post-exposure was related to changes in dust retention (Aim 4). RNA array analyses of tissues from these rats were also performed to ascertain if/what genes might have been significantly impacted by the high-dose exposure; the array data would, in turn, be used to provide insight into changes observed in all other endpoints being measured (re: Aims 1 and 3).

In light of the toxicity/lethality findings with the highest proposed WTC dust level, exposures using 33 mg dust/m^3 (approximating FR exposure levels of 250 mg/m^3 on-site 9/11-13/11) were performed, starting in Yr 3. Due to unexpected issues related to dust-induced damage to the lungs (i.e., upper airway bleeding), the original plan for 2-hr exposures on each of 3 consecutive days was again modified to 2 d and the system pressure reduced slightly for the second exposure. These modifications of the protocol alleviated the acute issues and thus rats could be exposed and then examined at various timepoints post-exposure (i.e., 7, 14, 28, 60, 120, 240, and 360 d). To accommodate the timelines, the first sets of rats exposed at this lower dose were delegated to the longest post-exposure timepoints; all other rats to cover the remaining timepoints were exposed in Yr 3-4 of the grant and then analyzed in tandem with all the other rats that had already been exposed in Yr 3.

Findings in Yr 2-3 Studies

The major findings of the Yr 2-3 studies were that a **single** 2-hr WTC dust exposure at 99 mg/m^3 (as occurred with First Responders on 9/11 after building collapses) resulted in:

- Significant changes in Mch responsivity (index for AHR);
- Significant time-dependent decreases in viability of lavaged (BAL) cells and increases in BAL total protein levels (indicating WTC dust-induced toxicities that were progressive);
- Significant changes (within 24 hr) in expression of key genes associated with inflammation, oxidative stress, immune system activation, and cell cycle in the lungs;
- Significant changes (within 1 hr) in lung weights (absolute/relative) that persisted; and,
- A conclusion that Ti and Al (but not Sb) were good lung markers of WTC dust exposure.

The limited major findings (by end of Yr 3) from the **two repeated (daily)** 2-hr WTC dust exposures at 33 mg/m^3 (as occurred with many FR on 9/12-13/11) resulted in (out to 30 d):

- Significant changes (within 24 hr) in lung weights that appeared to persist; and,
- Significant time-dependent decreases in viability of lavaged (BAL) cells and increases in BAL total protein levels (again indicating progressive WTC dust-induced toxicities).

Significance of Yr 2-3 Studies

The main significance of efforts this cycle was showing that a single exposure to the highest proposed dust level (99 mg WTC_{53}-/m^3) - as occurred during exposures to the original plume released when the builidings collapsed - led to: acute mortality in dust-exposed rats; validation of select (metal) *in situ* markers of WTC dust exposure; significant changes in airway hyper-responsivity (re: Aims 1 and 2); and, identification of alterations in the expression of key genes associated with inflammation, oxidative stress, immune system activation, and cell cycle in the lungs that were uniquely/significantly impacted by the single high-level dust exposure (re: Aim 3). The other major significant outcome was adoption of an exposure regimen (i.e., 2 daily 2-hr exposures to 33 mg WTC_{53}-/m^3) that would allow all the planned longitudinal studies/analyses to be intiated/performed in the remaining time for the grant. Several initial findings (i.e., out to 30 d post-exposure) indicated significant changes were induced in the lungs of rats even at this level of WTC dust. The fact the dusts introduced into the lungs of the rats were overwhelmingly comprised of supercoarse (i.e., > 85-90% were > 10 μm d_a) particles that were alkaline allowed us to conclude the *presence in the lungs of the alkaline portions of the WTC dusts* were likely a key factor underlying dust-induced changes in host AHR (re: Aim 2). Ultimately, data from these then-"ongoing" studies of rats exposed to the lower dust level would allow us to potentially confirm that the effects on many of the measured endpoints (including AHR) were also dose-related and provide support for the aim that sought to show that *increases in amounts of the larger d_a WTC particles in the lungs could contribute to increases in AHR* (re: Aim 1).

Progress Not Made during the Yr 2-3 Studies

The same major problem concerning inability to gain access to the micro-CT system remained unresolved. By this point it became clear the proposed off-site SLGPD analyses (longitudinal analyses in same rat hosts in over a 1-yr post-exposure period) would not be achiveable. Accordingly, the focus of the post-exposure studies shifted to examining endpoints associated with AHR (re: Aims 1-3) and/or with changes in lung structure etc. that could impact on WTC dust particle retention (re: Aim 4).

Yr 4 (and beyond [i.e., NCE])

Continuation of Exposures and Subsequent Longitudinal Post-exposure Analyses

In the final year of the grant, all remaining WTC dust exposures were done at the 33 mg dust/m^3 proposed level so that endpoints at each of the proposed post-exposure timepoints (i.e., 7, 14, 28, 60, 120, 240, and 360 d) could be analyzed. In this period, apart from many of the measures originally proposed, studies to ascertain effects of the exposures on: lung structures (i.e., examination of changes in airway ciliated cell populations) that could impact on any induced

changes in dust particle retention (re: Aim 4); lung physiology (i.e., examination of goblet cell presence/hyperplasia); as well as in host responsivity to allergens (i.e., asthmatic responses; re: AHR in Aims 1-3) were also undertaken. In addition, further studies of potential changes in gene expression and of induction of select key cytokine/modeling enzymes in the, as well as the persistence of these induced changes by this dose of WTC dust, were initiated.

Findings in Yr 4/NCE Studies

The major findings of the Yr 4 and during the NCE studies were that the **two repeated (daily)** 2-hr WTC dust exposures at 33 mg/m^3 resulted in:

- Onset of what appears to be a systemic neutropenia that initially persisted, then normalized;
- Significant changes in lung weights that seemed to persist;
- Significant changes in ciliated cell levels in upper airways that persisted/seemed to worsen with time post-exposure - this reflected a potential impact on subsequent retention of WTC dust itself/other co-inhaled Ground Zero particles;
- Significant retention of dusts in the lungs (up to 1 yr post-exposure; using Al, Ti markers to verify) evident well after a single set of exposures;
- Significant changes in goblet/mucin-bearing cell levels (markers for damage to airway epithelium) - the persistence of these changes remains to be determined;
- A finding that time-dependent changes in lavaged (BAL) cell viability and lavage total protein levels (indicating WTC dust-induced toxicities *in situ*) that were evident with the high dose level were not consistently evident with exposure to the lower low dust level; and,
- Inconclusive outcomes regarding dust-induced shifts in host responsivity to antigen challenge in lung (using repeated sampling of blood from/BUXCO analyses of single dedicated sets of rats/exposure group).

Progress Not Made during the Yr 4/NCE Studies

Disappointingly, the experimental design we used to assess potential WTC dust-induced changes in host responsivity to allergens (i.e., to reflect on asthmatic responses/AHR; re: Aims 1-3) did not allow us to obtain results that were conclusive. Further studies using rats (dedicated for each timepoint post-exposure) will permit isolation of lung tissues/lavages fluid in order to resolve this issue. In addition, our planned analyses of potential changes in gene expression and in induction of select key cytokine/modeling enzymes in the, as well as persistence of these induced changes, by the WTC dust was interrupted by "problems" at the laboratories of our project collaborators. There was no resolution to these problems for > 6 mo; as such, these biomaterials were still waiting to be analyzed at the time of this Final Progress Report. We have requested the materials promptly be returned to the PI's laboratories so they can be analyzed; all outcomes obtained will then be reported in upcoming publications.

Summary

Significant (Key) Findings. In these studies, rats were exposed to WTC dusts (collected 9/12-13/01) either once or on consecutive days (each exposure, 2 hr/d via intratracheal inhalation) and several biologic endpoints that would address the goals outlined in the Specific Aims then examined for (in some cases) up to a 1-yr post-exposure. For many endpoints, analyses from exposures of rats to the highest (99 mg WTC_{53}/m^3) and lowest (33 mg WTC_{53}/m^3) proposed doses were completed; a few results from the lower dose exposure are still pending (indicated as such below). The major findings from the studies (and where indicated, with which dust dose level) were that one or two WTC dust exposures resulted in significant:

- changes (starting within 1 hr) in lung weights (absolute/relative) that appeared to persist [at both dust levels];
- changes in methacholine responsivity (index for AHR) [seen at high dust level; not seen at low level];

- changes in goblet/mucin-bearing cell levels (markers for damage to airway epithelium) [seen at low dust level; high dust level tissues not yet examined];
- changes (time-dependent) in lavaged (BAL) cell viability, lavage total protein levels, and presence of select key cytokine/modeling enzymes (indicating progressive WTC dust-induced toxicities *in situ*) [at high dust level; not consistent with low dust level];
- changes in ciliated cell levels in upper airways that persisted/seemed to worsen with time post-exposure (reflective of potential impact on subsequent retention of WTC dust/other co-inhaled Ground Zero particles) [seen at low dust level; high dust level not examined];
- retention of dusts in the lungs (up to 1 yr post-exposure; using Al, Ti markers to verify) evident well after a single set of exposures;
- changes (≈24 hr) in expression of genes associated with inflammation, oxidative stress, immune system activation, and cell cycle in the lungs [seen at high dust level; low dust level and persistence outcomes pending];

The studies also permitted a determination to be made that Ti and Al (but not Sb) were good "markers" of WTC dust exposure [at high and low dust levels]. Results regarding potential dust-induced shifts in host responsivity to antigen challenge in the lung were inconclusive [only low dust level tested]; further studies are still needed to fully resolve this issue.

Conclusion/Translation of Findings. At this time, we do not feel the findings are fully complete (i.e., some data still pending) to allow us to be able to draw firm conclusions as to how the outcomes could be specifically applied to prevent workplace diseases and injuries among those who comprise First Responder populations (i.e., firefighters, police, rescue personnel). We still believe the data obtained so far (and to come) will provide valuable information about relationships between physicochemical properties of building collapse-/demolition-associated dusts and toxicologic effects in the lungs that will permit Investigators in Worker Safety\Emergency Planning and in Respiratory Medicine to develop, respectively, prospective action plans and focused treatments to protect respiratory health of firefighters/rescuers called to any future building collapses. We also feel the data provides a firm basis for future studies to examine the impact of the actual co-pollutant exposures that occurred at Ground Zero (i.e., dust and diesel exhaust fumes, dust and metal cutting fumes); grant applications addressing such exposures have been/will be submitted to CDC/NIOSH and other government funding agencies so we can ultimately attain an even more complete understanding of the bases for the increased incidence/severity of chronic pulmonary (and, increasingly, cardiovascular) disorders among the First Responders.

In we consider "How did this project lead to improvements in occupational safety and health?" at this point we can only address potential outcomes, i.e., findings, results, or recommendations that could impact workplace risk if they were used. Specifically, in the context of dusts from building collapses/demolition, it would seem imperative steps be taken to minimize the potential for entrainment of such dusts via mouth breathing. Clearly, Personal Protective Equipment (PPE) already exist for use by those who might be face these types of exposures; however, it is clear these PPE may need to be modified to minimize the discomfort that could cause individuals to remove the PPE during heavy labor under conditions of warm humid weather/ongoing on-site fires. In we consider "How can the findings of this study guide future investigations and research?", we feel the data obtained with the dust alone exposures permits us and other Investigators to move on to investigations of contributions to the chronic pulmonary (and, increasingly, cardiovascular) disorders among the First Responders from other major types of co-pollutant exposures that occurred at Ground Zero, specifically those from WTC dusts and diesel exhaust fumes, and from WTC dusts and metal cutting fumes.

Program Director/Principal Investigator (Last, First, Middle): Cohen, Mitchell D.

Inclusion Enrollment Report

This report format should NOT be used for data collection from study participants.

Study Title: WTC Dust Size and Alkalinity as Factors in First Responder Chronic Lung Ailments

Total Enrollment: 0 **Protocol Number:**

Grant Number: R01OH008280

PART A. TOTAL ENROLLMENT REPORT: Number of Subjects Enrolled to Date (Cumulative) by Ethnicity and Race				
Ethnic Category	**Females**	**Males**	**Sex/Gender Unknown or Not Reported**	**Total**
Hispanic or Latino				**
Not Hispanic or Latino				
Unknown (individuals not reporting ethnicity)				
Ethnic Category: Total of All Subjects*				*
Racial Categories				
American Indian/Alaska Native				
Asian				
Native Hawaiian or Other Pacific Islander				
Black or African American				
White				
More Than One Race				
Unknown or Not Reported				
Racial Categories: Total of All Subjects*				*

PART B. HISPANIC ENROLLMENT REPORT: Number of Hispanics or Latinos Enrolled to Date (Cumulative)				
Racial Categories	**Females**	**Males**	**Sex/Gender Unknown or Not Reported**	**Total**
American Indian or Alaska Native				
Asian				
Native Hawaiian or Other Pacific Islander				
Black or African American				
White				
More Than One Race				
Unknown or Not Reported				
Racial Categories: Total of Hispanics or Latinos**				**

* These totals must agree.
** These totals must agree.

Publications

Wang S, Prophete C, Soukup JM, Chen L, Costa M, Ghio A, Qu Q, **Cohen MD**, Chen H: [2010]. Roles of MAPK pathway activation during cytokine induction in BEAS-2B cells exposed to fine World Trade Center (WTC) dust. *Journal of Immunotoxicology* 7:298-307.

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Vaughan JM, Garrett BJ, C. Prophete C, Horton L, Sisco M, Skolup JM, Zelikoff J, Ghio A, Peltier RE, L.C. Chen LC, **Cohen MD**: [2014]. A novel system to generate WTC dust particles for inhalation exposures. *Journal of Exposure Science and Environmental Epidemiology* 24:105-112

Weiden MD, Naveed B, Kwon S, Segal LN, Kulkarni R, Comfort AL, Kasturiarachchi KJ, Prophete C, **Cohen MD**, Chen LC, Rom WN, Prezant DJ, Nolan A: [2012]. Comparison of WTC dust size on macrophage inflammatory cytokine release *in vivo* and *in vitro*. PLoS ONE 7:e40016.

Cohen MD, Vaughan JM, Garrett B, Prophete C, Horton L, Sisco M, Kodavanti U, Ward W, Peltier RE, Zelikoff J, Chen LC: [2014]. Acute high-level exposure to WTC particles alters expression of genes associated with oxidative stress and immune function in the lung. *Journal of Immunotoxicology* (online doi:10.3109/ 1547691X.2014.914609).

Cohen MD, Vaughan JM, Garrett B, Prophete C, Horton L, Sisco M, Ghio A, Zelikoff J, Chen LC: [2014]. Acute exposure to WTC dust particles impacts upon ciliated and goblet cells in lungs of rats. *Inhalation Toxicology* (submitted).

Cohen MD, Vaughan JM, Garrett B, Prophete C, Horton L, Sisco M, Ghio A, Chen LC: [2014]. Longitudinal effects of a series of "low- level" exposures to World Trade Center dusts on the lungs of rats. *Journal of Toxicology Environmental Health* (ready for submission).

Garrett BJ, Vaughan JM, Prophete C, Ghio A, Chen LC, **Cohen MD**: [2014]. Effects of acute high-level exposure to World Trade Center particles on rat bronchial airway function. *Inhalation Toxicology* (ready for submission).

Dissertation/Thesis

Vaughan, JM: [2011] A Fishing-line Generator to Deliver WTC Dust Particles for Inhalation Exposures, MS Thesis, New York University School of Medicine

Garrett B: [2011] Supercoarse World Trade Center Particle Effects on Rat Bronchial Airway Function, MS Thesis, New York University School of Medicine

Materials available for other investigators

Biologic materials from the rats (including several non-lung organs/tissues, blood/serum) were harvested at necropsy and have been archived at -80°C. These are available for use by investigators wanting to examine non-pulmonary effects from the exposures to the WTC dusts performed in the course of the funded project. These materials can be/have been accessed (as has been the case with samples of the original WTC dusts) by simple request (phone call, e-mail, etc.) from investigators within NYUSOM and from outside institutions. A chain-of-custody system is in place/will be used to document the selection/delivery of all requested samples. **Each requesting investigator is required to indicate the original NIOSH/CDC funding source/grant number in any publications that might arise from the work therein**.