FINAL REPORT

The Effect of Smoke from Burning Vegetative Residues on Airway Inflammation and Pulmonary Function in Healthy, Asthmatic, and Allergic Individuals

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Abstract

It is common practice worldwide to use open field burning to clear agricultural fields of rice straw and other vegetative residues, and for disease and pest control. This burning produces smoke, which contains respirable particulate and gaseous components. Currently, there is no specific information available on the direct effect of rice straw smoke (RSS) exposure on respiratory health effects, even though epidemiological data indicate that this issue requires investigation. The objective of this project was to investigate the effects of controlled exposure to RSS on human respiratory health. *Specific Aim One:* To determine the effect of a single exposure to RSS at two different concentrations on airway inflammation and spirometric pulmonary function. *Specific Aim Two:* To determine the effect of the RSS exposure format (single- vs. serial-day) on airway inflammation and spirometric pulmonary function. *Specific Aim Three:* To determine the effect of asthma and allergic rhinitis status on the airway inflammatory and spirometric pulmonary function responses to RSS exposure. The hypotheses of this project were: 1) that airway inflammation would be increased, and spirometric pulmonary function would be decreased, as a function of increased RSS concentration and dose; 2) that the RSS-induced changes in airway inflammation and spirometric pulmonary function would be increased in asthmatic and allergic individuals as compared to healthy individuals. This project consisted of three separate controlled human exposure experiments. Each experiment utilized 15 subjects; Experiment One used healthy individuals; Experiment Two used individuals with mild to moderate asthma; Experiment Three used individuals with allergic-rhinitis. The exposure conditions were single exposures to Filtered-Air (FA), and RSS at concentrations of 200 $\mu g/m^3$ (RSS-200), and 600 $\mu g/m^3$ (RSS-600), and three serial-day exposures to RSS at a concentration of 200 $\mu g/m^3$ (RSS-3x200). All exposures were of 30 min duration. Bronchoscopy was conducted 6-hr post-exposure, and spirometric pulmonary function was measured pre- and post-exposure, and pre-bronchoscopy. Results: *Cells:* Experiment One: In the bronchial fraction [(Bfx); first 15 ml of bronchoalveolar lavage (BAL)], for both RSS-600 and RSS-3x200, compared to FA, there was a significantly higher total leukocyte concentration, and for RSS-600, compared to FA, there was a significantly higher macrophage concentration. In the Bfx, for RSS-3x200, compared to both FA, and RSS-600, there was a significantly higher percent, and concentration of lymphocytes, and significantly lower percent of macrophages. Experiment Two: In the Bfx, for RSS-3x200, compared to RSS-600, there was a significantly higher neutrophil concentration. In the BAL, for RSS-3x200, compared to both FA, and RSS-600, there was a significantly higher lymphocyte concentration. Experiment Three: In the BAL, for RSS-600 compared to both FA, and RSS-3x200, there was a significantly lower percent, and concentration of epithelial cells. FA Condition: The Asthma subject group, compared to both the Healthy subject group, and the Allergic Rhinitis subject group; in the BAL had a significantly higher percent, and concentration of neutrophils. RSS-600 Condition: The Allergic Rhinitis subject group, compared to the Healthy subject group; in the BAL had a significantly higher percent, and concentration of lymphocytes. RSS-3x200 Condition: The Asthma subject group, compared to both the Healthy subject group, and the Allergic Rhinitis subject group; in the BAL had a significantly higher

percent, and concentration of neutrophils; and compared to the Allergic Rhinitis subject group, in the Bfx, had significantly higher percent, and concentration of neutrophils. The Allergic Rhinitis subject group, compared to the Healthy subject group, in BAL, had a significantly higher percent of neutrophils. *Cytokines*: Experiment One*:* In the Bfx, for RSS-3x200, compared to RSS-600, there was a significantly higher interleukin (IL)-8 concentration. Experiment Two*:* In the Bfx and BAL, for all of the conditions, there were no significant differences in the tumor necrosis factor-alpha (TNF-α), IL-8, or monocyte chemoattractant protein-1 (MCP-1) concentration. Experiment Three*:* In the Bfx and BAL, for all of the conditions, there were no significant differences in the TNF-α, IL-8, or MCP-1 concentration. *Spirometric pulmonary function*: For each of the three experiments, there were no physiologically relevant differences, either with-in or between conditions, or between experiments. The results of this project indicate that RSS is capable of inducing airway inflammation in healthy individuals, and in individuals with asthma, or allergic rhinitis.

Executive Summary

Introduction: It is common practice worldwide to use open field burning for the clearance of agricultural fields of rice straw and other vegetative residues. Rice is a major crop in California, particularly in the Central Valley region. Due to the large acreage of rice fields, there are high costs involved in both removing the rice straw post-harvest, and for pest and disease control. Burning of rice straw fields is used to attempt to deal with these issues. However, there is concern from the residential, scientific, and regulatory communities that this burning and subsequent smoke exposure in humans could result in adverse health effects. This burning produces smoke, which contains airborne respirable particulate and gaseous components. In situations where incorrect predictions (weather forecasts), or changes, in climatic conditions (wind direction, temperature inversions) occur, the smoke from these burns moves into inhabited areas, where it could induce adverse health effects. Also, workers involved in rice farming can be exposed to RSS in occupational settings, which could also result in adverse health effects. Currently, there is no specific information available on the direct effect of RSS exposure on respiratory health effects, even though epidemiological data indicate that this area requires investigation.

Objective and Specific Aims and Hypothesis: The overall objective of this project was to investigate the effects of inhaled RSS on human respiratory health. *Specific Aim One:* To determine the effect of an acute exposure to RSS at two different concentrations on airway inflammation and spirometric pulmonary function. *Specific Aim Two:* To determine the effect of total RSS exposure format (single vs. serial day) on airway inflammation and spirometric pulmonary function. *Specific Aim Three:* To determine the effect of asthma and allergic rhinitis status on the airway inflammatory and spirometric pulmonary function responses to RSS exposure. The hypotheses of this project were: 1) that airway inflammation, as indicated by cell distribution (macrophages, neutrophils), and cytokine protein expression [tumor necrosis factor-alpha (TNF-α), interleukin (IL)-8, monocyte chemoattractant protein-1 (MCP-1)], would be increased, and spirometric pulmonary function [forced vital capacity (FVC), forced expired volume in 1 sec (FEV_1), forced expired flow-rate between 25 to 75% of FVC (FEF_{25-75}), forced expired flow-rate between at 75% of FVC (FEF_{75})], decreased, as a function of increased smoke concentration and dose; 2) that the RSS-induced changes in airway inflammation and spirometric pulmonary function would be increased in asthmatic and allergic individuals as compared to healthy (non-asthmatic, non-allergic) individuals.

Materials and Methods: This project consisted of three separate controlled human exposure experiments. Each experiment utilized 15 subjects; *Experiment One* used healthy non-allergic non-asthmatics; *Experiment Two* used individuals with mild to moderate asthma; *Experiment Three* used individuals with allergic-rhinitis. The exposure conditions were single-day exposures to Filtered-Air (FA), and RSS at concentrations of 200 $\mu g/m^3$ (RSS-200), and 600 $\mu g/m^3$ (RSS-600), and a serial (3)-day exposure to RSS at a concentration of 200 $\mu g/m^3$ (RSS-3x200). The duration of all the exposures was 30 min. *Independent Variables:* The independent variables were the exposure conditions: 1) FA; single exposure; 2) RSS at 200 $\mu g/m^3$; single exposure;

RSS-200; 3) RSS at 600 μg/m^3; single exposure RSS-600; 4) RSS at 3x200 μg/m^3; three serial-day exposures (RSS-3x200); and the subject groups: 1) Healthy; 2) Asthma; 3) Allergic Rhinitis. *Dependent Variables:* The dependent variables measured were: 1) Cells in the airway (bronchoscopy for Bfx and BAL; 6 h post-exposure); 2) Cytokine/chemokine protein; 3) Spirometric pulmonary function; 4) Airway inflammation grading; 5) Symptoms.

Results: Cell Distribution: *Experiment One*: In the bronchial fraction [(Bfx); first 15 ml of bronchoalveolar lavage (BAL)], for both RSS-600 and RSS-3x200, compared to FA, there was a significantly higher total leukocyte concentration, and for RSS-600, compared to FA, there was a significantly higher macrophage concentration. In the Bfx, for RSS-3x200, compared to both FA, and RSS-600, there was a significantly higher percent, and concentration of lymphocytes, and significantly lower percent of macrophages. *Experiment Two:* In the Bfx, for RSS-3x200, compared to RSS-600, there was a significantly higher neutrophil concentration. In the BAL, for RSS-3x200, compared to both FA, and RSS-600, there was a significantly higher lymphocyte concentration. *Experiment Three:* In the BAL, for RSS-600 compared to both FA, and RSS-3x200, there was a significantly lower percent, and concentration of epithelial cells. Between Experiments*:* FA Condition: The Asthma subject group, compared to both the Healthy subject group, and the Allergic Rhinitis subject group; in the BAL had a significantly higher percent, and concentration of neutrophils; and compared to the Healthy subject group, in both the Bfx and BAL, had a significantly lower percent of macrophages. The Allergic Rhinitis subject group, compared to the Asthma subject group; in the BAL had a significantly higher percent of epithelial cells. RSS-600 Condition: The Allergic Rhinitis subject group, compared to the Healthy subject group; in the BAL had a significantly higher percent, and concentration of lymphocytes, and a significantly lower concentration of epithelial cells; and in the Bfx, had a significantly lower percent of macrophages. The Asthma subject group, compared to the Healthy subject group, in the Bfx had a significantly lower percent of macrophages, and in the BAL, had a significantly lower percent of epithelial cells. RSS-3x200 Condition: The Asthma subject group, compared to both the Healthy subject group, and the Allergic Rhinitis subject group; in the BAL had a significantly higher percent, and concentration of neutrophils; and compared to the Allergic Rhinitis subject group, in the Bfx, had significantly higher percent, and concentration of neutrophils. The Allergic Rhinitis subject group, compared to the Healthy subject group, in BAL, had a significantly higher percent of neutrophils. Cytokines: *Experiment One:* In the Bfx, for RSS-3x200, compared to RSS-600, there was a significantly higher IL-8 concentration. In the BAL, for RSS-3x200, compared to both RSS-200, and RSS-600, there was a significantly lower MCP-1 concentration. In both the Bfx and BAL, for all of the conditions, there were no significant differences in the TNF-α concentration. *Experiment Two:* In the Bfx and BAL, for all of the conditions, there were no significant differences in the TNF-α, IL-8, or MCP-1 concentration. *Experiment Three:* In the Bfx and BAL, for all of the conditions, there were no significant differences in the TNF-α, IL-8, or MCP-1 concentration. *Between Experiments:* There were no differences between experiments in TNF-α, IL-8, or MCP-1 concentration. Spirometric Pulmonary Function: For each of

the three experiments, there were no physiologically relevant differences, either with-in or between conditions, or between experiments, in FVC, FEV_1, FEF_{25-75}, or FEF_{75}.

Discussion: The results of the project support the airway inflammation component of the hypothesis, but not the spirometric pulmonary function component. The RSS exposure resulted in changes in the cell distribution, both within, and between, the three subject groups, but no measurable change in spirometric pulmonary function. The Asthma subject group, compared to both the Healthy and Allergic Rhinitis groups, exhibited pre-existing inflammation, having increased airway neutrophils in the FA condition. This increased number of neutrophils in the Asthma group was also present in the RSS-600 condition, indicating a greater overall inflammatory response, which could be a function of a either or both the pre-existing inflammation or the response to the RSS. The RSS-induced changes in cell distribution were present in both the Bfx and BAL, indicating that inhaled components of RSS are penetrating both the bronchial and alveolar regions of the airways. Several indices of inflammation, including increases in neutrophils, epithelial cells, and IL-8, indicate that the serial-day RSS exposure provided the highest inflammatory stimulus of the exposure conditions, specifically compared to the single 600 $\mu g/m^3$ exposure.

The results of this project indicate that RSS is capable of inducing airway inflammation in healthy individuals, and in individuals with asthma, or allergic rhinitis. Further experiments are required delineate the toxic component(s) of RSS, the threshold concentrations and doses for exposure regulation, and the effects on other susceptible populations, including individuals with cardiopulmonary disease.

Introduction

It is common practice worldwide to use open field burning for clearing, and disease and pest control, in agricultural fields of rice straw and other vegetative residues. Rice is a major crop in California, particularly in the Central Valley region. Due to the large acreage of rice fields, there are high costs involved in both removing the rice straw post-harvest, and for pest and disease control. Burning of rice straw fields is used to attempt to deal with these issues. However, there is concern from the residential, scientific, and regulatory communities that this burning and subsequent smoke exposure in humans could result in adverse health effects. This burning produces smoke, which contains airborne respirable particulate and gaseous components. In situations where incorrect predictions (weather forecasts), or changes, in climatic conditions (wind direction, temperature inversions) occur, the smoke from these burns moves into inhabited areas, where it could induce adverse health effects. Exposure of the general population to rice straw smoke (RSS) could occur in both outdoor and indoor environments, and could result in both acute and chronic adverse health effects. Also, workers involved in rice farming can be exposed to RSS in occupational settings, which could also result in adverse health effects. Currently, there is no specific information available on the direct effect of RSS exposure on respiratory health effects, even though epidemiological data indicate that this issue requires investigation.

Epidemiological Studies:

There are currently four studies in the published literature, which have investigated the association between ambient RSS levels and human population heath effects. In Butte County, California, during the period of 1983-1992, 690,000 acres of rice straw was burned (82% of the total planted acreage). During this period the maximum concentration of airborne particulate matter with a mean mass aerodynamic diameter (MMAD) < 10 μm (PM_{10}), was elevated to 636 $\mu g/m^3$. As a function of the amount of acres of rice stubble burned, there was a significant increase in the risk for hospitalization (using daily asthma hospitalizations), and asthma morbidity (Jacobs *et al.* 1997).

In Winnipeg, Canada, in October 1992, there was an air pollution episode, due to smoke from agricultural fields were straw and stubble were being burned. This episode produced markedly elevated levels of PM_{10}, to a maximum concentration of 200 $\mu g/m^3$, and of carbon monoxide, nitrogen dioxide, and volatile organic compounds (VOC). From participants on the on-going Lung Health Study, whom had mild to moderate airway obstruction [mean forced expiratory volume in one sec. (FEV_1) = 73% predicted], and airway hyper-reactivity; 42% had onset or worsened cough, wheezing, chest tightness, or shortness of breath, and 20% had trouble breathing during the episode. Importantly, participants with asthma or chronic bronchitis were most likely to be effected (Long *et al.* 1998).

In Niigata Prefecture, Japan, throughout the period 1994 to 1998, during the rice straw burning season, the maximum concentration of PM_{10}, was elevated to over 410 $\mu g/m^3$. During this period, there was an increase in the number of asthma attack visits to the emergency room, and asthma attack hospital admissions in children (Torigoe *et al.* 2000). There was also a significant correlation between the ambient concentration of PM_{10}, and the number of asthma attacks in children (Torigoe *et al.* 2000).

In the rural areas of Isfahan, Iran, during a rice farm residue burning episode in October 2000, the concentrations of total particulate and PM_{10} were doubled. Compared to the pre-smoke period, post-smoke there was an increase in recent asthma attacks, asthma medication use, sleep disruption due to dyspnea and cough, exercise-induced cough, and a decrease in FEV_1, FEV_1/forced vital capacity (FVC)% (FEV_1/FVC), peak expiratory flow rate (PEFR), and the forced expiratory flow rate between 25-75% of FVC (FEF_{25-75}) (Golshan *et al.* 2000).

In addition to ambient exposure in the general population, exposure to RSS is a health issue in occupational settings. In the one study investigating this issue, California rice farmers reported chronic cough which was associated with hours per year engaged in burning rice stubble, and the rice farmers, compared to the general population, had an increased prevalence of asthma (McCurdy *et al.* 1996).

In addition to rice straw, multiple other forms of biomass are burned, both intentionally and accidentally, for many reasons in regions throughout the world. The combustion of these biomass fuels also results in smoke to which humans are exposed. In many countries, wood, crop residues, and animal dung are used for both heating and cooking in domestic situations (Reviewed; Zelikoff *et al.* 2002). Also, in the USA and Canada there has been an increase in the use of wood burning for heating, potentially due to an increase in the cost of other energy sources (Reviewed; Zelikoff et al. 2002).

In South-East Asia, during the period 29-September to 27-October, 1997, biomass fires produced smoke to which local inhabitants were exposed. These fires increased the levels of PM_{10}, from a baseline mean of < 50 $\mu g/m^3$, to a maximum mean concentration of 125 $\mu g/m^3$, with a peak concentration of 216 $\mu g/m^3$. The level of PM_{10} was significantly associated with increased peripheral blood band neutrophils (an indicator of neutrophil precursors from bone marrow) (Tan *et al.* 2000), and interleukin (IL)-1β, IL-6, and granulocyte and macrophage-colony stimulating factor (GM-CSF) (van Eeden *et al.* 2001)

Studies of accidental and occupational smoke exposures indicate that changes in airway inflammation and spirometric pulmonary function can occur as a function of ambient smoke inhalation. A group of subjects with accidental acute smoke inhalation (defined as exposure to toxic products of combustion), had an increased percent of neutrophils in bronchoalveolar lavage (BAL) fluid, and decreased chemotactic migration of alveolar macrophages, compared to separate control groups of cigarette smokers and non-cigarette- smokers (Demarest *et al.*, 1979). Similarly, a group of fire victims, all cigarette smokers, with smoke inhalation injury, had an increased total cell count, and

percent of neutrophils in BAL, compared to a control group of cigarette smokers (Riyami *et al.* 1990). Further, chronic exposure to fire smoke, as measured in a group of fire fighters, none of whom were cigarette smokers, was associated with an increased percent of lymphocytes and fibronectin and hyaluronic acid concentration in BAL, compared to a control group of non-cigarette smokers (Bergstrom *et al.* 1997). Following smoke exposure during fire overhaul, a group of firefighters had an increase in IL-10, an anti-inflammatory cytokine, as measured in inducted-sputum, and an increase in Clara-cell protein and surfactant-associated protein in serum, indicating an increase in lung permeability (Burgess *et al.* 2002).

Spirometric pulmonary function has been monitored in wildland/forest firefighters exposed to smoke during work shifts. Cross-shift analyses showed there were significant decreases in FVC, FEV_1, FEF_{25-75} from pre-shift, to both mid-shift and post-shift (Betchley *et al.*, 1997). Similarly, cross-season analyses, showed that there were significant decreases in FEV_1 (Betchley *et al.*, 1997), and FVC, FEV_1, and FEF_{25-75}, and an increase in non-specific airway responsiveness (Liu et al., 1992) from pre- to post season.

As respirable particulate matter is a major component of RSS, data concerning the association of ambient particulate matter and respiratory and general health are also relevant in assessing the possible adverse health effects of RSS exposure. Epidemiological studies indicate that there are positive associations between the level of ambient particulate matter air pollution and population health effects. For PM_{10}, there are positive associations between particulate levels and decreased spirometric pulmonary function (Pope *et al.,* 1991; Stern *et al.,* 1989; Pope and Kranner, 1993); increased number of asthma attacks (Whittemore and Korn, 1980; Schenker, 1993); increased asthma medication usage (Pope *et al.,* 1991); increased emergency room visits for respiratory illness; increased hospital admissions (Greenburg *et al.,* 1967; Knight *et al.,* 1989; Martin, 1964; Samet *et al.,*1981); and increased daily mortality (Dockery *et al.,* 1993, Schwartz, 1993). These positive associations between PM_{10} exposure and health effects have been found at particle concentrations below the current USA national standard of 150 $\mu g/m^3$ (24 h average).

Controlled Human Exposure Experiments:

Currently there are no known studies, which have assessed human airway or pulmonary responses as a function of exposure to RSS under controlled laboratory conditions, either in healthy individuals, or in potentially susceptible individuals including those with asthma or allergic rhinitis. In a case study, using an semi-controlled exposure, a subject with chronic asthma, was exposed to RSS produced from burning approximately 300 g of rice straw, while the subject was situated approximately 5 m away, for approximately 5 min (Torigoe *et al.* 2000). In this subject, this exposure decreased peak expiratory flow (PEF) from pre-exposure (640 l/min), at both 20 min (380 l/min), and 10 h (400 l/min) post-exposure, despite inhaled bronchodilator medication being taken at both 25 min and 7 h post-exposure (Torigoe *et al.* 2000). The

exposure induced an asthma attack, which continued for 48 h after the exposure (Torigoe *et al.* 2000).

Like RSS, environmental tobacco smoke (ETS), is a product of combustion of vegetative matter, the constituents of which include toxic particles (typically < 1.0 μm in diameter), and multiple toxic gases (NCI, 1999). A multitude of chemical constituents of both the particle and gas phases of RSS and ETS are common in both smokes (CARB, 1996; CEPA, 1977). Controlled human exposures to ETS have resulted in no change in cellular or protein indices of inflammation in individuals with asthma (Nowak *et al.* 1997), but do result in a decrease in exhaled nitric-oxide, an indicator of inflammation (Yates *et al.* 2001). Controlled human exposures to ETS, result in inconsistent changes in spirometric pulmonary function in healthy individuals, and decreases of up to 20% in spirometric pulmonary function in individuals with asthma (Reviewed; NCI, 1999).

Given that particulate matter is a major component of RSS, data from experiments investigating the effect of inhaled particles and aerosols are relevant in the assessment of the potential pulmonary effects of RSS. Controlled exposure of healthy subjects to concentrated ambient air particles has resulted in an increase in neutrophils in BAL, with no associated change in spirometric pulmonary function (Ghio *et al.* 2000). Controlled exposures to sodium chloride or zinc ammonium sulfate in non-asthmatic subjects (Kleinman *et al.*, 1985), or to ammonium nitrate (Kleinman *et al.*, 1980), or highly pure carbon black (Anderson *et al.*, 1992), in both non-asthmatic and asthmatic individuals resulted in no changes in spirometric pulmonary function. Similarly, exposure of asthmatic subjects to sodium bisulfate, ammonium sulfate, or ammonium bisulfate did not change spirometric pulmonary function, with the exception of ammonium bisulfate at a concentration of 450 $\mu g/m^3$, which resulted in a decrease in specific airway conductance (Utell *et al.*, 1983). Although these studies did not show any changes in spirometric pulmonary function due to the particle exposure, no attempts were made to investigate whether airway inflammation was induced. Assessment of the correlation between acute ozone-induced changes in FVC and FEV_1 and subsequent airway inflammation have shown either, no correlation, or a negative correlation, between indices of spirometric pulmonary function and airway inflammation (Schelegle *et al.,* 1991, Aris *et al.,* 1993, Balmes *et al.,* 1996). It is therefore crucial to assess inflammation at the cellular and biochemical levels, as well to perform spirometric pulmonary function tests to determine health effects of inhaled particles.

Rice Straw Smoke Composition:

Rice straw smoke is a mixture of respirable particles and gases, and the characteristics of the smoke at any specific time is a function of the:

1) composition of the rice straw; which is controlled by factors including the rice variety, and the growing conditions, both of which will effect the chemical composition and total water content.
2) straw burning conditions; including combustion temperature and ambient temperature, humidity, and airflow (wind).
3) time the smoke has been in ambient conditions; which will effect total water content, particle and gas concentrations, and particle size (disintegration, aggregation)

Particle Composition:

In RSS generated from a controlled burn designed to simulate open-field burns, the particulate matter included the following components and distribution: carbon 39.4%; nitrogen 1.1%, and sulfur 1.0% (weight) (CARB, 1996). The major component, carbon, was present in both the inorganic and organic forms, each with its respective solubility. Inhaled particles can have toxic effects in the airway epithelium by virtue of initial impact and time of residence. These toxic effects can be modified as a function of the chemical composition of the particle, particle solubility which effects residence time, and toxic components independently producing airway inflammation. As carbon is insoluble in the airway, this component of the inhaled RSS particulate matter will not dissolve in the airway and will require clearance by phagocytosis involving macrophages and neutrophils, and/or by direct mucocillary clearance processes associated with the respiratory tract lining. An extended residence time could potentially increase the inflammatory effects of the particles. Additional description of the particulate component of RSS is given in Part II of this report.

Particle Size:

In RSS generated from a controlled burn designed to simulate open-field conditions, the particle size distribution was: 91% (cumulative mass fraction) < 7.96 μm dia.; 85% < 4.24 μm; 79% < 2.24 μm; 77% < 1.29 μm; 72% < 0.76 μm; 66% < 0.40 μm (CARB, 1996). Air pollutant particles can be categorized into three groups by approximate MMAD ranges; ultra-fine = < 0.2 μm, fine = 0.2-2.5 μm, and coarse 2.5-30 μm. Particle size is the primary characteristic of inhaled particles that determines both deposition location and regional number of particles (Schlesinger, 1989). Ultra-fine particles have high number concentrations for a given mass concentration, compared to fine and coarse particles, this high number being proposed as the major reason for the potentially greater inflammatory effects of ultra-fine particles (Utell *et al.*, 1997). Ultra-fine and fine particles sized < 0.5 μm have the potential to penetrate into the more distal areas of the pulmonary tract, and therefore have a larger total surface area on which to deposit as compared to particles > 0.5 μm. However, in the size range 0.2-0.5 μm, higher deposition rates do not occur as particles in this range air transported similarly to

a gas, and are mainly exhaled and therefore do not deposit in the pulmonary tract (Schlesinger, 1989). Coarse particles are deposited primarily in the large airways (Schlesinger, 1989). Given the range of the particle size distribution, particles in inhaled RSS will be expected to deposit throughout the respiratory tract. However, as the majority of particles are in the fine and ultra-fine sizes (approx 70% of mass), a large fraction would be expected to be deposited in the distal airways and alveoli. Additional description of the particulate component of RSS is given in Part II of this report.

Gaseous Components:

The major gaseous components of RSS are carbon monoxide, carbon dioxide, nitric oxide, nitrogen oxides, and sulfur dioxide (CARB, 1996). Of these, carbon monoxide inhibits oxygen transport in the body, binding to hemoglobin (to form carboxyhemoglobin), with approximately 230 times the affinity that oxygen binds to hemoglobin (to form oxyhemoglobin); nitrogen dioxide results in airway inflammation in healthy individuals, as indicated by an increase in neutrophils in the bronchial fraction [(Bfx); first 15 ml of BAL] (Solomon *et al.*, 1997); and sulfur dioxide produces bronchoconstriction in asthmatic individuals (Jaeger *et al.*, 1979; Shepard *et al.*, 1980). Additional description of the gaseous component of RSS is given in Part II of this report.

Particle and Gas Concentrations:

The current USA national standard for ambient PM_{10} is 150 $\mu g/m^3$ for a 24-h average, and 50 $\mu g/m^3$ for an annual average. The California State standard is 50 $\mu g/m^3$ for a 24-h average, and 20 $\mu g/m^3$ for an annual average. As particle concentration directly affects the total inhaled dose of particles, for any specific exposure time, the particle concentration is a primary factor in the potential toxicity of inhaled particles. The concentration of particles from RSS in the open environment is controlled by many factors, which include the initial concentration, disintegration, aggregation, dispersion, and the suspension time. During a smoke episode in Sacramento County in November 1994, the PM_{10} level reached an hourly peak of 182 $\mu g/m^3$, and the baseline PM_{10} level during this period of approximately 20-30 $\mu g/m^3$ (CARB, 1997). In Butte County, during the period of 1983-1992 the maximal daily PM_{10} concentration was 636 $\mu g/m^3$, and the mean baseline PM_{10} level during this period was 34.3 $\mu g/m^3$ (Jacobs *et al.* 1997). Therefore, for controlled exposure experiments, RSS concentrations of 200 $\mu g/m^3$ and 600 $\mu g/m^3$ would provide exposures that are within the range of relevant ambient concentrations. Also, based on controlled human exposures to another ambient smoke, environmental tobacco smoke, the particle concentrations of 200 $\mu g/m^3$ and 600 $\mu g/m^3$ to be used in this project approximate indoor and outdoor ambient particle concentrations (NCI, 1999). Importantly these concentrations would be expected to be appropriate for subject safety for acute exposures. In situations where the RSS concentration is determined by the particle concentration, the concentrations of the gases in the smoke will be a function of the straw composition and burning conditions, for the specific particle concentration. Additional description of the particulate and gaseous components of RSS is given in Part II of this report.

Asthma and Allergy Status and Rice Straw Smoke Exposure:

Under ambient conditions, both healthy individuals, and individuals with pre-existing airway patho-physiology are exposed to RSS. Individuals with asthma and allergic rhinitis, have pre-existing airway inflammation and hyper-reactivity, and compromised spirometric pulmonary function, which could produce a higher risk for RSS-induced changes in these factors. Therefore, it is expected that individuals with asthma and allergic rhinitis will elicit increased inflammatory and spirometric pulmonary function responses to RSS, as compared with healthy individuals.

Indices of Airway Inflammation:

Changes in differential leukocyte cell counts (specifically increased neutrophils and macrophages), in BAL are used as the primary measure of airway inflammation following air pollutant exposures (Balmes *et al.*, 1996; Solomon *et al.*, 2000). Activation of alveolar and airway macrophages following phagocytosis of deposited particles may play an important role in mediating the recruitment of other inflammatory leukocytes and therefore effect inflammation and cellular immunity. Assessment of the phagocytic activity and respiratory burst activity of macrophages provides measures of cellular function important for assessment of particle and gas exposures. The following biochemical components measured in the fluid phase of BAL are indicative of airway inflammation and/or injury: tumor necrosis factor-alpha (TNF-α), is an early-phase pro-inflammatory cytokine; IL-8 is a potent neutrophil chemoattractant; macrophage chemoattactant protein-1 (MCP-1) is a phagocyte chemoattractant.

The timing of the measurement of these indices of airway inflammation is important so that a potential change in a variable is not missed. Inhaled toxin-induced changes in cells and cytokines in BAL have been measured at; 6 h (Sundeep *et al.* 2000), and 18 h (Rudell *et al.* 1999), post-exposure to diesel exhaust; 18 h post-exposure to concentrated ambient particles (Giho *et al.* 2000); at 1 h, 6 h, and 24 h post-exposure to ozone in healthy individuals (Schelege *et al.* 1991); and 18 h post-exposure to ozone in individuals with asthma (Balmes *et al.* 1996). Although the exact sequence is unknown, inhaled toxin-induced changes in airway pro-inflammatory cytokines are expected to occur prior to changes in cell distribution. As the exact time-dynamics of the cell and cytokine responses to RSS are unknown, a 6 h post-exposure measurement time would provide a reasonable interval for measurement of both the potential cell and cytokines changes.

Objective and Specific Aims:

The overall objective of this project was to investigate the effects of inhaled RSS on human respiratory health.

Specific Aim One:

To determine the effect of an acute exposure to RSS at two different concentrations on airway inflammation and spirometric pulmonary function.

Specific Aim Two:

To determine the effect of total RSS exposure format (single vs. serial day) on airway inflammation and spirometric pulmonary function.

Specific Aim Three:

To determine the effect of asthma and allergic rhinitis status on the airway inflammatory and spirometric pulmonary function responses to RSS exposure.

Hypotheses

The hypotheses of this project were: 1) that airway inflammation, as indicated by cell distribution (macrophages, neutrophils), and cytokine protein expression (TNF-α, IL-8, MCP-1), would be increased, and spirometric pulmonary function (FVC, FEV_1, FEF_{25-75}, FEF_{75}), decreased, as a function of increased smoke concentration and dose; 2) that the RSS-induced changes in airway inflammation and spirometric pulmonary function would be increased in asthmatic and allergic individuals as compared to healthy (non-asthmatic, non-allergic) individuals.

Materials and Methods

Design:

This project consisted of three separate controlled human exposure experiments. Each experiment utilized 15 subjects; Experiment One used healthy individuals with no asthma or allergic rhinitis; Experiment Two used individuals with mild to moderate asthma; Experiment Three used individuals with allergic-rhinitis. The exposure conditions were single exposures to Filtered-Air (FA), and RSS at concentrations of 200 $\mu g/m^3$ (RSS-200), and 600 $\mu g/m^3$ (RSS-600), and three serial-day exposures to RSS at a concentration of 200 $\mu g/m^3$ (RSS-3x200). The serial-day exposure was used to provide a different total dose, at the same concentration, for comparison to RSS-200; and the same total dose, at a different concentration, for comparison to RSS-600. The duration of all the exposures was 30 min.

For all three experiments, each subject attended the laboratory for one characterization session, and subsequently for three or four exposure and bronchoscopy sessions. The characterization session was used to collect physical and pulmonary characteristics, and to familiarize each subject with the procedures of the experiment. Each of the experiments utilized a repeated measures design, with each subject completing each condition within the experiment. The order of the experimental conditions was counter-balanced/randomized within each experiment.

Controls:
For each of the three experiments, a control exposure condition of FA was used. To allow recovery from preceding sessions, a minimum of three weeks separated each of the exposure conditions within each experiment.

Independent Variables:
The independent variables were:
The exposure conditions:

1) FA; single exposure
2) RSS at 200 $\mu g/m^3$; single exposure; RSS-200
3) RSS at 600 $\mu g/m^3$; single exposure RSS-600
4) RSS at 3x200 $\mu g/m^3$; three serial-day exposures (RSS-3x200)

The subject groups:

1) Healthy
2) Asthma
3) Allergic Rhinitis

The initial data from Experiment One and Experiment Three indicated that, for the RSS-200 single exposure condition, compared to FA, there were no differences in the Bfx/BAL cell distribution or in spirometric pulmonary function. Therefore, for Experiment One RSS-200 was conducted in 13 subjects; for Experiment Two RSS-200 was not conducted (Experiment Two was completed last chronologically due to subject recruitment); and in Experiment Three RSS-200 was conducted in 8 subjects.

Dependent Variables:
The dependent variables measured were:

1) Cells; in Bfx and BAL; total and differential cell counts (macrophages, lymphocytes, neutrophils, eosinophils, epithelial cells)
2) Cytokine protein: in Bfx and BAL; TNF-α, IL-8, MCP-1
3) Spirometric pulmonary function: FVC, FEV_1, FEF_{25-75}, FEF_{75}
4) Airway inflammation grading (visual)
5) Symptoms (general and respiratory)

Subjects:

All subjects were informed of the risks of the experiment and provided informed consent prior to participation. The procedures for this experiment were approved by the University of California, San Francisco, Institutional Review Board, the Committee on Human Research.

All subjects completed a medical history questionnaire, were current non-smokers, had no history of excessive smoking, and had no serious health problems. Female subjects were not pregnant throughout the project. Subjects had no respiratory-tract illness in the three weeks preceding, or during, each session. Subjects were characterized by physical characteristics, spirometric pulmonary function, non-specific airway reactivity, and allergy skin test.

Experiment One:

Subjects were healthy, and specifically had no history, or current symptoms of asthma or allergic rhinitis. All subjects had non-specific airway reactivity of > 10 mg/ml methacholine. Subjects were characterized by physical and pulmonary characteristics (Table 1.).

Experiment Two:

All subjects had mild to moderate asthma, and were other-wise healthy. Asthma status was determined using the guidelines of the National Asthma Education Program (National Asthma Education Program Expert Panel, 1997). All subjects had non-specific airway reactivity of < 10 mg/ml methacholine. Subjects were characterized by physical and pulmonary characteristics (Table 2.).

Experiment Three:

All subjects had allergic-rhinitis, and were other-wise healthy. Allergic-rhinitis status was determined using allergen skin testing results and clinical symptoms. All subjects had non-specific airway reactivity of > 10 mg/ml methacholine. Subjects were characterized by physical and pulmonary characteristics (Table 3.).

Controls and Medications:
Subjects abstained from caffeine for 8 h prior to each session, and were instructed not to take any medication with known or potential, anti-inflammatory or bronchoactive properties, for specific periods, before or during the exposure and bronchoscopy periods. Subjects abstained from all inhaled steroids for a minimum of two weeks prior to all testing sessions.

Equipment and Procedures:

Laboratory:

All sessions, excluding bronchoscopy (Refer to Bronchoscopy section), were conducted in the Human Exposure Laboratory at the Lung Biology Center, San Francisco General Hospital Campus, University of California San Francisco.

Spirometric Pulmonary Function:

Spirometry for the determination of indices of pulmonary function; FVC, FEV_1, FEF_{25-75}, FEF_{75}, was conducted using a dry, rolling-seal spirometer (Anderson Instruments; Spirotech Division, Model No. S400), using standardized procedures (Crapo *et al.*, 1995). Spirometry was conducted for subject characterization, non-specific airway reactivity, immediately pre-and post-exposure, and pre-bronchoscopy.

Non-Specific Airway Reactivity:

Non-specific airway reactivity was determined by the FEV_1 response to inhalation of nebulized (Devilbiss, Model No. 646) phosphate-buffered saline (PBS) and doubling concentrations of methacholine in PBS (0.313, 0.625, 1.25, 2.5, 5.0, 10.0 mg ml) delivered via a dosimeter (Rosenthal) at the rate of 0.01 ml breath (Kranner *et al.*, 1994). Non-specific airway reactivity was determined for subject characterization.

Allergy Skin Test:

Epicutaneous skin-prick testing with nine local aeroallergens (DP plus *aspergillus fumigatus*, birch mix, chinese elm, cat, dog, mountain cedar, mugwort sage, olive tree, perennial rye) and controls of saline/50% glycerol and histamine were performed on the volar forearm to determine atopic status. Sensitivity was be defined as a >2 x 2 mm skin wheal response.

Exposure Chamber:

The exposure sessions were conducted in a custom-built steel and glass exposure chamber (Nor-Lake Inc., Model No. W00327-3R), which is 2.5 m x 2.5 m x 2.4 m in size, and has an average airflow rate of 300 ft^3 min. The chamber air supply is sourced from ambient air, which is filtered by passing through purifying (Purafil Model No. 6239), and high efficiency particle (Aeropac Model No.53 HEPA 95) filters. The filtered air is dehumidified by passing through a drier (Cargocaire Engineering Corp.). HC-575), and the air temperature is decreased with a chilled-water coil. Subsequently, temperature and humidity are increased with steam (Nortec Model No. NHMC-050), to obtain the pre-set temperature (20 ^{O}C) and relative humidity (50%) conditions in the chamber. The temperature and relative humidity inside the chamber are monitored [LabView 2; (3-min intervals)] and controlled throughout the exposures (Johnson Controls, Model No. DSC 8500).

Rice Straw Smoke Generation and Exposure System:

The design, construction, installation, and characterization, of the system for the RSS generation and controlled human exposures were a major component of this project. This unique, purpose-built system was completed through a Sub-Contract with the University of California, Davis. Due to the detail of this system, it is described in Part II of this report.

Particle and Gas Phase Measurement:

The total particle concentration was measured at the subjects breathing zone using a filter (Pallflex; 0.22 μm), sampling at 14 l min. The filter mass was determined pre- and post-sampling (Micro-systems). Particle concentration samples were collected for the complete 30 min of each exposure (Table 4., Table 5., Table 6.). The particle size distribution was determined, for RSS characterization only, using a voltage regulated particle sorter (SMPS). The methods and results for the particle size measurements are described in Part II of this report.

The chamber concentration of carbon monoxide (CO), was monitored (Dasibi; Model No. 1006) throughout the initial approximately 20 RSS exposure sessions, for RSS-200, RSS-600, and RSS-3x200. The maximum CO concentration averaged approximately 1.5 ppm, with < 5 peaks of 2.5 ppm. Due to the low CO concentration, monitoring was discontinued.

Exposures:

All exposures were conducted with the subject seated at rest. Subject wore nose-clips throughout the exposure to ensure oral breathing. For subject safety, expired peak flow was measured, pre- and post-exposure, and at the 10 and 20 min intervals of the exposure. All exposures were of 30 min duration.

Pulmonary Ventilation:

Expired ventilation was calculated from tidal volume and breathing frequency measured using a pneumotachograph (Fleish, Model No. 3). Expired ventilation was measured for 60 s during both the 0-2 min and 28-30 min periods of the exposure.

Bronchoscopy:

The bronchoscopies were conducted in a dedicated room at San Francisco General Hospital. Vital signs were measured pre- and post-bronchoscopy. Throughout the procedure, intravenous access was maintained, and arterial hemoglobin:oxygen percent saturation, the electrocardiograph, and blood pressure were monitored. Atropine, to decrease airway secretions, and if required, midazolam, to maintain subject comfort, was administered intravenously. The posterior pharynx was anesthetized using a 1% lidocaine spray, and 4% lidocaine-soaked cotton-tipped plegets applied to the mucosa over the ninth cranial nerve. Supplemental oxygen was delivered via a nasal cannula at 2 l/min. The bronchoscope (Pentax, Model No. FB 18x), tipped with lidocaine jelly, was introduced through the mouth, and the larynx and airways were anesthetized using 1% lidocaine solution as required. The bronchoscope was directed and wedged into the right middle lobe orifice (2 x 50 ml lavage), and subsequently into the lingula (1 x 50 ml lavage). The lavages were conducted using 0.9% saline heated to $37^{O}C$. The first 15 ml of lavage fluid returned was designated as the bronchial fraction (Bfx). The lavage fluids were immediately centrifuged at 200 g for 15 minutes (Girofuge model No. 1805), and the supernatant separated and recentrifuged at 1800 g for 15 minutes to remove any cellular debris. The supernatant was then frozen and stored at –80 ^{O}C for biochemical analysis. The bronchoscopy was conducted 6 h post-exposure.

For Experiment Two, as the subjects in this experiment had asthma, 30 min before the bronchoscopy each subject under-went a standard inhaled nebulized albuterol procedure, to minimize the chance of bronchoconstriction during the procedure.

Cell Counts:

Total cells were counted in un-spun aliquots of Bfx and BAL using a hemacytometer (Fisher Scientific, Cat. No. 0267110). Differential cell counts were conducted on slides prepared using a cytocentrifuge at 200 g for 5 min (Shandon Southern Products Ltd., Model No. Cytospin 2) and stained in Diff-Quik (Diff-Quik, Baxter, Cat. No. B4132-1). All differential leukocytes cell counts were expressed as a percent of total leukocytes (macrophages, lymphocytes, neutrophils, eosinophils), and differential epithelial cell counts were expressed as a percent of total leucocytes + epithelial cells. Two readers each performed all cell counts in duplicate.

Biochemical Assays:

In both the Bfx and BAL supernatant, the protein levels were determined using ELISA for TNF-α (R&D Systems, Quantikine, High Sensitivity), IL-8 (R&D Systems, QuantiGlo), and MCP-1 (BioSource International).

Airway Grading:

During each bronchoscopy the visual airway inflammation in the airways was graded by the bronchoscopist on a scale of: 0 = Normal, 1 = Mildly inflamed, 2 = Moderately inflamed, 3 = Severely inflamed.

Symptoms:

Subject self-graded symptoms of; anxiety, chest discomfort or chest tightness, chest pain on deep inspiration, cough, eye irritation, headache, nasal irritation, nausea, phlegm or sputum production, shortness of breath, throat irritation, wheezing; were graded on a scale of; 0 = None, 1 = Minimal (symptom is barely noticeable), 2 = Mild (symptom is present but not annoying), 3 = Moderate (symptom is somewhat annoying), 4 = Severe (symptom is very annoying and/or limits performance. Symptoms were graded immediately pre- and post-exposure.

Statistical Analysis:

The sample size of 15 subjects for each experiment was calculated to achieve >95% power, at alpha = 0.05 to detect a 15% absolute (approx 100% relative) increase in neutrophils in BAL following zinc oxide fume exposure (Kuschner et al., 1997). The zinc oxide data were utilized as there are currently no data for controlled human RSS inhalations, and the zinc oxide fume could have inflammation-inducing characteristics similar to RSS.

The data for the majority of dependent variables in this project were not normally distributed, therefore, non-parametric methods were utilized. For the paired comparisons with-in each of the three experiments, both with-in and between, the exposure conditions, the Wilcoxon Signed-Rank test was used. For the unpaired comparisons across the three experiments the Mann-Whitney test was used. For all analyses differences were assigned as statistically significant at an alpha of <0.05.

Table 1. Experiment One (Healthy subject group): Individual subjects physical, spirometric pulmonary function, and airway responsiveness characteristics

Subj.	Gender	Age (yr)	Height (cm)	Mass (kg)	FVC (l)	FEV_1 (l)	FEV_1/FVC (%)	NSAR PC_{20} (mg ml)
1	M	35	178	66.4	4.99	3.99	80	>10
2	F	47	173	61.8	4.29	3.37	79	>10
3	M	44	185	70.9	5.66	4.61	81	>10
4	F	23	173	80.9	4.57	4.07	89	>10
5	M	41	178	78.2	4.46	3.74	84	>10
6	F	24	163	63.6	2.93	2.75	94	>10
7	M	31	170	65.5	4.53	3.28	72	>10
8	M	25	175	61.4	5.75	5.05	88	>10
9	M	26	180	90.9	6.34	4.90	77	>10
10	M	25	183	77.3	7.00	5.89	84	>10
11	M	30	168	69.1	4.12	3.38	82	>10
12	F	24	155	54.5	3.20	3.00	94	>10
13	M	28	178	109.1	4.73	4.05	86	>10
14	M	32	185	81.8	6.53	5.62	86	>10
15	F	24	155	56.8	2.99	2.68	90	>10
Mean ± SD	(F=5) (M=10)	30.6 7.8	173 10	72.5 14.3	4.81 1.3	4.03 1.0	84 6	>10

Abbreviations: FVC = forced vital capacity; FEV_1 = forced expired volume in 1 s; NSAR PC_{20} = non-specific airway reactivity, methacholine provocative concentration at which FEV_1 decreased 20%, maximum dose = 10 mg ml

Table 2. Experiment Two (Asthma subject group): Individual subjects physical, spirometric pulmonary function, and airway responsiveness characteristics

Subj.	Gender	Age (yr)	Height (cm)	Mass (kg)	FVC (l)	FEV_1 (l)	FEV_1/FVC (%)	NSAR PC_{20} (mg ml)
1	F	23	158	59.1	3.77	3.49	93	< 10
2	F	30	178	80.9	4.76	3.76	79	0
3	M	34	170	79.5	4.27	3.57	84	5
4	F	27	163	56.8	3.89	3.09	79	1.25
5	M	44	183	81.8	5.07	3.95	81	< 10
6	F	28	155	51.4	3.51	2.95	84	1.25
7	F	22	158	54.5	3.85	3.08	80	0.625
8	F	36	155	49.1	2.73	2.00	73	0.625
9	F	32	168	59.1	3.31	2.70	82	5.0
10	F	29	183	100.0	5.08	4.28	84	0.31
11	F	40	165	78.6	2.58	2.08	81	0.31
12	F	22	173	84.1	4.03	3.53	88	0.5
13	F	45	150	52.7	2.25	1.75	78	0.625
14	F	35	165	136.4	3.17	2.52	79	2.5
15	F	21	168	63.6	3.77	3.03	80	1.0
Mean	(F=13)	31.2	166	72.5	3.74	3.05	82	1.5
± SD	(M=2)	7.8	10.1	23.3	0.9	0.7	4.6	1.7

Abbreviations: FVC = forced vital capacity; FEV_1 = forced expired volume in 1 s; NSAR PC_{20} = non-specific airway reactivity, methacholine provocative concentration at which FEV_1 decreased 20%, maximum dose = 10 mg ml

Table 3. Experiment Three (Allergic Rhinitis subject group): Individual subjects physical, spirometric pulmonary function and airway responsiveness characteristics

Subj.	Gender	Age (yr)	Height (cm)	Mass (kg)	FVC (l)	FEV_1 (l)	FEV_1/FVC (%)	NSAR PC_{20} (mg ml)
1	F	36	173	72.7	4.19	3.33	79	>10
2	M	22	173	67.3	5.04	3.93	78	>10
3	M	39	170	72.7	4.56	3.96	87	>10
4	F	28	165	60.0	4.43	3.80	86	>10
5	F	25	165	55.9	4.12	3.69	90	>10
6	F	22	163	59.5	3.59	2.99	83	>10
7	M	25	180	68.2	5.72	4.83	84	>10
8	M	54	175	81.4	4.05	3.23	80	>10
9	M	28	175	70.5	4.90	4.40	90	>10
10	F	28	160	72.7	3.93	3.23	82	>10
11	F	37	160	69.1	3.95	3.23	82	>10
12	F	23	173	64.1	5.38	4.75	88	>10
13	M	29	188	84.1	6.16	4.21	68	>10
14	F	31	160	58.2	3.78	3.08	81	>10
15	F	48	163	59.1	3.26	2.62	80	>10
Mean ± SD	(F=9) (M=6)	31.7 9.5	170 8	67.9 8.7	4.47 0.8	3.69 0.7	83 5	>10

Abbreviations: FVC = forced vital capacity; FEV_1 = forced expired volume in 1 s; NSAR PC_{20} = non-specific airway reactivity, methacholine provocative concentration at which FEV_1 decreased 20%, maximum dose = 10 mg ml

Table 4. Experiment One (Healthy subject group): Exposure Particle Concentrations

	Exposure Condition					
	FA	RSS-200	RSS-600	RSS-3x200		
				Exp.-1	Exp.-2	Exp.-3
[Particle] ($\mu g/m^3$)	51	196	546	217	184	212
±	20	74	207	93	81	69

Values are mean ± SD. Abbreviations: FA = filtered air; RSS-200 = rice straw smoke at 200 $\mu g/m^3$; RSS-600 = rice straw smoke at 600 $\mu g/ m^3$; RSS-3x200 = 3 serial-day exposures to rice straw smoke at 200 $\mu g/m^3$; Exp.-1 = Exposure-1; Exp.-2 = Exposure-2; Exp.-3 = Exposure-3

Table 5. Experiment Two (Asthma subject group): Exposure Particle Concentrations

	Exposure Condition				
	FA	RSS-600	RSS-3x200 Exp.-1	RSS-3x200 Exp.-2	RSS-3x200 Exp.-3
[Particle] ($\mu g/m^3$)	41	614	271	193	201
±	39	125	115	11	43

Values are mean ± SD. Abbreviations: FA = filtered air; RSS-600 = rice straw smoke at 600 $\mu g/m^3$; RSS-3x200 = 3 serial-day exposures to rice straw smoke at 200 $\mu g/m^3$; Exp.-1 = Exposure-1; Exp.-2 = Exposure-2; Exp.-3 = Exposure-3

Table 6. Experiment Three (Allergic Rhinitis subject group): Exposure Particle Concentrations

	Exposure Condition					
	FA	RSS-200	RSS-600	RSS-3x200 Exp.-1	RSS-3x200 Exp.-2	RSS-3x200 Exp.-3
[Particle] ($\mu g/m^3$)	48	194	519	197	187	189
±	50	41	166	61	61	68

Values are mean ± SD. Abbreviations: FA = filtered air; RSS-200 = rice straw smoke at 200 $\mu g/m^3$; RSS-600 = rice straw smoke at 600 $\mu g/m^3$; RSS-3x200 = 3 serial-day exposures to rice straw smoke at 200 $\mu g/m^3$; Exp.-1 = Exposure-1; Exp.-2 = Exposure-2; Exp.-3 = Exposure-3

Results

Cell Distribution:

Experiment One:

In the Bfx, for both RSS-600 and RSS-3x200, compared to FA, there was a significantly higher total leukocyte concentration (Table 8.), and for RSS-600, compared to FA, there was a significantly higher macrophage concentration (Table 8.). In the Bfx, for RSS-3x200, compared to both FA, and RSS-600, there was a significantly higher percent (Table 7.), and concentration of lymphocytes (Table 8.), and significantly lower percent of macrophages (Table 8.). In the BAL, for RSS-3x200, compared to RSS-600, there was a significantly lower concentration of eosinophils (Table 8.).

Experiment Two:

In the Bfx, for RSS-3x200, compared to RSS-600, there was a significantly higher neutrophil concentration (Table 10.). In the BAL, for RSS-3x200, compared to both FA, and RSS-600, there was a significantly higher lymphocyte concentration (Table 10.).

Experiment Three:

In the BAL, for RSS-600 compared to both FA, and RSS-3x200, there was a significantly lower percent (Table 11.), and concentration of epithelial cells (Table 12.).

Between Experiments:

FA Condition: The Asthma subject group, compared to both the Healthy subject group, and the Allergic Rhinitis subject group; in the BAL had a significantly higher percent (Table 7.; Table 9.; Table 11.), and concentration of neutrophils (Table 8.; Table 10.; Table 12.); and compared to the Healthy subject group, in both the Bfx and BAL, had a significantly lower percent of macrophages (Table 7.; Table 9.). The Allergic Rhinitis subject group, compared to the Asthma subject group; in the BAL had a significantly higher percent of epithelial cells (Table 9.; Table 11.).

RSS-600 Condition: The Asthma subject group, compared to the Healthy subject group, in the Bfx had a significantly lower percent of macrophages, and in the BAL, had a significantly lower percent of epithelial cells (Table 7.; Table 9.). The Allergic Rhinitis subject group, compared to the Healthy subject group; in the BAL had a significantly higher percent (Table 7.; Table 11.), and concentration of lymphocytes, and a significantly lower concentration of epithelial cells (Table 8.; Table 12.); and in the Bfx, had a significantly lower percent of macrophages (Table 7.; Table 11.).

RSS-3x200 Condition: The Asthma subject group, compared to both the Healthy subject group, and the Allergic Rhinitis subject group; in the BAL had a significantly higher percent (Table 7.; Table 9.; Table 11.), and concentration of neutrophils (Table 8.; Table

10.; Table 12.); and compared to the Allergic Rhinitis subject group, in the Bfx, had significantly higher percent (Table 9.; Table 11.), and concentration of neutrophils (Table 10.; Table 12.). The Allergic Rhinitis subject group, compared to the Healthy subject group, in BAL, had a significantly higher percent of neutrophils (Table 7.; Table 11.).

Cytokines:

Experiment One:

In the Bfx, for RSS-3x200, compared to RSS-600, there was a significantly higher IL-8 concentration (Table 13.). In the BAL, for RSS-3x200, compared to both RSS-200, and RSS-600, there was a significantly lower MCP-1 concentration (Table 13.). In both the Bfx and BAL, for all of the conditions, there were no significant differences in the TNF-α concentration (Table 13.).

Experiment Two:

In the Bfx and BAL, for all of the conditions, there were no significant differences in the TNF-α, IL-8, or MCP-1 concentration (Table 14.).

Experiment Three:

In the Bfx and BAL, for all of the conditions, there were no significant differences in the TNF-α, IL-8, or MCP-1 concentration (Table 15.).

Between Experiments:

There were no differences between experiments in TNF-α, IL-8, or MCP-1 concentration (Table 13., Table 14., Table 15.).

Spirometric Pulmonary Function:

Experiment One, Experiment Two, Experiment Three:

For each of the three experiments, there were no physiologically relevant RSS-induced differences, either with-in or between conditions, in FVC, FEV_1, FEF_{25-75}, or FEF_{75} (Table 16, Table 16 A, Table 17, Table 17 A, Table 18, Table 18 A). There were several statistically significant differences in measures of spirometric pulmonary function, however, these differences were at a level (minimal) that would not be expected to have any physiological relevance. Further, the differences between the pre- and post-exposure values, compared to the pre-bronchoscopy values could be due to diurnal variation, as these measurements were taken between approximately 6:00 to 9:00 am and 1:00 to 4:00 pm, respectively, at time periods that have differences in spirometric pulmonary function variables (Randell et al. 1997).

Between Experiments:

The Asthma subject group, compared to both the Healthy subject group, and the Allergic Rhinitis subject group, had significantly lower values for the majority of indices of spirometric pulmonary function, both for characterization and for all measurement times (Table 16, Table 16 A, Table 17, Table 17 A, Table 18, Table 18 A).

Airway Grading:

Experiment One:

In both RSS-600 and RSS-3x200, compared to FA, and RSS-3x200, compared to RSS-200, there were significant increases in the airway grading score (Data not presented).

Experiment Two:

There were no differences in the airway grading scores in this experiment (Data not presented).

Experiment Three:

In RSS-600, compared to FA, there was a significant increase in the airway grading score (Data not presented).

Airway Grading System:
Due to the preliminary and categorical type of system used for airway grading in this project, these data are not presented. A more sensitive and objective system is currently being developed for use in future smoke-exposure projects.

Symptoms:

For each of the three experiments, there were no significant differences, either with-in or between conditions, or between experiments, in symptoms grades (Data not presented).

Table 7. Experiment One (Healthy subject group): Leukocyte and epithelial cell differential percent in bronchial fraction, and bronchoalveolar lavage, post-exposure to filtered air, and three conditions of rice straw smoke

Cell	Exposure Condition							
	FA		RSS-200		RSS-600		RSS-3x200	
	Bfx	BAL	Bfx	BAL	Bfx	BAL	Bfx	BAL
Macrophages (%)								
Median	91.5 [A]	90.9	98.4	90.1	90.7 [B]	91.1	87.5 [A B]	89.3
25-75% range	88.9-93.4	87.9-94.4	87.1-95.7	81.6-93.8	87.8-94.3	82.6-93.2	85.0-90.0	84.5-93.5
Neutrophils (%)								
Median	3.2	2.1	3.2	2.1	2.2	1.8	3.6	1.5
25-75% range	2.3-5.8	1.4-3.7	1.6-5.2	1.2-2.9	1.1-4.8	0.8-3.1	2.8-5.0	0.8-2.0
Lymphocytes (%)								
Median	4.4 [A]	5.3	4.7	6.1	5.1 [B]	7.0	8.0 [A B]	8.0
25-75% range	0.9-6.0	1.9-9.6	0.9-8.1	2.4-12.2	2.2-7.6	3.4-13.3	6.4-9.9	4.6-12.7
Eosinophils (%)								
Median	0.3	0.5	0.3	0.4	0.5	0.3	0.0	0.3
25-75% range	0.0-0.5	0.0-0.6	0.0-0.7	0.0-0.8	0.3-0.9	0.3-0.7	0.0-0.8	0.0-0.7
Epithelial Cells (%)								
Median	8.0	5.8	8.2	6.7	9.8	5.0	7.5	5.0
25-75% range	5.5-14.4	3.3-9.6	6.8-11.9	4.7-11.0	6.7-14.5	3.8-11.0	2.5-12.3	2.7-6.9

Values are median and 25-75% range. Epithelial cells = percent of total non-squamous cells; all other cell types = percent of leukocytes. [A B] = with-in each variable values with same letter are significantly different ($P < 0.05$). Abbreviations: FA = filtered air; RSS-200 = rice straw smoke at 200 μg/m^3; RSS-600 = rice straw smoke at 600 μg/m^3; RSS-3x200 = 3 serial-day exposures to rice straw smoke at 200 μg/m^3; BFx = bronchial fraction; BAL = bronchoalveolar lavage.

Table 8. Experiment One (Healthy subject group): Leukocyte and epithelial cell concentration in bronchial fraction, and bronchoalveolar lavage, post-exposure to filtered air, and three conditions of exposure to rice straw smoke

Cell	Exposure Condition							
	FA		RSS-200		RSS-600		RSS-3x200	
	Bfx	BAL	Bfx	BAL	Bfx	BAL	Bfx	BAL
Total Leukocytes (x10^4 ml)								
Median	11.3 $^{A\ B}$	13.8	13.5	13.6	14.5 ^{A}	15.8	16.0 ^{B}	14.0
25-75% range	9.4-16.5	12.7-15.3	10.3-15.8	9.7-16.0	11.3-18.4	11.7-23.2	9.4-21.6	10.2-17.7
Macrophages (x10^4 ml)								
Median	9.4 ^{A}	12.3	12.2	11.5	12.9 ^{A}	14.3	14.5	12.5
25-75% range	8.6-14.6	11.1-14.6	9.4-14.8	8.5-14.5	10.1-17.5	10.0-20.8	8.3-18.1	8.4-15.9
Neutrophils (x10^4 ml)								
Median	0.4	0.3	0.5	0.2	0.4	0.2	0.6	0.2
25-75% range	0.3-0.7	0.2-0.6	0.3-0.6	0.2-0.5	0.3-0.7	0.1-0.5	0.3-1.0	0.1-0.3
Lymphocytes (x10^4 ml)								
Median	0.4 ^{A}	0.8	0.6	0.9	0.5 ^{B}	1.2	1.2 $^{A\ B}$	1.0
25-75% range	0.2-1.1	0.4-1.3	0.1-1.1	0.3-1.3	0.3-1.3	0.6-1.5	0.8-1.8	0.7-1.5
Eosinophils (x10^4 ml)								
Median	0.0	0.1	0.0	0.1	0.1	0.1 ^{A}	0.0	0.0 ^{A}
25-75% range	0.0-0.1	0.0-0.1	0.0-0.1	0.0-0.1	0.0-0.1	0.0-0.2	0.0-0.1	0.0-0.1
Epithelial Cells (x10^4 ml)								
Median	2.0	1.3	2.0	0.8	2.3	1.5	2.3	0.8
25-75% range	1.3-2.4	1.0-1.8	1.8-4.2	0.5-1.4	1.4-3.5	0.9-2.2	1.4-2.7	0.3-1.9

Values are median and 25-75% range. Epithelial cells = percent of total non-squamous cells; all other cell types = percent of leukocytes. $^{A\ B}$ = with-in each variable values with same letter are significantly different ($P < 0.05$). Abbreviations: FA = filtered air; RSS-200 = rice straw smoke at 200 μg/m^3; RSS-600 = rice straw smoke at 600 μg/m^3; RSS-3x200 = 3 serial-day exposures to rice straw smoke at 200 μg/m^3; BFx = bronchial fraction; BAL = bronchoalveolar lavage.

Table 9. Experiment Two (Asthma subject group): Leukocyte and epithelial cell differential percent in bronchial fraction, and bronchoalveolar lavage, post-exposure to filtered air, and two conditions of exposure to rice straw smoke

Cell	Exposure Condition					
	FA		RSS-600		RSS-3x200	
	Bfx	BAL	Bfx	BAL	Bfx	BAL
Macrophages (%)						
Median	88.4	87.2	87.4	87.5	86.0	84.5
25-75% range	85.8-91.2	83.0-90.0	81.7-90.7	83.3-90.6	80.5-89.1	81.5-88.4
Neutrophils (%)						
Median	4.3	4.7	4.5	2.6	5.2	3.7
25-75% range	2.3-6.8	2.4-6.5	3.3-5.4	1.2-5.9	3.3-7.0	2.3-6.2
Lymphocytes (%)						
Median	3.9	6.7	6.2	6.8	6.9	11.0
25-75% range	3.2-8.9	3.3-11.8	4.8-11.5	5.4-11.0	5.2-10.9	6.0-15.2
Eosinophils (%)						
Median	0.3	0.3	0.6	0.0	0.0	0.0
25-75% range	0.2-0.7	0.0-1.2	0.0-0.8	0.0-0.8	0.0-0.3	0.0-0.5
Epithelial Cells (%)						
Median	4.5	4.3	6.8	3.0	8.0	5.8
25-75% range	3.4-12.2	2.9-5.9	2.7-11.9	1.3-5.5	3.9-11.8	3.3-7.2

Values are median and 25-75% range. Epithelial cells = percent of total non-squamous cells; all other cell types = percent of leukocytes. Abbreviations: FA = filtered air; RSS-200 = rice straw smoke at 200 $\mu g/m^3$; RSS-600 = rice straw smoke at 600 $\mu g/m^3$; RSS-3x200 = 3 serial-day exposures to rice straw smoke at 200 $\mu g/m^3$; BFx = bronchial fraction; BAL = bronchoalveolar lavage.

Table 10. Experiment Two (Asthma subject group): Leukocyte and epithelial cell concentration in bronchial fraction, and bronchoalveolar lavage, post-exposure to filtered air, and two conditions of exposure to rice straw smoke

Cell	Exposure Condition					
	FA		RSS-600		RSS-3x200	
	Bfx	BAL	Bfx	BAL	Bfx	BAL
Total Leukocytes ($x10^4$ ml)						
Median	12.5	16.5	12.8	13.3	17.0	14.3
25-75% range	11.2-15.7	11.3-23.4	8.9-15.5	11.1-15.9	10.8-21.3	11.6-22.8
Macrophages ($x10^4$ ml)						
Median	11.4	13.8	11.3	11.3	14.3	12.5
25-75% range	9.8-14.2	9.6-20.9	7.3-12.8	9.5-13.5	9.2-16.5	9.7-19.6
Neutrophils ($x10^4$ ml)						
Median	0.6	0.8	0.5 [A]	0.4	0.8 [A]	0.5
25-75% range	0.4-1.0	0.3-1.1	0.4-0.7	0.2-0.8	0.6-1.0	0.4-1.2
Lymphocytes ($x10^4$ ml)						
Median	0.6	0.8 [A]	0.7	1.2 [B]	1.0	1.6 [A B]
25-75% range	0.4-0.9	0.7-1.6	0.5-2.0	0.6-2.1	0.7-1.6	0.9-2.1
Eosinophils ($x10^4$ ml)						
Median	0.0	0.0	0.1	0.0	0.0	0.0
25-75% range	0.0-0.1	0.0-0.3	0.0-0.2	0.0-0.2	0.0-0.1	0.0-0.1
Epithelial Cells ($x10^4$ ml)						
Median	1.0	1.0	1.3	1.0	1.8	1.0
25-75% range	0.5-2.7	0.8-1.3	0.9-2.0	0.5-1.4	0.9-3.6	0.4-2.3

Values are median and 25-75% range. Epithelial cells = percent of total non-squamous cells; all other cell types = Percent of leukocytes. [A B] = with-in each variable values with same letter are significantly different ($P < 0.05$). Abbreviations: FA = filtered air; RSS-200 = rice straw smoke at 200 $\mu g/m^3$; RSS-600 = rice straw smoke at 600 $\mu g/m^3$; RSS-3x200 = 3 serial-day exposures to rice straw smoke at 200 $\mu g/m^3$; BFx = bronchial fraction; BAL = bronchoalveolar lavage.

Table 11. Experiment Three (Allergic Rhinitis subject group): Leukocyte and epithelial cell differential percent in bronchial fraction, and bronchoalveolar lavage, post-exposure to filtered air, and three conditions of exposure to rice straw smoke

Cell	Exposure Condition							
	FA		RSS-200		RSS-600		RSS-3x200	
	Bfx	BAL	Bfx	BAL	Bfx	BAL	Bfx	BAL
Macrophages (%)								
Median	85.3	89.2	88.5	86.9	88.1	87.0	86.8	87.7
25-75% range	83.7-91.0	85.6-92.1	83.0-92.1	83.3-90.8	85.3-89.6	81.9-90.2	84.4-92.2	85.8-89.7
Neutrophils (%)								
Median	3.1	2.2	3.7	2.4	3.5	2.1	2.3	2.1
25-75% range	2.3-5.0	1.5-3.1	2.8-4.3	1.8-3.8	2.1-5.2	1.5-2.9	2.1-3.5	1.8-2.9
Lymphocytes (%)								
Median	8.1	8.4	7.7	8.8	8.3	10.1	8.1	9.1
25-75% range	5.1-11.0	5.1-12.5	4.1-12.8	5.7-14.4	5.8-9.9	7.3-14.5	4.7-12.1	6.5-11.6
Eosinophils (%)								
Median	0.3	0.0	0.2	0.4	0.3	0.3	0.3	0.3
25-75% range	0.0-0.9	0.0-0.4	0.0-1.0	0.2-0.9	0.0-1.1	0.0-1.6	0.2-1.1	0.0-0.6
Epithelial Cells (%)								
Median	8.5	7.8 [A]	15.3	4.6	7.0	3.8 [A B]	10.8	6.8 [B]
25-75% range	7.6-10.9	4.3-9.6	5.0-27.6	2.8-7.4	5.1-9.5	2.2-5.2	5.3-13.1	4.5-10.1

Values are median and 25-75% range. Epithelial cells = percent of total non-squamous cells; all other cell types = percent of leukocytes. [A B] = with-in each variable values with same letter are significantly different ($P < 0.05$). Abbreviations: FA = filtered air; RSS-200 = rice straw smoke at 200 μg/m^3; RSS-600 = rice straw smoke at 600 μg/m^3; RSS-3x200 = 3 serial-day exposures to rice straw smoke at 200 μg/m^3; BFx = bronchial fraction; BAL = bronchoalveolar lavage.

Table 12. Experiment Three (Allergic Rhinitis subject group): Leukocyte and epithelial cell concentration in bronchial fraction, and bronchoalveolar lavage, post-exposure to filtered air, and three conditions of exposure to rice straw smoke

Cell	Exposure Condition							
	FA		RSS-200		RSS-600		RSS-3x200	
	Bfx	BAL	Bfx	BAL	Bfx	BAL	Bfx	BAL
Total Leukocytes								
($x10^4$ ml)	13.8	13.8	16.9	14.7	15.0	15.0	16.5	15.5
25-75% range	11.7-18.8	13.2-18.0	12.2-20.2	12.4-18.8	11.8-22.3	10.6-21.7	12.5-19.7	10.4-22.7
Macrophages ($x10^4$ ml)								
Median	13.2	12.6	14.1	14.1	13.6	12.7	14.3	14.2
25-75% range	9.7-16.8	10.9-15.3	9.4-17.7	10.1-20.0	10.0-18.1	8.8-18.8	10.6-18.0	9.0-19.7
Neutrophils ($x10^4$ ml)								
Median	0.4	0.4	0.5	0.3	0.4	0.3	0.4	0.3
25-75% range	0.3-1.0	0.2-0.5	0.4-0.7	0.2-0.8	0.4-0.7	0.2-0.3	0.3-0.7	0.2-0.7
Lymphocytes ($x10^4$ ml)								
Median	1.2	1.3	1.4	1.3	1.1	1.4	1.2	1.2
25-75% range	0.6-1.9	0.8-2.2	0.6-2.1	0.9-2.0	0.8-1.4	1.2-1.9	0.9-1.7	1.1-1.9
Eosinophils ($x10^4$ ml)								
Median	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.1
25-75% range	0.0-0.2	0.0-0.1	0.0-0.2	0.0-0.2	0.0-0.2	0.0-0.3	0.0-0.1	0.0-0.2
Epithelial Cells ($x10^4$ ml)								
Median	2.3	1.3 [A]	3.3	1.3	2.3	0.8 [A B]	2.3	1.5 [B]
25-75% range	1.7-2.9	1.3-1.8	2.0-5.7	0.9-2.3	1.5-2.8	0.3-1.3	1.8-3.8	0.8-2.9

Values are median and 25-75% range. Epithelial cells = percent of total non-squamous cells; all other cell types = percent of leukocytes. [A B] = with-in each variable values with same letter are significantly different ($P < 0.05$). Abbreviations: FA = filtered air; RSS-200 = rice straw smoke at 200 $\mu g/m^3$; RSS-600 = rice straw smoke at 600 $\mu g/m^3$; RSS-3x200 = 3 serial-day exposures to rice straw smoke at 200 $\mu g/m^3$; BFx = bronchial fraction; BAL = bronchoalveolar lavage.

Table 13. Experiment One (Healthy subject group): Cytokine concentration in bronchial fraction, and bronchoalveolar lavage, post-exposure to filtered air, and three conditions of exposure to rice straw smoke

Cell	Exposure Condition							
	FA		RSS-200		RSS-600		RSS-3x200	
	Bfx	BAL	Bfx	BAL	Bfx	BAL	Bfx	BAL
TNF-α (pg ml)								
Median	0.07	0.09	0.07	0.09	0.07	0.08	0.08	0.09
25-75% range	0.05-0.08	0.06-0.10	0.06-0.11	0.07-0.13	0.04-0.11	0.07-0.11	0.06-0.13	0.08-0.11
IL-8 (pg ml)								
Median	14.3	11.1	19.8	12.7	15.9 [A]	12.0	34.9 [A]	7.7
25-75% range	10.6-48.4	5.2-14.2	12.8-37.5	7.8-16.0	12.1-29.7	8.2-13.3	16.4-66.0	3.6-13.7
MCP-1 (pg ml)								
Median	15.0	14.4	24.4	16.2 [A]	18.6	14.8 [B]	13.4	8.4 [A B]
25-75% range	10.4-23.8	8.1-20.0	12.2-34.2	9.1-37.9	12.1-36.6	8.7-23.5	9.2-21.7	7.0-17.7

Values are median and 25-75% range. [A B] = with-in each variable values with same letter are significantly different ($P < 0.05$).
Abbreviations: FA = filtered air; RSS-200 = rice straw smoke at 200 μg/m^3; RSS-600 = rice straw smoke at 600 μg/m^3; RSS-3x200 = 3 serial-day exposures to rice straw smoke at 200 μg/m^3; BFx = bronchial fraction; BAL = bronchoalveolar lavage; TNF-α = tumor necrosis factor-alpha; IL-8 = interleukin-8; MCP-1; monocyte chemotatic protein-1.

Table 14. Experiment Two (Asthma subject group): Cytokine concentration in bronchial fraction, and bronchoalveolar lavage, post-exposure to filtered air, and two conditions of exposure to rice straw smoke

Cell	Exposure Condition					
	FA		RSS-600		RSS-3x200	
	Bfx	BAL	Bfx	BAL	Bfx	BAL
TNF-α (pg ml)						
Median	0.05	0.08	0.07	0.07	0.07	0.08
25-75% range	0.04-0.09	0.05-0.09	0.05-0.09	0.06-0.12	0.05-0.08	0.07-0.12
IL-8 (pg ml)						
Median	13.1	9.2	15.8	11.6	19.3	9.6
25-75% range	7.5-27.8	6.6-12.8	9.6-28.6	6.4-19.9	9.8-34.5	6.1-12.8
MCP-1 (pg ml)						
Median	18.7	17.0	33.8	22.3	21.6	18.0
25-75% range	16.2-23.0	12.8-23.7	17.2-46.4	15.5-25.1	15.2-28.5	14.5-22.0

Values are median and 25-75% range. Abbreviations: FA = filtered air; RSS-200 = rice straw smoke at 200 μg/m^3; RSS-600 = rice straw smoke at 600 μg/m^3; RSS-3x200 = 3 serial-day exposures to rice straw smoke at 200 μg/m^3; BFx = bronchial fraction; BAL = bronchoalveolar lavage; TNF-α = tumor necrosis factor-alpha; IL-8 = interleukin-8; MCP-1; monocyte chemotatic protein-1.

Table 15. Experiment Three (Allergic Rhinitis subject group): Cytokine concentration in bronchial fraction, and bronchoalveolar lavage, post-exposure to filtered air, and three conditions of exposure to rice straw smoke

Cell	Exposure Condition							
	FA		RSS-200		RSS-600		RSS-3x200	
	Bfx	BAL	Bfx	BAL	Bfx	BAL	Bfx	BAL
TNF-α (pg ml)								
Median	0.08	0.11	0.07	0.08	0.08	0.09	0.08	0.09
25-75% range	0.05-0.10	0.05-0.16	0.05-0.10	0.05-0.15	0.05-0.12	0.06-0.14	0.04-0.10	0.07-0.12
IL-8 (pg ml)								
Median	22.7	10.9	16.5	8.2	17.9	10.0	27.9	10.7
25-75% range	12.8-31.5	7.0-21.4	10.0-28.8	6.5-10.4	9.7-27.7	4.7-12.6	14.5-44.1	7.6-14.8
MCP-1 (pg ml)								
Median	21.9	19.4	26.8	20.0	23.5	12.0 [A]	17.2	15.0 [A]
25-75% range	13.8-44.6	10.9-40.0	21.5-35.6	13.2-26.1	16.4-33.4	9.6-26.8	11.8-25.8	6.6-23.0

Values are median and 25-75% range. [A B] = with-in each variable values with same letter are significantly different ($P < 0.05$).
Abbreviations: FA = filtered air; RSS-200 = rice straw smoke at 200 μg/m^3; RSS-600 = rice straw smoke at 600 μg/m^3; RSS-3x200 = 3 serial-day exposures to rice straw smoke at 200 μg/m^3; BFx = bronchial fraction; BAL = bronchoalveolar lavage; TNF-α = tumor necrosis factor-alpha; IL-8 = interleukin-8; MCP-1; monocyte chemotatic protein-1.

Table 16. Experiment One (Healthy subject group): Spirometric pulmonary function, pre- and post-exposure, and pre-bronchoscopy, in filtered air, and two single-exposure conditions of exposure to rice straw smoke

	Exposure Condition								
	FA			RSS-200			RSS-600		
	Pre-Exp.	Post-Exp.	Pre-Bronch.	Pre-Exp.	Post-Exp.	Pre-Bronch.	Pre-Exp.	Post-Exp.	Pre-Bronch.
FVC (l)									
Median	4.59	4.53	4.58	4.52	4.45	4.43	4.73	4.57	4.60
25-75% range	4.27-5.54	4.23-5.52	4.21-5.38	3.82-5.69	3.71-5.59	3.79-5.46	4.27-5.41	4.19-5.32	4.31-5.44
FEV_1 (l)									
Median	3.73	3.77	3.94	3.55	3.61	3.44	3.82	3.79	3.91
25-75% range	3.31-4.74	3.31-4.73	3.19-4.97	3.12-4.64	3.08-4.74	3.12-4.56	3.27-4.71	3.22-4.54	3.27-4.58
FEF_{25-75} (l s)									
Median	3.79	4.32	4.45	3.68	4.04	4.22	4.13	4.20	4.27
25-75% range	3.05-4.73	3.18-4.92	3.58-5.27	3.20-4.52	3.42-4.82	3.27-5.03	3.29-4.84	3.46-5.01	3.38-4.98
FEF_{75} (l s)									
Median	1.64	1.82	2.19	1.73	1.97	1.93	1.77	1.81	2.11
25-75% range	1.37-2.51	1.52-2.51	1.63-2.73	1.25-2.35	1.47-2.27	1.62-2.32	1.54-2.37	1.65-2.66	1.46-2.58

Values are median and 25-75% range. Abbreviations: FA = filtered air; RSS-200 = rice straw smoke at 200 $\mu g/m^3$; RSS-600 = rice straw smoke at 600 $\mu g/m^3$; Pre-Exp. = pre-exposure; Post-Exp. = post-exposure; Pre-Bronch. = pre-bronchoscopy; FVC = forced vital capacity; FEV_1 = forced expired volume in 1 s; FEF_{25-75} = forced expired flow-rate at 25-75% FVC; FEF_{75} = forced expired flow-rate at 75% FVC.

Table 16 A. Experiment One (Healthy subject group): Spirometric pulmonary function, pre- and post-exposure, and pre-bronchoscopy, in serial-exposure to rice straw smoke

	Exposure Condition						
	RSS-3x200; Exp.-1		RSS-3x200; Exp.-2		RSS-3x200; Exp.-3		
	Pre-Exp.	Post-Exp.	Pre-Exp.	Post-Exp.	Pre-Exp.	Post-Exp.	Pre-Bronch.
FVC (l)							
Median	4.58	4.59	4.58	4.57	4.56	4.53	4.52
25-75% range	4.25-5.27	4.26-5.40	4.27-5.45	4.21-5.26	4.23-5.29	4.25-5.21	4.15-5.29
FEV_1 (l)							
Median	3.92	3.90	3.95	3.93	3.88	3.97	3.82
25-75% range	3.15-4.50	3.24-4.66	3.20-4.59	3.27-4.43	3.24-4.38	3.30-4.41	3.26-4.49
FEF_{25-75} (l s)							
Median	4.01	4.44	4.44	4.43	3.98	3.92	3.95
25-75% range	3.28-5.03	3.33-4.90	3.42-5.02	3.39-5.08	3.18-4.79	3.35-4.83	3.58-5.11
FEF_{75} (l s)							
Median	2.01	1.95	2.03	2.00	1.71	2.12	1.86
25-75% range	1.38-2.49	1.45-2.57	1.42-2.53	1.51-2.56	1.32-2.28	1.46-2.48	1.63-2.62

Values are median and 25-75% range. Abbreviations: RSS-3x200 = 3 serial-day exposures to rice straw smoke at 200 $\mu g/m^3$; Pre-Exp. = pre-exposure; Post-Exp. = post-exposure; Pre-Bronch. = pre-bronchoscopy; FVC = forced vital capacity; FEV_1 = forced expired volume in 1 s; FEF_{25-75} = forced expired flow-rate at 25-75% FVC; FEF_{75} = forced expired flow-rate at 75% FVC.

Table 17. Experiment Two (Asthma subject group): Spirometric pulmonary function, pre- and post-exposure, and pre-bronchoscopy, in filtered air, and one single-exposure condition of exposure to rice straw smoke

	Exposure Condition					
	FA			RSS-600		
	Pre-Exp.	Post-Exp.	Pre-Bronch.	Pre-Exp.	Post-Exp.	Pre-Bronch.
FVC (l)						
Median	3.80	3.75	3.65	3.73	3.84	3.56
25-75% range	3.09-4.09	3.16-4.16	3.19-4.09	2.91-4.23	3.04-4.13	3.13-4.15
FEV_1 (l)						
Median	3.01	3.04	3.05	2.86	2.82	2.94
25-75% range	2.52-3.62	2.61-3.61	2.54-3.74	2.26-3.56	2.40-3.45	2.37-3.65
FEF_{25-75} (l s)						
Median	2.58	2.78	3.04	2.39	2.50	2.50
25-75% range	2.36-3.36	2.53-3.46	2.35-3.60	1.94-3.77	2.00-3.41	2.05-3.48
FEF_{75} (l s)						
Median	1.33	1.31	1.45	1.31	1.33	1.27
25-75% range	1.02-1.56	1.12-1.70	1.04-1.79	0.82-1.74	0.85-1.70	0.79-1.79

Values are median and 25-75% range. Abbreviations: FA = filtered air; RSS-200 = rice straw smoke at 200 $\mu g/m^3$; RSS-600 = rice straw smoke at 600 $\mu g/m^3$; Pre-Exp. = pre-exposure; Post-Exp. = post-exposure; Pre-Bronch. = pre-bronchoscopy; FVC = forced vital capacity; FEV_1 = forced expired volume in 1 s; FEF_{25-75} = forced expired flow-rate at 25-75% FVC; FEF_{75} = forced expired flow-rate at 75% FVC.

Table 17 A. Experiment Two (Asthma subject group): Spirometric pulmonary function, pre- and post-exposure, and pre-bronchoscopy, in serial-exposure to rice straw smoke

	Exposure Condition						
	RSS-3x200; Exp.-1		RSS-3x200; Exp.-2		RSS-3x200; Exp.-3		
	Pre-Exp.	Post-Exp.	Pre-Exp.	Post-Exp.	Pre-Exp.	Post-Exp.	Pre-Bronch.
FVC (l)							
Median	3.76	3.64	3.83	3.87	3.85	3.77	3.77
25-75% range	3.17-4.37	3.05-4.36	3.08-4.34	3.07-4.24	3.31-4.27	3.11-4.14	3.07-4.24
FEV_1 (l)							
Median	3.00	2.96	2.94	3.09	3.01	2.94	2.92
25-75% range	2.49-3.71	2.45-3.71	2.33-3.65	2.50-3.60	2.67-3.59	2.53-3.59	2.54-3.72
FEF_{25-75} (l s)							
Median	2.66	2.86	2.84	2.86	2.84	2.69	2.96
25-75% range	1.96-3.54	2.09-3.77	1.87-3.47	2.17-3.61	2.13-3.62	2.34-3.64	2.39-3.94
FEF_{75} (l s)							
Median	1.50	1.32	1.42	1.37	1.32	1.41	1.56
25-75% range	1.00-1.81	0.93-1.75	0.79-1.65	0.99-1.63	1.06-1.69	1.14-1.70	1.14-1.80

Values are median and 25-75% range. Abbreviations: RSS-3x200 = 3 serial-day exposures to rice straw smoke at 200 $\mu g/m^3$; Pre-Exp. = pre-exposure; Post-Exp. = post-exposure; Pre-Bronch. = pre-bronchoscopy; FVC = forced vital capacity; FEV_1 = forced expired volume in 1 s; FEF_{25-75} = forced expired flow-rate at 25-75% FVC; FEF_{75} = forced expired flow-rate at 75% FVC.

Table 18. Experiment Three (Allergic rhinitis subject group): Spirometric pulmonary function, pre- and post-exposure, and pre-bronchoscopy, in filtered air, and two single-exposure conditions of exposure to rice straw smoke

	Exposure Condition								
	FA			RSS-200			RSS-600		
	Pre-Exp.	Post-Exp.	Pre-Bronch.	Pre-Exp.	Post-Exp.	Pre-Bronch.	Pre-Exp.	Post-Exp.	Pre-Bronch.
FVC (l)									
Median	4.16	4.12	4.17	4.55	4.54	4.48	4.30	4.33	4.24
25-75% range	3.86-5.00	3.85-4.90	3.69-4.90	4.14-5.12	4.11-5.09	4.23-4.95	3.90-5.02	3.90-4.95	3.81-5.12
FEV_1 (l)									
Median	3.61	3.59	3.67	3.87	3.87	3.88	3.65	3.69	3.70
25-75% range	3.15-4.02	3.13-3.99	3.13-4.11	3.44-4.27	3.41-4.29	3.46-4.19	3.17-3.87	3.20-3.83	3.12-4.18
FEF_{25-75} (l s)									
Median	3.67	3.60	3.45	4.31	4.04	4.36	3.53	3.24	3.62
25-75% range	2.80-4.06	3.13-4.28	3.10-4.53	3.15-4.92	3.19-4.97	3.40-4.94	2.75-4.33	2.86-4.15	3.19-4.53
FEF_{75} (l s)									
Median	1.58	1.66	1.73	2.15	1.92	2.16	1.57	1.74	1.79
25-75% range	1.24-2.06	1.37-2.22	1.39-2.31	1.53-2.46	1.59-2.47	1.69-2.65	1.29-2.12	1.31-1.97	1.35-2.26

Values are median and 25-75% range. Abbreviations: FA = filtered air; RSS-200 = rice straw smoke at 200 $\mu g/m^3$; RSS-600 = rice straw smoke at 600 $\mu g/m^3$; Pre-Exp. = pre-exposure; Post-Exp. = post-exposure; Pre-Bronch. = pre-bronchoscopy; FVC = forced vital capacity; FEV_1 = forced expired volume in 1 s; FEF_{25-75} = forced expired flow-rate at 25-75% FVC; FEF_{75} = forced expired flow-rate at 75% FVC.

Table 18 A. Experiment Three (Allergic Rhinitis subject group): Spirometric pulmonary function, pre- and post-exposure, and pre-bronchoscopy, in serial-exposure to rice straw smoke

	Exposure Condition						
	RSS-3x200; Exp.-1		RSS-3x200; Exp.-2		RSS-3x200; Exp.-3		
	Pre-Exp.	Post-Exp.	Pre-Exp.	Post-Exp.	Pre-Exp.	Post-Exp.	Pre-Bronch.
FVC (l)							
Median	4.30	4.23	4.30	4.24	4.27	4.21	4.25
25-75% range	3.84-5.02	3.73-5.06	3.82-4.97	3.79-4.90	3.72-5.04	3.77-5.04	3.73-5.05
FEV_1 (l)							
Median	3.69	3.57	3.61	3.61	3.65	3.65	3.52
25-75% range	3.09-3.88	3.13-3.98	3.02-3.94	3.12-4.04	3.04-4.06	3.06-4.07	3.12-4.18
FEF_{25-75} (l s)							
Median	3.31	3.32	3.40	3.34	3.22	3.32	3.39
25-75% range	2.91-4.55	3.16-4.16	2.68-4.15	3.07-4.29	2.86-4.32	2.83-4.44	3.11-4.02
FEF_{75} (l s)							
Median	1.64	1.68	1.52	1.61	1.47	1.47	1.75
25-75% range	1.36-2.29	1.33-2.07	1.27-2.17	1.44-2.03	1.37-2.32	1.30-2.38	1.40-2.03

Values are median and 25-75% range. Abbreviations: RSS-3x200 = 3 serial-day exposures to rice straw smoke at 200 $\mu g/m^3$; Pre-Exp. = pre-exposure; Post-Exp. = post-exposure; Pre-Bronch. = pre-bronchoscopy; FVC = forced vital capacity; FEV_1 = forced expired volume in 1 s; FEF_{25-75} = forced expired flow-rate at 25-75% FVC; FEF_{75} = forced expired flow-rate at 75% FVC.

Table 19. Summary of changes in cell distribution and cytokine protein concentration for all exposure conditions, with-in and between, the Healthy, Asthma, and Allergic Rhinitis groups

	Healthy				Asthmatic			Allergic Rhinitis			
	FA	RSS-200	RSS-600	3x200	FA	RSS-600	3x200	FA	RSS-200	RSS-600	3x200
Healthy											
FA			↓ Mac.% Bfx ↑ [TLC] Bfx ↑ [Mac.] Bfx ↑ [Lym.] Bfx	↑ [TLC] Bfx ↑ Lym.% Bfx ↑ [Lym.] Bfx	↓ Mac.% Bfx ↓ Mac.% BAL ↑ Neut% BAL ↑ [Neut.] BAL						
RSS-200				↓ [MCP-1] BAL							
RSS-600				↓ Mac.% Bfx ↑ Lym.% Bfx ↑ [Lym.] Bfx ↓ [Eos.] BAL ↑ [IL-8] Bfx ↓ [MCP-1] BAL		↓ Mac.% Bfx ↓ Epi.% BAL				↓ Mac.% Bfx ↑ Lym.% BAL ↑ [Lym.] BAL ↓ [Epi.] BAL	
RSS-3x200							↑ Neut% BAL ↑ [Neut.] BAL				↑ Neut% BAL
Asthma											
FA							↑ [Lym.] BAL	↑ Epi.% BAL			
RSS-600							↑ [Neut.] Bfx ↑ [Lym.] BAL				
RSS-3x200											
Allergic Rhinitis											
FA					↑ Neut.% BAL ↑ [Neut.] BAL					↓ Epi.% BAL ↓ [Epi.] BAL	
RSS-200											
RSS-600											
RSS-3x200							↑ Neut% Bfx ↑ [Neut.] Bfx			↓ Epi.% BAL ↓ [Epi.] BAL	

Abbreviations: FA = filtered air; RSS-200 = rice straw smoke at 200 μg/m^3; RSS-600 = rice straw smoke at 600 μg/m^3; RSS-3x200 = 3 serial-day exposures to rice straw smoke at 200 μg/m^3; ↑= increase; ↓ = decrease; % = differential percent; [ ] = concentration; TLC = total leukocyte count; Mac. = macrophages; Lym. = lymphocytes; Neut. = neutrophils; Eos. = eosinophils; Epi. = epithelial cells; IL-8 = interleukin-8; MCP-1 = monocyte chemoattractant protein; BFx = bronchial fraction; BAL = bronchoalveolar lavage. The direction of change indicated by the arrows refers to the change in the condition in the horizontal titles, compared to the condition listed in the vertical titles.

Discussion

This project was designed to test the hypothesis that airway inflammation, as indicated by cell distribution and cytokine protein expression, would be increased, and spirometric pulmonary function, decreased, as a function of increased RSS concentration and dose, and in individuals with either asthma, or allergic rhinits, as compared to healthy individuals. The results of the project support the airway inflammation component of this hypothesis, but not the spirometric pulmonary function component. The RSS exposure resulted in changes in the cell distribution, both within, and between, the three subject groups, but no measurable change in spirometric pulmonary function.

The RSS-induced increases in total leukocytes, macrophages, neutrophils, and lymphocytes, and IL-8, in the current study are in accord with those from resulting from accidental and occupational smoke exposures which include increased neutrophils in BAL, and decreased chemotactic migration of alveolar macrophages (Demarest *et al.*, 1979), increased total cell count and neutrophils in BAL (Riyami *et al.* 1990), increased lymphocytes and fibronectin and hyaluronic acid concentration in BAL (Bergstrom *et al.* 1997), increased IL-10 in induced-sputum, and an increase in Clara-cell protein and surfactant-associated protein in serum (Burgess *et al.* 2002). Additionally, exposure to forest fire smoke increases neutrophils (Tan *et al.* 2000), and pro-inflammatory cytokines (IL-1β, IL-6, GM-CSF) (van Eeden *et al.* 2001), in peripheral blood. Due to the large range of exposures (fuel source, smoke concentration, exposure duration, exercise), used in these studies it is not possible to make direct comparisons of dose-response changes in cells or proteins involved in inflammation, however, it appears that there are smoke-induced changes in indices of inflammation that are common across different smoke exposures.

The RSS-induced changes in airway cell distribution included increases in macrophages, neutrophils, and lymphocytes. As particulate matter is a major component of RSS, it was expected that both macrophages and neutrophils would be recruited into the airway to remove these particles. As controlled exposure to concentrated ambient air particles results in an increase in neutrophils in the airway (Ghio *et al.* 2000), it is probable that the particulate component of RSS was responsible for, at least part, of the increase in inflammatory cells observed in the current experiments. It is also possible that the increase in macrophages and neutrophils in the airway were in response to the gas phase of the inhaled RSS, as these cells are increased in the airway in response to ozone (Christian *et al.* 1996), and nitrogen dioxide (Solomon *et al.* 2000), and are involved in general inflammatory processes. Further experiments using separate exposures to the particle phase only, and gas phases only, are required to determine the contribution of each of these components to the inflammatory response.

The RSS-induced changes in cell distribution were present in both the Bfx and BAL, indicating that inhaled components of RSS are penetrating both the bronchial and alveolar regions of the airways. It is possible that the particle and gas phases of the RSS could be independently active in the different regions, and further experiments

partitioning the particle and gas phases are required to investigate this issue. A finding of interest in this project was the overt visual inflammation observed in the RSS-600 and RSS-3x200 conditions. This finding warrants further investigation, using objective, quantitative methods.

Several indices of inflammation, including increases in neutrophils, epithelial cells, and IL-8, indicate that the serial-day RSS exposure provided the highest inflammatory stimulus of the exposure conditions, specifically compared to the single 600 $\mu g/m^3$ exposure. It is possible that the anti-inflammatory mechanisms involved in controlling the inflammatory response to the single dose of RSS were further stressed in response to the serial-day exposures. These anti-inflammatory pathways would be expected to include tumor growth factor-beta (TGF-β), and IL-10, both of which are of interest in further investigation of the activation and balance of pro- versus anti-inflammatory cytokines which are activated by inhaled smoke.

The Asthma subject group, compared to both the Healthy and Allergic Rhinitis groups, exhibited pre-existing inflammation, having increased airway neutrophils in the FA condition. This increased number of neutrophils in the Asthma group was also present in the RSS-600 condition, indicating a greater overall inflammatory response, which could be a function of either or both the pre-existing inflammation or the response to the RSS. The lack of response to the RSS in the Allergic Rhinitis group could have been due, in part, to the absence of exposure/stimulation of the nasal mucosa, as all subjects wore noseclips during the exposures to ensure oral breathing. It is currently unclear if stimulation of the upper airways is required for inflammatory responses in the lower airway in individuals with allergic rhinitis. It is also possible that the reason for there not being a more pronounced inflammatory response in both the Asthma and Allergic Rhinitis groups was that pre-existing airway inflammation could actually provide an environment that allows a more rapid and directed response to inhaled toxins. Instead of pre-disposing the individual to a greater inflammatory response, the primed cellular and cytokine systems, could negate a potential toxin more efficiently than a more dormant system. Further investigation of anti-inflammatory process is required to determine the potential capacity for this to occur.

There was no physiologically relevant effect of RSS exposure on any indices of spirometric pulmonary function or subject symptoms in any of the subject groups or exposure conditions in this experiment. The finding of cellular and cytokine protein inflammatory response in the absence of any change in neither spirometric pulmonary function or subject symptoms, indicates that adverse RSS-induced health effects can occur in situations where healthy individuals are unaware of the exposure. The finding of no RSS-induced change in spirometric pulmonary function, in association with inflammation, in the Healthy subject group is in agreement with results of controlled exposures to ETS (NCI, 1999), and particles (Ghio *et al.* 2000) in healthy subjects. However, data from ETS exposures in individuals with asthma indicate that the current exposure could have induced decreases in spirometric pulmonary function (NCI, 1999). It is likely that the lower concentrations and duration of exposure used in the current

experiments, compared to those used in the ETS experiments, are the reason for the absence of any change.

The results of this project can be, in part, generalized to other forms of biomass smoke to which humans are exposed. The current data provide an indication of levels of biomass smoke that result in airway inflammation, which could be applied to other sources of smoke, including forest fires, and residential heating and cooking fires. The increase in use of wood and other biomass for fuel throughout the world (Reviewed; Zelikoff *et al.* 2002), would be expected to lead to an increase in exposure of both healthy and susceptible humans to biomass smoke. Further information is needed to identify the similarities and differences between various types of biomass smoke, and the specific toxic effects on healthy and susceptible humans.

Summary and Conclusions

The results of this project indicate that RSS is capable of inducing airway inflammation in healthy individuals, and in individuals with asthma, or allergic rhinitis. The pattern of RSS-induced changes in cell distribution and cytokine proteins were different between the three subject groups, however, each group did exhibit some cell or protein changes indicative of inflammation and/or injury in the airway. This is the first series of controlled human exposure experiments investigating the effects of RSS on human respiratory health, and specifically airway inflammation. Further experiments are required delineate the toxic component(s) of RSS, the threshold concentrations and doses for exposure regulation, and the effects on other susceptible populations, including individuals with cardiopulmonary disease.

Recommendations

Although the primary independent variables used in this project did show a change as a function of RSS exposure, other variables could have provided further information on the RSS-induced inflammatory response; including sub-sets of cell types (lymphocytes), multiple pro- and anti-inflammatory cytokines, and gene expression.

The exposure chamber and smoke generation system used in this study were designed to generate smoke from the burning of various vegetative matter. This vegetative matter could include, but would not be limited to, other field residues, agricultural prunings, forest waste/biomass, and residential wood burning fuels. Also, this system will allow exposures to be conducted in specific subject groups, and using controlled concentrations, durations, and doses.

Table 20. Table of abbreviations

Exposure conditions:
FA = filtered air
RSS-200 = rice straw smoke at 200 μg m^3
RSS-600 = rice straw smoke at 600 μg m^3;
RSS-3x200 = 3 serial-day exposures to rice straw smoke at 200 μg m^3;

Bronchoscopy:
Bfx = bronchial fraction (first 15 ml of BAL)
BAL = bronchoalveolar lavage

Biochemical:
TNF-α = tumor necrosis factor-alpha
IL-8 = interleukin-8
MCP-1 = monocyte chemoattactant protein-1

Spirometric pulmonary function:
FVC = forced vital capacity
FEV_1 = forced expired volume in 1 s
FEF_{25-75} = forced expired flow-rate at 25-75% FVC
FEF_{75} = forced expired flow-rate at 75% FVC.

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