Closeout Report
A) FINAL PROGRESS REPORT

Title Page

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Table of Contents

List of Terms & Abbreviations

bronchoalveolar lavage fluid (BAL)
chronic obstructive pulmonary disease (COPD)
concentrated animal feeding operations (CAFOs)
epidermal growth factor receptor (EGFR)
hog barn dust extract (HDE)
intercellular adhesion molecule (ICAM-1)
interleukin 6 (IL-6)
interleukin 8 (IL-8)
lysophosphatidic acid (LPA)
protein kinase C (PKC)
tumor necrosis factor-alpha (TNF-alpha)

Abstract

Exposure to organic dusts is a cause of airway disease, including chronic obstructive pulmonary disease (COPD). As many as 20% of COPD cases are attributed to occupational exposures. In rural areas, an important source of dust exposure occurs in hog concentrated animal feeding operations (CAFOs). Persons exposed to hog CAFOs have airway inflammation and an increased incidence of COPD. Although many substances are present in hog barn dust that induce inflammation, actual mechanisms leading to COPD are not well defined. Understanding mechanisms of hog barn dust-induced airway disease is relevant in developing both targeted treatment and prevention strategies.

Epithelial cells within the airways respond to inhaled agents with the release of cytokines that recruit and activate inflammatory cells and expression of molecules that serve as receptors and ligands for interactions with other cells. Previously, we observed that hog barn dust extract (HDE) augments human airway epithelial protein kinase C (PKC) activation, resulting in IL-8 and IL-6 release and increased ICAM-1 expression, mediating inflammatory cell adhesion to airway epithelium *in vitro*.

The objective of this proposal was to define mechanisms by which hog barn dust activates epithelial cell PKC and the role of PKC in airway inflammation associated with chronic bronchitis occurring in confinement facility workers and to determine the role of hog barn dust-related lysophosphatidic acid (LPA), an important lipid mediator, in modulating dust effects on PKC and inflammatory responses. We addressed our objective with these specific aims:

1) Determine the biochemical nature and specific identity of factor(s) in HDE that activate epithelial cell PKC and identify the specific PKC isoenzymes activated by HDE and these factors.
2) Establish how HDE-associated lysophosphatidic acid (LPA) modulates HDE-induced epithelial cell PKC activity and IL-8/IL-6 release.
3) Identify mechanisms by which HDE augmentation of epithelial cell PKC *in vitro* mediates recruitment and adhesion of inflammatory cells to airway epithelium *in vitro.*
4) Determine how HDE modulates airway epithelial PKC activation and inflammatory responses *in vivo* utilizing an animal model of exposure, including testing the potential role of LPA.

The results of our studies support that several components of dusts found in hog CAFOs contribute to airway inflammation in workers in these facilities. Although endotoxin (from Gram-negative bacteria in the environment) is an important constituent of such dusts and a constituent of dusty environments that can be monitored, our data provides evidence that endotoxin alone is not the only important component of dusts in terms of respiratory disorders. We have observed that substances from Gram-positive bacteria (peptidoglycan) and proteases within the dust contribute substantially to airway epithelial inflammation. Better understanding of factors in dust that elicit airway inflammation is critical to enhancing the design of monitoring and prevention strategies for animal confinement facilities to protect the respiratory health of workers.

We have created a mouse model with dust from hog confinement facilities (using intranasal exposure of dust extract) that demonstrates several features that are similar to respiratory disease seen in workers. This model shows a brisk initial inflammatory response to the dust extract, the development of some tolerance to the dust with repeated exposure, but significant lung inflammation with continued exposure. This animal model is important in ongoing work that allows us to study the mechanisms by which dust causes inflammation and to then test specific interventions to see if they improve the inflammation.

Our data has provided new insights into the basic mechanisms by which dusts from confinement facilities cause inflammation in airways. We have demonstrated that dusts of swine confinement facilities activate specific isoforms of an important cell-signaling molecule protein kinase C (PKC) that regulates inflammatory mediator release of airway epithelial cells and inflammatory cell interactions with the epithelial cells. By defining the role of this important cell-signaling molecule in dust-induced airway inflammation, we can explore potential therapeutic interventions for airway disease focused on mediating the effect of dust on this pathway.

Section 1

Highlights/Significant Findings

Our data supports that several components of dusts found in swine confinement facilities contribute to airway inflammation in those working in these confinement facilities. Although endotoxin (from Gram-negative bacteria in the environment) is an important constituent of such dusts and a constituent of dusty environments that can be monitored, our data provides evidence that endotoxin alone is likely not the only important component of dusts in terms of respiratory disorders. We have observed that substances from Gram-positive bacteria (peptidoglycan) and proteases within the dust contribute substantially to airway epithelial inflammation. Better understanding dust factors that elicit airway inflammation is critical to enhancing the design of monitoring and prevention strategies for animal confinement facilities to protect the respiratory health of workers in these environments.

We have created a mouse model with dust from swine confinement facilities (using intranasal exposure of dust extract) that demonstrates several features that are similar to respiratory disease seen in workers. This model shows a brisk initial inflammatory response to the dust extract, the development of some tolerance to the dust with repeated exposure, but significant lung inflammation despite the tolerance with continued exposure. This animal model is important in ongoing work that allows us to study the mechanisms by which dust causes inflammation and to then test specific interventions to see if they improve the inflammation.

Our data has provided new insights into the basic mechanisms by which dusts from confinement facilities cause inflammation in airways. We have demonstrated that dusts of swine confinement facilities activate specific isoforms of an important cell-signaling molecule protein kinase C (PKC) that regulates inflammatory mediator release of airway epithelial cells and inflammatory cell interactions with the epithelial cells. By defining the role of this important cell-signaling molecule in dust-induced airway inflammation, we can explore potential therapeutic interventions for airway disease focused on mediating the effect of dust on this pathway.

Translation of Findings

Our finding that endotoxin in dust from swine confinement facilities is not solely responsible for airway inflammation sets the groundwork for continued definition of the role of other components of the dust (molecules from Gram-positive bacteria and proteases) that should be monitored in order to provide the safest environment for workers in swine confinement facilities and likely other concentrated animal feeding operations (CAFOs). We expect to further define the importance of the various components of CAFO dusts. The long-term goal is to determine if monitoring of specific components is useful in terms of predicting respiratory symptoms and disease of workers and if strategies aimed at reducing these components are effective in improving the environment in terms of respiratory disease for workers.

Our findings regarding the cellular processes regulated by dust exposure and our animal model of dust exposure set the stage for testing whether specific therapies can be used to treat workers with respiratory disease in the future.

Outcomes/Relevance/Impact

This study has provided new information about cellular processes by which dust from swine CAFOs causes inflammation in the airways of workers. In addition, this study has provided further information about the potential role of a variety of components in dust besides endotoxin that may be important in respiratory disease in workers. Furthermore, we have created an animal model of dust exposure that will allow us to test whether specific future therapeutic interventions may have value in treating workers with respiratory disease. The findings from this study provide potential outcomes in terms of determining components of the environment in CAFOs that should be more closely monitored with the long-term goal of better prevention strategies for respiratory disease in CAFO workers. Similarly, our findings using cells and the mouse model

provide potential outcomes in terms of developing new therapeutic strategies for airway disease of CAFO workers.

Section 2

Scientific Report

Background: Exposure to organic dusts is a cause of airway disease, including chronic obstructive pulmonary disease (COPD). As many as 20% of COPD cases are attributed to occupational exposures. In rural areas, an important source of dust exposure occurs in hog confinement barns. Persons exposed to hog barns have airway inflammation and an increased incidence of COPD. Although many substances are present in hog barn dust that induces inflammation including endotoxins, actual mechanisms leading to COPD are not well defined. Understanding mechanisms of hog barn dust-induced airway disease is relevant in developing both targeted treatment and prevention strategies.

Epithelial cells respond to inhaled agents with the release of cytokines that recruit and activate inflammatory cells and expression of molecules that serve as receptors and ligands for interactions with other cells. We previously observed that hog barn dust extract (HDE) augments human airway epithelial protein kinase C (PKC) activation, resulting in IL-8 and IL-6 release and increased ICAM-1 expression, mediating inflammatory cell adhesion to airway epithelium *in vitro*. At the start of this project, we had preliminary data using an intranasal exposure to HDE in mice that HDE lead to an increase in airway epithelial PKC activation and inflammatory responses *in vivo*. We had also observed that epithelial cell exposure to HDE results in an increase of the lipid mediator lysophosphatidic acid (LPA). Treatment with phospholipase B to inactivate LPA inhibits HDE-stimulated IL-6 and IL-8 release. The role of LPA induced by hog barn dust in directing airway inflammation is not known.

The objective of this proposal was to define mechanisms by which hog barn dust activates epithelial cell PKC and the role of PKC in airway inflammation associated with chronic bronchitis/COPD occurring in confinement facility workers and to determine the role of hog barn dust-related LPA, an important lipid mediator, in modulating dust effects on PKC and inflammatory responses.

Specific Aims: 1) Determine the biochemical nature and specific identity of factor(s) in HDE that activate epithelial cell PKC and identify the specific PKC isoenzymes activated by HDE and these factors.
2) Establish how HDE-associated lysophosphatidic acid (LPA) modulates HDE-induced epithelial cell PKC activity and IL-8/IL-6 release.
3) Identify mechanisms by which HDE augmentation of epithelial cell PKC *in vitro* mediates recruitment and adhesion of inflammatory cells to airway epithelium *in vitro.*
4) Determine how HDE modulates airway epithelial PKC activation and inflammatory responses *in vivo* utilizing an animal model of exposure, including testing the potential role of LPA.

Procedures/Methodology: To study the effects of HDE on airway epithelial cell inflammatory responses, we used human airway epithelial cells in culture. We have utilized predominantly BEAS-2B human airway epithelial cells (cell line) in submerged cell culture system. We have confirmed all key observations with primary human bronchial epithelial cells in a similar culture system. The epithelial cell inflammatory responses investigated have been primarily IL-6, IL-8, and TNF-alpha release (utilizing ELISA methods) and ICAM-1 expression of epithelial cells utilizing flow cytometry methods. PKC activity including PKC-alpha and PKC-epsilon specific activity was measured. We have also investigated inflammatory cell adhesion (predominantly human neutrophils) to airway epithelial cells *in vitro*.

We have also used an *in vivo* model of HDE exposure. Mice receive intranasal instillation of 12.5% dust extract or saline control once (single exposure) or once daily for 2 weeks (repetitive exposure). Animal food intake and body weight is monitored to ensure that the changes in lung pathology are not due to weight loss as opposed to actual treatment effects. We have not found changes in dust-exposed wildtype mice. The

parameters measured in mice have included bronchoalveolar lavage fluid (BAL) for cell numbers, cell types, and cytokines; histology; airway hyper-responsiveness; and epithelial cell PKC activity.

Results and Discussion: Regarding specific aim 1 and the nature of factor(s) in dust and dust extract, we have observed that there are multiple substances that have yet to be specifically identified that influence airway epithelial cell function including cytokine release (IL-6/8 and TNF-alpha) and PKC activity. In collaboration with Dr. Myron Toews (co-Investigator), we fractionated HDE using Sephadex G-100 columns. We have found multiple components within HDE that influence IL-8, IL-6, as well as epidermal growth factor-receptor (EGFR) binding and phosphorylation of airway epithelial cells (see role of EGF in results from Aim 2 below). Some fractions are proteinase K sensitive and others are not. Some fractions that augment airway epithelial cell IL-6 and IL-8 release are dependent on EGFR activation and others are not. Our studies to date with mass spectroscopy analysis of these fractions have demonstrated several interesting substances including proteases. Thus, we subsequently analyzed several swine confinement dusts extracts by zymography and demonstrated that HDE contains protease activity and this activity is sensitive to protease inhibitors (PMSF, AEBSF). Treatment of airway epithelial cells with various exogenous proteases (trypsin, thrombin, MMP-2/9, porcine pancreatic elastase) or with HDE induces release of IL-6/8 and TNF-alpha. Pretreatment of HDE with PMSF or AEBSF significantly attenuates the HDE-induced release of IL-6/8 and TNF-alpha. In addition, HDE-induced cytokine release can be inhibited by pretreatment with protease activated receptor antagonists. We presented data regarding the presence of proteases including porcine pancreatic elastase in HDE and its role in mediating airway epithelial cell cytokine release as a poster at the May 2010 meeting of the American Thoracic Society meeting. A manuscript is currently being prepared and these studies have provided important preliminary data for our ongoing NIOSH-sponsored work.

In collaboration with Dr. Jill Poole (NIEHS KO8 awardee mentored by Dr. Romberger), we have performed a number of additional analyses of our swine CAFO dusts. Semi-quantitative elemental analysis by inductively coupled plasma-mass spectrometry revealed trace metals (B, Mg, Ti, Mn, Fe, Co, Ni, Cu, Rb, Mo, Zn). However, dust that is heat-inactivated at 120°C (a process that inactivates the biologics and leaves the metals intact) did not result in ust-induced inflammatory or immune changes, indicating that metals and particulates are likely not playing a critical role. Bacterial counts of the dust revealed that approximately 98% of the colonies were Gram-positive bacteria (Staphylococcus, Bacillus, Streptomycetes, Enterococci), while the remaining 2% were Gram-negative bacteria. Mass spectrometry analysis demonstrated that swine CAFO dust had 10 times more muramic acid (reflecting peptidoglycan typically from Gram-positive bacteria) than house dust, elevated levels of 3-hydroxy fatty acids indicating endotoxin (two-fold) and no difference in ergosterol (reflecting fungi). These data further support the potential importance of Gram-positive bacteria in swine CAFOs that has also been suggested by others.

Regarding Aim 2, our preliminary data suggesting that HDE-associated lysophosphatidic acid (LPA) modulates HDE-induced epithelial cell PKC activity and IL-8/IL-6 release have not proven correct. However, pursuit of this aim (comparing effects of LPA and HDE) has led to interesting observations regarding the effect of HDE on epidermal growth factor receptors (EGFRs) of airway epithelial cells. Cell membrane receptors (such as EGFRs) by which HDE initiates release of IL-6/8 and TNF-alpha are not clearly identified. Because several other inhaled agents induce airway epithelial cell responses through EGFR activation, we hypothesized that HDE would activate EGFRs and that EGFRs would be required for some of the responses to HDE. Indeed, exposure of BEAS-2B cells to HDE caused EGFR phosphorylation and downstream ERK activation, and the EGFR-selective kinase inhibitor, AG1478, blocked both responses. Both AG1478 and EGFR-neutralizing antibody reduced HDE-stimulated IL-6 and IL-8 release by about half. Similar EGFR phosphorylation and requirement for maximal IL-6 and IL-8 release were found with primary isolates of human bronchial epithelial cells. Because HDE-stimulated IL-6 and IL-8 release involve the Ca^{2+}-dependent PKCα, we hypothesized that HDE would induce intracellular Ca^{2+} mobilization. HDE exposure induced intracellular Ca^{2+} mobilization in Beas-2B cells, but this response was neither mimicked by EGF nor inhibited by AG1478. Thus,

HDE activates EGFRs and their downstream signaling, and EGFR activation is required for some but not all airway epithelial cell responses to HDE. This data has recently been submitted as a manuscript.

Regarding Aim 3 to identify mechanisms by which HDE augmentation of epithelial cell PKC *in vitro* mediates recruitment and adhesion of inflammatory cells to airway epithelium *in* vitro and in collaboration with Dr. Wyatt (co-Investigator), we have reported on the sequential activation of PKC isoforms in regulating cytokine release. HDE stimulates PKCα activity by 1 h, and within 6 h the activity returns to baseline. PKCα-specific inhibitor or PKCαDN cells, abolish this HDE-mediated effect. Both IL-6 and IL-8 release are likewise diminished under these conditions compared to normal human airway epithelial cells, and treatment with TNFα neutralizing antibody does not further inhibit cytokine release. In contrast, PKCε activity was enhanced by 6 h after HDE treatment. TNFα blockade abrogated this effect. HDE-stimulated IL-6, but not IL-8 release in PKCεDN cells. The concentration of TNFα released by HDE-stimulated airway epithelial cells is sufficient to have a potent cytokine-eliciting effect. A time course of TNFα release suggests that TNFα is produced after PKCα activation, but before PKCε. These results suggest a temporal ordering of events responsible for the release of cytokines, which initiate and exacerbate inflammatory events in the airways of people exposed to agricultural dust (reference # 4 in Publications section).

We have also performed experiments that have demonstrated that HDE augments neutrophil adhesion to airway epithelial cells *in vitro* (outlined in Specific Aim 3). We have observed that airway epithelial cells exposed to HDE support greater neutrophil adhesion that is concentration- and time-dependent. Pretreatment of neutrophils with HDE (instead of airway epithelial cells) does not alter neutrophil adhesion to cultured airway epithelial cells, demonstrating the importance of HDE influence on airway epithelial cells in this adhesion assay. The effect of HDE on neutrophil adhesion to airway epithelial cells is substantially inhibited by pretreatment with two PKC isoform pharmacological inhibitors (PKC-alpha inhibitor, Gö6976 and PKC-epsilon inhibitor RO-318220). Furthermore, airway epithelial cells expressing inactive PKC-alpha or PKC-epsilon did not support enhanced neutrophil adhesion when exposed to HDE, also demonstrating the role of these two PKC isoforms in mediating the HDE regulation of neutrophil adhesion to airway epithelial cells. We have previously reported that HDE enhances the expression of ICAM-1 of airway epithelial cells and neutrophil adhesion to airway cells was inhibited by ICAM-1 antibody. These experiments confirm that HDE exposure augments inflammatory cells, specifically neutrophil, adhesion to airway epithelial cells that is mediated by specific PKC isoforms (alpha and epsilon). These data were presented as an oral presentation at the May 2009 American Thoracic Society meeting. Additional studies this past year have demonstrated that HDE is augmenting TNF-alpha converting enzyme activity that also contributes to the neutrophil adhesion to airway epithelial cells and can be attenuated by exposure to ethanol. An abstract to the 2011 American Thoracic Society meeting will be submitted and a manuscript is under preparation on this most recent data.

Regarding Specific Aim 4, we have now published the effects of single versus repetitive dust-induced airway inflammation in mice by the intranasal exposure method. Mice were exposed to swine facility dust extract (DE) or saline once and once daily for 1 and 2 weeks. Dust exposure resulted in increased bronchoalveolar lavage fluid neutrophils and macrophages after single and repetitive exposures. Lavage fluid TNFα, IL-6, KC, and MIP-2 were significantly increased after single and repetitive dust exposures, but were dampened in 2-week dust-exposed mice as compared to single exposure. Dust exposure induced protein kinase C (PKC) alpha and epsilon activation in isolated tracheal epithelial cells, but was dampened with repetitive exposures. *Ex vivo* stimulation of alveolar macrophages from 2 week animals demonstrated reduced cytokine responsiveness and phagocytic ability. Significant lung pathology occurred with heterogeneity of mixed mononuclear cellular aggregates (T- and B-lymphocytes, phagocytes) after repetitive dust exposure, a novel observation. Airway hyper-responsiveness to methacholine occurred after single dust exposure, but resolved after 2 weeks. Collectively, intranasal exposure to DE results in significant lung inflammatory and pathologic responses marked by a modulated innate immune response to single and repetitive dust exposures that is associated with PKC activity (reference # 5 in Publications section). This mouse model will be used in

our ongoing NIOSH work to determine whether strategies aimed at manipulating specific cytokines, PKC activation, and protease activity will be useful in an *in vivo* setting.

To further delineate mechanisms by which dust from swine confinement facilities regulates cellular inflammatory processes and in collaboration with Dr. N. Parinandi (subcontract at Ohio State University) in conjunction with Aim 1, we examined swine dust (SD)-induced reactive oxygen species (ROS) formation by the RAW 264.7 and human monocyte-derived macrophages. SD (0.25-1 mg/ml), following 2h of treatment, induced maximal intracellular ROS generation (as measured by the DCFDA fluorescence) by both cell systems. Superoxide dismutase (SOD 100 units) and SOD mimetic (MnTBAP, 5μM) significantly attenuated SD-induced ROS generation by the macrophages. Diphenyleneiodonium (50 μM) and oxipurinol (100 μM) completely blocked SD-induced ROS formation by the cells, followed by significant but partial inhibition of the same offered by rotenone (100 μM), suggesting the role of NADPH oxidase and xanthine oxidase to a greater extent and respiratory electron transport to a lesser extent in SD-induced ROS generation by the macrophages. N-acetylcysteine (5 mM) and desferal (1 mM) significantly attenuated SD-induced ROS generation by the cells, indicating the role of thiol-redox and intracellular iron, therein. These data indicated that SD-induced ROS formation by the macrophages was mediated by NADPH oxidase, xanthine oxidase, and respiratory electron transport, involving thiol-redox and intracellular iron. We also demonstrated that SD induced MIP-1a/CCL3 and IL-8 cytokine release from macrophages, implicating the role of inflammation in the process. Furthermore, N-acetylcysteine, the thiol-redox antioxidant, attenuated the SD-induced cytokine secretion by the macrophages, suggesting the role of oxidative stress in mediating the cytokine secretion by macrophages. Although the role of ROS in airway epithelial cells has not been extensively studied with Dr. Parinandi, we have not found N-acetylcysteine or desferal to be effective in attenuating airway epithelial cell IL-6 and IL-8 release, suggesting that HDE regulates various types of cells using different mechanisms.

Because of this project, we established a new collaboration with Dr. Bidasee at UNMC. The purpose of the collaboration was to assess whether HDE contains components that are capable of binding to and modulating the activity of type 1 ryanodine receptor Ca2+-release channel (RyR1), a key regulator of skeletal muscle function. HDE collected from confinement facilities were extracted with chloroform, filtered, and rotary evaporated to dryness. Residues were resuspended in hexane-chloroform (20:1) and precipitates, referred to as HBDorg, were air-dried and studied further. In competition assays, HBDorg dose-dependently displaced [3H]ryanodine from binding sites on RyR1 with an IC50 of 1.5±0.1 microg/ml (Ki=0.4±0.0 microg/ml). In single-channel assays using RyR1 reconstituted into a lipid bilayer, HBDorg exhibited three distinct dose-dependent effects: first it increased the open probability of RyR1 by increasing its gating frequency and dwell time in the open state, then it induced a state of reduced conductance (55% of maximum) that was more likely to occur and persist at positive holding potentials, and finally it irreversibly closed RyR1. In differentiated C2C12 myotubes, addition of HBD triggered a rise in intracellular Ca2+ that was blocked by pretreatment with ryanodine. Because persistent activation and/or closure of RyR1 results in skeletal muscle weakness, these new data suggest that HDE is responsible, at least in part, for the muscle ailment reported by hog confinement workers. These data have been published (reference #1 in Publications section).

We have collaborated extensively with Dr. Jill Poole whose research focus is on determining mechanisms by which CAFO dust exposure influences immune function including adaptation responses. With her, we have observed that repeat HDE exposure modulates inflammatory mediator production in human monocytes independent of endotoxin, consistent with an adaptation response. In addition, the ability of PKC to be reactivated contributes to this response. We have also observed that repetitive organic dust exposure significantly decreases markers of antigen presentation and host defense function in monocyte-derived macrophages.

Conclusions: This project has provided data that dust in swine CAFOs has multiple components that are capable of inducing inflammatory mediators from airway epithelial cells including IL-6, IL-8 and TNF-alpha. Although endotoxin is recognized as a biological component of dust that results in inflammation and has been

monitored is some settings, our data supports that other components of dust are also important in inducing inflammation that can result in respiratory disease in workers. This suggests that monitoring of other components including components of Gram-positive bacteria may also have value in terms of better identifying environments within CAFOs that are likely to be associated with respiratory disease.

Our data have demonstrated that PKC isoforms (alpha and epsilon) are critically involved in dust extract stimulation of inflammatory cytokines of airway epithelial cells *in vitro* as well as interactions between epithelial cells and neutrophils. In addition, we have developed an animal model of dust exposure that supports that our *in vitro* observations are relevant to an *in vivo* setting. Our mechanistic studies including use of the animal model have set the stage for us to continue translational studies aimed at determining how we can mediate dust-induced PKC activation in the airway with a goal of defining novel therapeutic approaches to lung disease in CAFO workers.

Publications

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Under review: Dodmane PR, Schulte NA, Heires AJ, Band H, Romberger DJ, Toews ML. Airway Epithelial EGF Receptor Mediates Hogbarn Dust-Induced Cytokine Release But Not Ca2+ Response. (submitted to Am J Respir Cell Mol Biol.)

Inclusion of Gender and minority study subjects
Not applicable.

Inclusion of children
Not applicable.

Materials available for other investigators
Published materials and methods are available to other investigators.

FINANCIAL STATUS REPORT

(Short Form)

(Follow instructions on the back)

1 Federal Agency and Organizational Element to Which Report is Submitted	2 Federal Grant or Other Identifying Number Assigned By Federal Agency	OMB Approval No.	Page	of
DHHS/CDC/NIOSH	5 R01 OH008539-04		1	1 pages

3 Recipient Organization (Name and complete address, including ZIP code)

University of Nebraska Medical Center
Sponsored Programs Accounting
985100 Nebraska Medical Center
Omaha, NE 68198-5100

4 Employer Identification Number	5 Recipient Account Number or Identifying Number	6. Final Report	7. Basis
1470049123A6	34-5237-2006-004	☐ Yes ☒ No	☒ Cash ☐ Accrual

8 Funding/Grant Period (*See instructions*) From: (Month, Day, Year)	To: (Month, Day, Year)	9. Period Covered by this Report From: (Month, Day, Year)	To: (Month, Day, Year)
8/1/2006	7/31/2010	8/1/2009	7/31/2010

10 Transactions	I Previously Reported	II This Period	III Cumulative
a. Total outlays	958,110.67	343,524.90	1,301,635.57
b. Recipient share of outlays	-		-
c. Federal share of outlays	958,110.67	343,524.90	1,301,635.57
d. Total unliquidated obligations			-
e. Recipient share of unliquidated obligations			-
f. Federal share of unliquidated obligations			-
g. Total Federal share *(sum of lines c and f)*			1,301,635.57
h. Total Federal funds authorized for this funding period			1,360,000.00
i. Unobligated balance of Federal funds *(Line h minus line g)*			58,364.43

11 Indirect Expense	a. Type of Rate *(Place "X" in appropriate box)* ☐ Provisional ☒ Predetermined ☐ Final ☐ Fixed				
	b. Rate	c. Base	d. Total Amount		e. Federal Share
	47.00%	838,399.81	394,047.91		394,047.91

12 *Remarks: Attach any explanations deemed necessary or information required by Federal sponsoring agency in compliance with governing legislation*

University of Nebraska Medical Center, under Expanded Authority, will carry forward the unobligated balance of $58,364.43

13 Certification: **I certify to the best of my knowledge and belief that this report is correct and complete and that all outlays and unliquidated obligations are for the purposes set forth in the award documents.**

Typed or Printed Name and Title		Telephone (Area code, number and extension)
Alan Reeves	Accountant, Sponsored Programs Accounting	(402) 559-5818
Signature of Authorized Certifying Official		Date Report submitted
Alan Reeves		10/21/2010

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Prescribed by OMB Circulars A-102 and A-110

Form Approved Through 06/30/2012
OMB No. 0925-0001

Department of Health and Human Services

Final Invention Statement and Certification

(For Grant or Award)

DHHS Grant or Award No.

5R01OH008539-04

A. We hereby certify that, to the best of our knowledge and belief, all inventions are listed below which were conceived and/or first actually reduced to practice during the course of work under the above-referenced DHHS grant or award for the period

08/01/2006 *original effective date* through 07/31/2010 *date of termination*.

B. Inventions (Note: If no inventions have been made under the grant or award, insert the word *"NONE"* under Title below.)

NAME OF INVENTOR	TITLE OF INVENTION	DATE REPORTED TO DHHS
None		
(Use continuation sheet if necessary)		

C. Signature — This block ***must*** be signed by an official authorized to sign on behalf of the institution.

Title
Director, Sponsored Programs Administration

Typed Name
Deborah K. Vetter

Signature
Deborah K. Vetter

Date
10/29/10

Name and Mailing Address of Institution

University of Nebraska Medical Center
987835 Nebraska Medical Center
Omaha, NE 68198-7835

HHS 568 (Rev. 06/09)