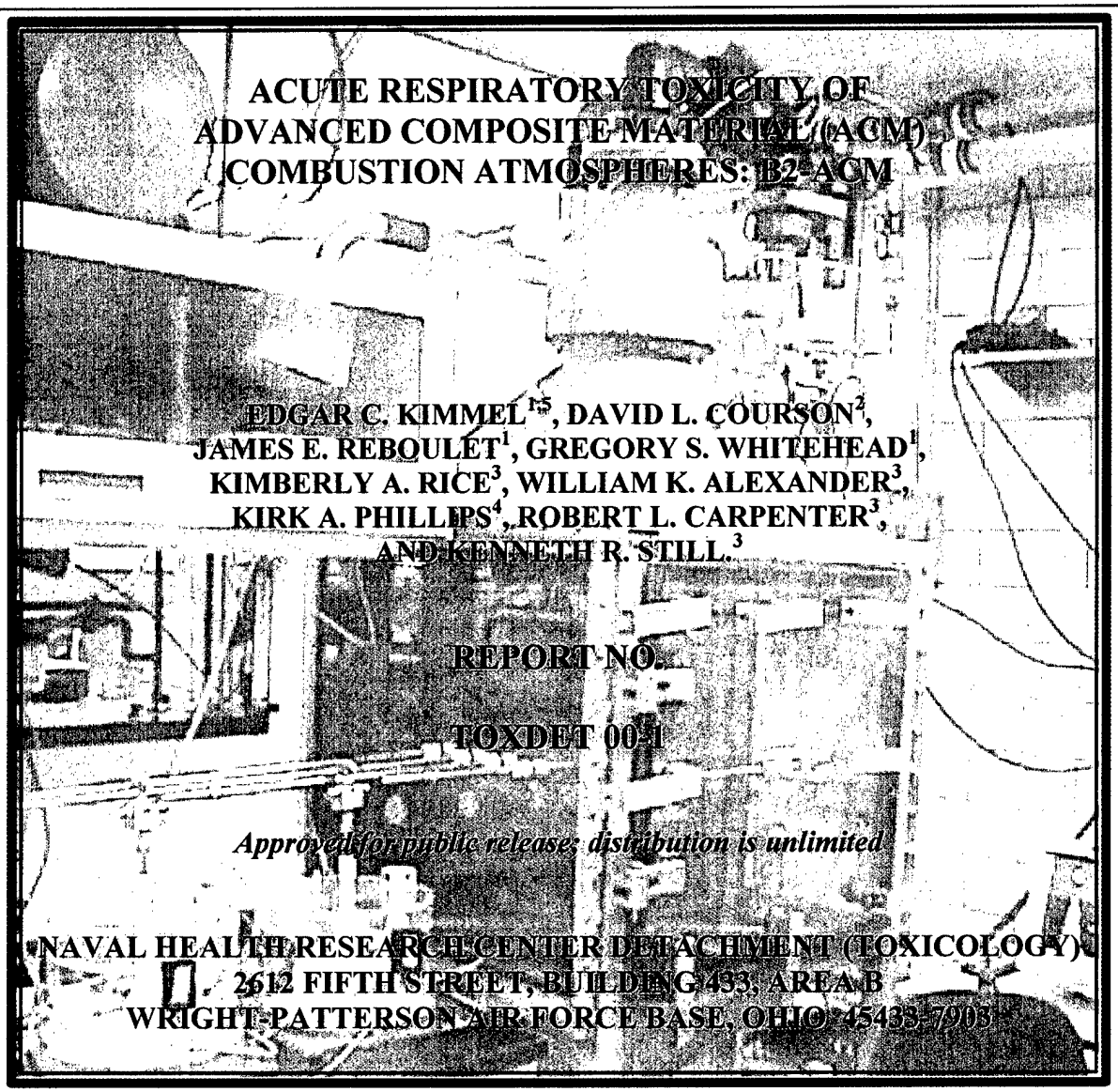
Naval Health Research Center Detachment (Toxicology)



ACUTE RESPIRATORY TOXICITY OF ADVANCED COMPOSITE MATERIAL (ACM) COMBUSTION ATMOSPHERES: B2 ACM

EDGAR C. KIMMEL[1,5], DAVID L. COURSON[2], JAMES E. REBOULET[1], GREGORY S. WHITEHEAD[1], KIMBERLY A. RICE[3], WILLIAM K. ALEXANDER[3], KIRK A. PHILLIPS[4], ROBERT L. CARPENTER[3], AND KENNETH R. STILL.[3]

REPORT NO.

TOXDET 00-1

Approved for public release; distribution is unlimited

NAVAL HEALTH RESEARCH CENTER DETACHMENT (TOXICOLOGY)
2612 FIFTH STREET, BUILDING 433, AREA B
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433-7903

1. Geo-Centers, Inc, at NHRC/TD
2. ManTech Environmental Technology, Inc. at NHRC/TD
3. Naval Health Research Center Detachment (Toxicology) – (NHRC/TD)
4. 509 ADOS/AGGB, USAF – Whiteman, AFB
5. To whom correspondence should be addressed at
NHRC/TD Bldg. 433
2612 5th St.
Wright-Patterson AFB, OH 45459

20000317 043

PREFACE

This document contains the results of an investigation of lethality and pulmonary pathophysiology produced in laboratory rats by acute exposure to smoke generated from combustion of advanced composite material (ACM). Various types of ACM are currently being used the construction and retrofit of numerous military systems and vehicles. The type of ACM under investigation was material commonly used in the construction of the B2 bomber (B2-ACM) and was provided by Northrop Grumman Corp. The report is intended to provide information pertinent to the evaluation of the potential health risks to humans exposed to B2-ACM smoke. This work was conducted at the Naval Health Research Center Detachment Toxicology (NHRC/TD) under the direction of CAPT Kenneth R. Still, MSC, USN, Officer-in-Charge NHRC/TD. The research was requested by Capt Kirk A. Phillips, BSC, USAF, Bioenvironmental Engineering Flight Commander 509 ADOS/SGGB. This work was sponsored by the 509 ADOS/SGGB and the Naval Medical Research and Development Command under Work Unit # 63706N-M00095.004.1714.

The opinions contained herein are those of the authors and are not to be construed as official or reflecting the view of the Department of Defense, the Department of the Navy or the Naval Services at large.

Animal handling procedures used in this study were subject to review and approval by the Animal Care and Use Committee located at Wright-Patterson AFB and the Airforce Surgeon General. The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Research, National Research Council, DHHS, National Institutes of Health Publication 85-23, 1985, and the Animal Welfare Act of 1966, as amended.

TABLE OF CONTENTS

SECTION	PAGE
Executive Summary	3
Abstract	5
Glossary of Terms and Abbreviations	6
List of Tables	7
List of Figures	8
Introduction	11
Methods and Materials	13
Results	22
Discussion	28
Conclusion	33
References	35
Tables	41
Figures	43

EXECUTIVE SUMMARY

PROBLEM

Advanced composite material (ACM) is a term used to describe a variety of products composed primarily of fabrics woven of carbon, aramid, and/or glass fibers that are impregnated with and imbedded in epoxy resins. Several different types of ACM are currently used or are being proposed for use in the construction and retrofit of military, as well as civilian, vehicles and systems. They are widely used in a variety of military aircraft. ACMs are generally more combustible than are the materials they are replacing. The health risks associated with exposure to atmospheres generated by combustion of ACMs are not known. Consequently, there is heightened concern over potential health risks associated with aircraft mishaps that result in exposure to smoke generated by burning ACM. There are myriads of possible exposure scenarios associated with aircraft mishap fires that are dependent on several factors including the type of ACM burned. The present investigation focused upon the type of ACM used in the construction of B2 stealth bomber wing components (B2-ACM).

OBJECTIVE

The current investigation was undertaken to gather information on the lethality and respiratory toxicity from acute exposure to B2-ACM smoke, which is needed for health protection of Air Force, and civilian personnel associated with aircraft crash sites. There are myriad exposure scenarios associated with aircraft mishap fires. Each is dependent on situational details including the type of ACM burned. In order to be specific in our research, the current investigation focussed on the lethality and respiratory toxicity from acute exposure to B2-ACM smoke. This study directly considers the health consequences to non-emergency response personnel who may not have respiratory protection available and is applicable to establishing the cordon boundaries used to isolate a crash site.

RESULTS

This investigation shows that burning B2-ACM smoke is a hazardous substance and that moderately high doses can severely affect the respiratory tract causing respiratory distress and death. Preliminary results from data to be published in a subsequent report suggest that exposure to smoke concentrations too low to be visible have significant impact on breathing and upper airway function and that a threshold for these effects may exist.

CONCLUSION

Acute exposure to B2-ACM smoke can be fatal. Dispersion of smoke generated from burning approximately 20 grams of B2-ACM into about 0.7 m^3 of air produces smoke that is life threatening if breathed for 1 to 2 hours. Inhalation of B2-ACM smoke at this and lower concentrations causes changes in lung function indicative of long-term, debilitating lung disease. There is substantial health risk accrued to individuals who may be unable to escape the vicinity of an aircraft mishap which involves burning ACM within a few hours. The results of this investigation indicate that acute exposure to B2-ACM smoke may result in long-term respiratory

debilitation and that certain individuals may develop non-specific respiratory hypersensitization, which could be lethal. It is strongly recommended that these possibilities be subject of further investigation in order to understand, fully, the health risks associated with B2-ACM smoke exposure.

ABSTRACT

Exposure for 2 hr to smoke generated from pyrolysis of 100 g of B2-ACM was lethal to experimental animals. Surviving animals showed elevated carboxyhemogobin levels, severe respiratory acidosis, and diminished oxygen transport in the blood, the combination of which was deemed the cause of death. These animals also showed signs of pulmonary inflammatory response. Two days post exposure, animals exhibited pulmonary pathophysiology indicative of restrictive lung disease. The pattern of pulmonary dysfunction at 14 days post exposure was similar, with some measures of lung function returning to normal while others remained significantly different from normal values. Although no deaths occurred, similar patterns of pulmonary dysfunction were evident in animals exposed for 1 hr to smoke from pyrolysis of 55 or 100 g of B2-ACM. These animals also had elevated carboxyhemogobin levels, decreased blood pH, and diminished oxygen capacity immediately post exposure however these symptoms were not as severe as those in animals exposed for 2 hr to smoke generated from pyrolysis of 100 g of B2-ACM. Quantitative analysis of a few selected smoke gases (CO, CO_2, NOx, SO_2) and aerosol particulate consistently accounted for over 80 % of the amount of B2-ACM that actually burned. Calculation of the nominal concentration of the smoke correlated well with the chemical analysis, the bulk amount of B2-ACM used to generate the smoke, and the pyrolysis rate. Consequently these estimates of smoke concentration could be used for dose-response determinations which, in turn, provide a suitable basis for risk assessment.

KEY WORDS AND PHRASES

B2-ACM smoke, Pulmonary pathophysiology, Inhalation Toxicity.

GLOSSARY OF TERMS AND ABBREVIATIONS

Note: Common chemical and measurement abbreviations are not included.

ACM – Advanced Composite Material
AR – Airway Reactivity
B2-ACM – One of the ACM types used in the construction of the B2 stealth bomber.
BAL – Bronchoalveolar lavage
Cdyn – Dynamic compliance
Cqst – Quasistatic compliance
DLco – Single breath carbon monoxide diffusing capacity
EES – End expiratory sample
ELPI – Electronic Low Pressure Impactor
ERV – Expiratory reserve volume
f – Breathing frequency
FEF – Forced expiratory flow
    FEF10 – FEF at 10% of FVC (90% expired)
    FEF25 – FEF at 25% of FVC (75% expired)
    FEF50 – FEF at 50% of FVC (50% expired)
FEV – Forced expiratory volume
    $FEV_{0.05}$ – FEV in 0.05 seconds
    $FEV_{1.0}$ – FEV in 1.0 seconds
FRC – Functional residual capacity
FV – Flow - volume curve
FVC – Forced vital capacity
IES – Initial expiratory sample
MMAD – Mass median aerodynamic diameter
MMEF – Mid mean expiratory flow (average FEF between 25 and 75% FVC)
PAM – Pulmonary alveolar macrophage
Pao – Pressure at airway opening
pCO_2 – Partial pressure of carbon dioxide
Pes – Esophageal pressure
PMN – polymorphnuclear leukocyte
pO_2 – Partial pressure of oxygen
Ppl – Plural pressure
PV – Pressure - volume curve
R_L – Lung resistance
RV – Residual volume
SEM – Standard error of the mean
SwRI – Southwest Research Institute
TLC – Total lung capacity
Ve – Minute ventilation
Vt - Tidal volume
σg – Geometric standard deviation

LIST OF TABLES

TABLE 1. B2-ACM Nominal Exposure Concentrations.

TABLE 2. Exposure Mass Balance

LIST OF FIGURES

Figure 1. Schematic Diagram of the Exposure System.

Figure 2. Schematic Diagram of the Sampling System.

Figure 3. Schematic Diagram of the SwRI Furnace.

Figure 4. Thermal Gravimetric Spectrum of a Small (1 mg), B2-ACM Sample.

Figure 5. Carbon Dioxide Concentration in B2-ACM Smoke Exposures.

Figure 6. Carbon Monoxide Concentration in B2-ACM Smoke Exposures.

Figure 7. Oxygen Concentration in B2-ACM Smoke Exposures.

Figure 8. Nitrogen Oxide(s) Concentration in B2-ACM Smoke Exposures.

Figure 9. Sulfur Dioxide Concentration in B2-ACM Smoke Exposures.

Figure 10. HydrogenCyanide Concentration in B2-ACM Smoke Exposures.

Figure 11. Aerosol Concentration in B2-ACM Smoke Exposures.

Figure 12. Aerosol Size Distribution in B2-ACM Smoke Exposures.

Figure 13. Typical B2-ACM Smoke Aerosol Particle.

Figure 14. Energy Dispersive X-Ray Spectrum from a Single Aerosol Particle.

Figure 15. Cluster of B2-ACM Smoke Aerosol, Chain Aggregate Particles.

Figure 16. Energy Dispersive X-Ray Spectrum from a Chain Aggregate Particle.

Figure 17. Typical B2-ACM Smoke Aerosol Fiber.

Figure 18. Energy Dispersive X-Ray Spectrum from a Smoke Aerosol Fiber.

Figure 19. Theoretical Aerosol Deposition in the Pulmonary Region of the Lung.

Figure 20. Animal Body Weight to 14 Days Post Exposure.

Figure 21. Venous Blood Gas, Carbonate, and pH Values.

Figure 22. Venous Blood Hemoglobins.

Figure 23. Bronchoalveolar Lavage Fluid Mobile Cell Population and Type.

Figure 24. Particle Laden Pulmonary Alveolar Macrophages – 100g-2hr Group.

Figure 25. Tidal Volume

Figure 26. Minute Ventilation.

Figure 27. Dynamic Compliance.

Figure 28. Quasistatic Compliance.

Figure 29. Lung Resistance.

Figure 30. Lung Volumes.

Figure 31. Percent of Total Lung Capacity Represented by Functional Residual Capacity.

Figure 32. Percent of Functional Residual Capacity Represented by Residual Volume.

Figure 33. Mid-mean Expiratory Flow Normalized to Forced Vital Capacity.

Figure 34. Forced Expiratory Flow at 50% Forced Vital Capacity Normalized to Forced Vital Capacity.

Figure 35. Forced Expiratory Flow at 25% Forced Vital Capacity Normalized to Forced Vital Capacity.

Figure 36. Forced Expiratory Flow at 10% Forced Vital Capacity Normalized to Forced Vital Capacity.

Figure 37. Forced Expiratory Volume at 0.05 Second.

Figure 38. Forced Expiratory Volume at 0.1 Second.

Figure 39. Single Breath Carbon Monoxide Diffusing Capacity.

INTRODUCTION

The use of advanced composite materials (ACMs) as substitutes for metallic components in the construction and retrofit of military and civilian aircraft, as well as other vehicles, is increasing. ACMs offer a superior strength-to-weight ratio and a higher resistance to corrosion compared to metallic components. Particularly important to military applications is the fact that ACMs reflect less electromagnetic energy than metals and thus are less detectable by radar. However, because ACMs are more combustible than their metallic counterparts they present a source of airborne contaminants in smoke generated from fire related aircraft mishaps. Potential health risks posed to both emergency response personnel and the general population by exposure to smoke from burning ACMs are not well characterized.

In 1999 there were several mishaps resulting in the destruction of 25 US Air Force aircraft (Gideon, 2000), many of which contained ACM components. In the past 15 years there have been at least three incidents in which adverse health effects have been reported in personnel exposed to smoke or residue from downed aircraft containing ACM structural components. In 1997, 22 rescue workers exposed to smoke from an F-117A fighter downed near Baltimore, MD were taken to the hospital, within hours of exposure, with complaints of labored breathing, headache and nausea (Baltimore Sun, 1997, Reuters News Service, 1997). Workers recovering the engine of a RAF GR.5 Harrier which crashed in Denmark in 1990, suffered from eye and skin irritation which prompted the use of industrial respiratory and eye protection equipment with equivocal results (JDW, 1991). Workers complained of eye irritation, sore throat, and chest pains despite this protection. Two of four individuals exposed at the crash site of an F-18 fighter complained of respiratory problems and diminished exercise capacity several days after exposure. One of these individuals showed an abnormal one-second, forced expiratory volume (FEV_1) and increased airway reactivity in response to histamine challenge. The abnormal FEV_1 persisted for 5 months (Seibert, 1990).

The term ACM refers, collectively, to a variety of engineered materials that are composed of continuous fiber fabrics impregnated with and imbedded in a polymer matrix to form reinforced plys which then are bonded together in layers to form laminates (FFA, 1997). High-strength glass, carbon/graphite, tungsten/boron, and aramid materials (e.g. Kevlar ®, DuPont Chemical) are materials used to form the fiber mesh in various types of

ACMs. Thermosetting resins, such as epoxy, cyanate esters, bismaleimides, polyesters and vinyl esters are used to form the polymer matrix in various ACMs used for structural components in aircraft. All of these materials are highly flammable and have high heat release rates when burned. Because of lower heat release rates upon combustion, phenolic resin based ACMs are preferred for aircraft interior composite structures. Thermoplastic resins such as polyetherketones and polysulfones are used, without fiber reinforcement, for form molded parts and to strengthen thermoset resins for some applications. By in large epoxy resins are the most common polymers used in ACMs.

Smoke is a complex mixture of gases, vapors, and particulate matter the exact composition of which is dependent on the material being burned and combustion kinetics. Therefore the inhalation toxicity of a given smoke also is a function of the material being burned and the combustion kinetics. The broad range of possible compositions of ACM suggests that there is a broad spectrum of possible untoward responses that may be elicited by exposure to their smoke. Likewise, there may be a variety of untoward responses elicited by exposure to smoke from a given ACM formulation, which are dependent on smoke concentration, duration of exposure and other factors. The present investigation was undertaken to evaluate the acute respiratory toxicity of short-term exposure smoke from a single ply carbon/graphite/epoxy ACM used in the construction of the B2 bomber.

METHODS AND MATERIALS

Test Material

Northrop Grumman corporation provided single ply carbon/graphite/epoxy coupons of B2-ACM measuring 10 cm by 10 cm by approximately 0.3 cm thick for this study (ref # B01718, ASDL #146, Northrop/Grumman Corp. – Dan Ellison, personal communication). To simulate the extensive damage to ACM that reportedly occurs upon high impact (Flight International, 1991) these coupons were first quartered and then ground in a laboratory cutting mill (Fritsch Model # LC-124 Gilson Co., Worthington, OH). A coarse, 6 by 6 mm sieve was used in the mill and splintered fragments that typically measured 2 to 5 mm in width and 20 to 40 mm in length were produced by this process. De-lamination of the fiber mesh and the epoxy did occur, however this process did not effect the total fraction of B2-ACM that burned at test temperatures nor was the combustion rate substantially effected despite the increase in surface area to mass ratio.

Animals

180 male F-344 rats weighing between 280 and 310 g (at exposure) were obtained commercially (Charles River Laboratories, Raleigh, NC) for this investigation. Newly arrived animals were subject a veterinary examination and a 10-day quarantine period prior to use. The animals were housed individually in plastic, shoebox type rodent cages over adsorbent bedding for the duration of the investigation. Food and water were provided *ad-libitum* and the animals were maintained on a 12 hr diurnal cycle. Prior to exposure and during the post exposure observation period the animals were maintained in a fully, AALAC accredited facility.

Experimental Design

Initially, 12 animals were used in a range finding pilot investigation to estimate survivability. Five groups of 28 animals each were exposed to B2-ACM smoke for periods of 1 or 2 hours. Exposures were conducted using 10, 55 or 100 g of milled B2-ACM. These weights refer to the amount of B2-ACM loaded into the furnace and are not to be construed as smoke exposure concentrations. For convenience, the term load is used to distinguish between these quantities of un-combusted B2-ACM and their corresponding nominal and/or measured smoke

concentrations. Nominal concentration refers to the concentration of smoke calculated by dividing the amount of B2-ACM burned in a given time by the volume of air passed through the furnace during that time. If this volume of air was smaller than the chamber volume in which the smoke was trapped then chamber volume was used to make this calculation. Exposures using 10 and 55 g loads were conducted for 1 hr, whereas exposures using a 100 g load lasted for either 1 or 2 hr. Not all animals survived the 2 hr exposure therefore two separate exposures were conducted in order to have a sufficient number of animals to satisfy post exposure evaluation requirements. A control group, also of 28 animals, was exposed to room air for 1 hour. The following terms are used throughout to identify the exposure groups:

- Control - room air exposure for 1 hour.
- 10g-1hr - 10 g load, 1 hour exposure.
- 55g-1hr - 55 g load, 1 hour exposure.
- 100g-1hr - 100 g load, 1 hour exposure.
- 100g-2hr(1) - 1st 100 g load, 2 hour exposure.
- 100g-2hr(2) - 2nd 100 g load, 2 hour exposure.

Evaluations of respiratory and pulmonary responses were performed on 6 animals from each group either immediately after exposure or on days 2 or 7 post exposure. Ten animals from each group were required for day 14 post exposure examinations.

Exposure System

Main elements of the exposure system consisted to a 690 L whole-body inhalation exposure chamber (Kimmel, et al., 1997) and quartz glass radiant furnace purchased from Southwest Research Institute (SwRI - San Antonio, TX) which is similar in design to the furnace developed by H.W. Stacy (Gad and Anderson, 1990). The SwRI furnace was modified to operate as a flow through device. Because the effective capacity of the furnace was limited (100 g for milled B2-ACM) the combustion times for various loads of B2-ACM did not exceed 8.5 min. However exposure periods were substantially longer, consequently the exposure system was designed to be operated initially in a continuous flow (dynamic) mode during pyrolysis and then switched to static mode operation once pyrolysis was complete (Kimmel and Reboulet, 1998). By trapping the smoke within the exposure chamber in this manner, exposure periods longer in duration than the combustion times could be investigated without the necessity having

a prohibitively large furnace or a furnace equipped with a continuous test material feed mechanism. The system was configured so that main chamber airflow could be shunted directly to the exhaust system without passing through the exposure chamber volume this allowed uninterrupted system flow when switching between dynamic and static operation. For the same purpose, flow through the furnace was likewise equipped with a by-pass loop. Flow through the furnace was introduced into the chamber inlet flow via a counter current mixing device. A series of electronically activated, pneumatically controlled shut off valves (see Figure 1) was used to change system operational mode. Main chamber inlet and exhaust flow were actively supplied via blowers (inlet, model 2000-00-C, EG&G Rotron, Saugerties, NY; exhaust, model HS-2 Mystaire Scrubber, Misonix Inc, Farmington, NY). Both flows were controlled with metering valves and differential pressure producing devices were installed in both the exhaust and inlet piping to measure flow. Total flow through the system (exhaust flow) was set at 90 L/min. To facilitate mixing of the furnace output in the total chamber flow stream the chamber inlet flow was adjusted to 10 L/min. A 10 L/min jet of air was passed over the sample surface to simulate turbulence found in open fires. Primary furnace flow (approximately 70 L/min) was achieved by passive aspiration of air through openings in the furnace. Adjusting the aspiration pressure across the furnace between 3.8 and 4.5 cm H_2O sub-ambient set primary furnace flow rate. The furnace was equipped with a load cell (model 6005D, Automatic Timing Controls, Inc., King of Prussia, PA) for measurement of sample mass loss during pyrolysis. The output of the load cell was recorded in real-time using a personal computer based data acquisition system (sensor card, UPC601-U, Validyne Engineering, Northridge, CA; software, Labtech Pro, Laboratory Technologies Corp, Wilmington, MA). Simultaneous measurement and integration of system total flow was used to determine the volume of air into which the smoke generated by pyrolysis of a known mass of B2-ACM was delivered. With adjustment for chamber volume into which the smoke was delivered the nominal smoke concentration was determined.

The SwRI furnace consists of a 14.2 cm diameter, quartz glass cylinder, 30 cm long, with a removable 143/65 standard taper plug on one end for loading of samples (Figure 2). The opposite end has a 29/25 standard taper fitting through which spark source electrodes are passed. A rectangular (5 x 30 cm) chimney is located on one side of the cylinder (top of the furnace) through which smoke passes. The bottom of the furnace has a small orifice (1.2 cm diameter) through which a rod connecting the sample platform to the load cell passes. Four parabolic

heating lamps (model 5305, Research Inc., Minneapolis, MN) powered by a 240 v, 60 amp line are focused upon the sample holder. The lamp assembly was cooled with water provided by a re-circulating chiller (model RC20, Brinkman Instruments, Westbury, NY). Combustion temperatures were taken using a type K, columel/alumel, thermocouple (Omega Engineering, Stamford, DT) located just above the surface of the sample. The temperature profile during pyrolysis was recorded with a digital thermometer (model 680, Omega Engineering, Stamford, CT). At maximum setting the lamps produce temperatures ranging between 680 and 740 C^{o} with 700 C^{o} being typical. The rate at which maximum temperature was achieved could not be determined accurately due to thermocouple lag time, however smoke generation began instantaneously upon starting the heating element.

Smoke Characterization

In addition to calculation of nominal smoke concentration, specific analyses were made for several gases commonly found in combustion atmospheres as well as oxygen (O_2). Exposure chamber concentration of carbon monoxide (CO) and carbon dioxide (CO_2), and were monitored continuously using non-dispersive, wavelength specific infrared spectrometers (models 880 and 880A respectively, Rosemont Instruments, Inc., La Habra, CA). O_2 concentration also was monitored continuously using an O_2 specific electrochemical analyzer (model 326RA, Teledyne Analytical Instruments, City of Industry, CA). These instruments were connected in series, thus a single sample flow rate of 1 L/min served for all three analyses. Oxides of nitrogen (NOx) were measured with a chemoluminescent analyzer (model 10AR, Thermo-Environmental Instruments Inc., Franklin, MA). Five-minute samples were taken at the start of the exposure and once each subsequent 20-minute interval. Sample flow rate was 1 L/min. Sulfur dioxide (SO_2) samples were taken on the same schedule and a flow rate of 0.5 L/min for pulsed fluorescence spectrophotometric analysis (model 43C, Thermo-Environmental Instruments Inc., Franklin, MA). Hydrogen cyanide (HCN) concentration was determined electrochemically (model RM-28, Interscan Corp., Chatsworth, CA) in the 10 g load smoke. However substantial interference from other smoke constituents precluded analysis for HCN at higher smoke concentrations. In-line cartridge, absolute filters were placed in sample lines to remove smoke particulates from the samples. All analyzers were calibrated with known gas standards just prior to each exposure.

Sampling from a sealed chamber operating in a static mode would have resulted in an unacceptable decrease in chamber pressure. Therefore the sampling system was equipped with a feed back flow, that was delivered to the chamber exhaust plenum (Figure 3). Flow in the feed back loop was balance with sample flow rates so that the chamber was always maintained at a slightly sub-ambient pressure (0.5 to 1.0 cm H_2O). Gas sample flows caused a 10% dilution of smoke concentration in the chamber.

Several analyses were made of the particulate fraction of the B2-ACM smoke. Mass concentration was determined by gravimetric analysis of aerosol particles collected on absolute filters (type A/E 47mm glass fiber, Gelman Laboratory, Ann Arbor, MI). Leak free, isokinetic filter holders (In-Tox Products, Albuquerque, NM) were used at a sample flow rate of 5 L/min. Sample duration was 0.5 or 1 min depending on smoke concentration. Filter samples were taken at 5, 30, and 55 min for 1 hr exposures, additional samples at 90 and 115 min were added for 2 hour exposures. A point-to-plane electrostatic precipitator (ESP, model 02-1700, In-Tox Products, Albuquerque, NM) was used to collect particles on carbon planchettes (Earnest Fullam, Inc., Albany, NY) for scanning electron microscopy. Particle size distribution was determined with a multistage, electrical low-pressure cascade impactor (ELPI, Dekati Ltd., Tampere Finland). Impactor flow rate was 9 L/min, at an outlet absolute pressure of 73 torr, which allowed the collection of nanometer size particles (Keskinen et al., 1992). The ELPI can be used for near real-time analysis of aerosol distributions and is suitable for monitoring dynamic changes in aerosol size distribution that frequently occur in combustion atmospheres (Kymalainen et al., 1996). A 3 minute sample was adequate for complete purging of the impactor with sample. Impactor samples were taken twice during the exposures, once 10 minutes into the exposure and once 10 minuets prior to the end of exposure for both the 1 and 2 hour exposures. As with the gas sampling, the particle analysis sampling system was configured with a balanced flow feed back loop system to minimize the effect of sample collection on chamber pressure. Aerosol sampling procedures caused a 2.8 % dilution of smoke in the chamber.

Respiratory Function

Six animals per exposure group were sacrificed immediately after exposure for collection of blood samples (approx. 0.5 mL each) from the inferior vena cava. Partial pressure of dissolved oxygen and carbon dioxide (pO_2 and pCO_2 respectively), carbonate and hydrogen ion

concentrations (HCO_3 and pH, respectively) were obtained with a blood gas analyzer (model 1620, Instrumentation Laboratory, Lexington, MA) from mixed venous blood. Likewise CO-oximetry (model 682, Instrumentation Laboratory, Lexington, MA) was used to obtain fractions of hemoglobin bound to oxygen, carbon monoxide, nitrogen species or in reduced form (O_2Hb, COHb, RHb, and metHb respectively).

On days 2, 7 and 14 post exposure 6 animals from each exposure group underwent a battery of pulmonary function tests (pfts) using modifications of previously described (Diamond and O'Donnell, 1977; Sabo et al., 1984; Kimmel and Diamond, 1984; Kimmel et al., 1985; Whitehead et al., 1999) whole-body plethysmographic techniques. Animals were anesthetized with ethyl carbamate (urethane, i.p. 0.75g/kg) and a tracheal catheter (2 mm I.D., volume = 0.1 ml) was inserted prior to testing. Measurements of ventilation, tidal volume (Vt), breathing frequency (*f*), and minute ventilation (Ve); and pulmonary mechanics measurements, dynamic compliance (Cdyn) and pulmonary resistance (R_L); functional residual capacity (FRC – Boyles Law method, DuBois et al., 1956, Palecek, 1969.) were performed on spontaneously breathing animals. Pressure-volume (PV) curve measurements and forced expiratory flow-volume (FV) were performed after apnea had been induced by hyperventilation. Volume history was established just prior to these measurements by inflation of the lungs to total lung capacity (TLC), which was defined as the lung volume at an airway opening pressure (Pao) of 30 cm H_2O. A 1.5 L volume displacement plethysmograph (model ply 3114, Buxco Electronics, Sharon, CT) was used for these measurements. A 1 cm diameter, screen pneumotachograph (6 layer #325 wire mesh, Small Parts, Inc. Miami Lakes, FL) port located in the plethysmograph side wall was used to measure respiratory flows and volumes. The pressure differential produced by flow across the pneumotachograph was measured (model S0X1, SynSem Inc., Malpitas, CA) and the signal was integrated electronically (Max II, Maneuver Signal Generator, Buxco Electronics, Sharon, CT) to obtain volume. Pao was measured (model TRD4510, Buxco Electronics, Sharon, CT) at the plethysmograph breathing port and valve assembly that was used to perform PV and FV maneuvers. Total dead space in the tracheal catheter and valve assembly combined was 0.8 ml and was factored into subsequent calculations. Plural pressure (Ppl) required for calculation of indices pulmonary mechanics, dynamic compliance (Cdyn) and pulmonary resistance (R_L) was estimated by measuring esophageal pressure (Pes) using a pressure transducer (model TRD0113, Buxco Electronics, Sharon, CT) connected to a water

filled esophageal catheter. Signals to the maneuver control valves, data derived from various transducers, and calculation of the various lung function parameters were performed automatically via personal computer interface with the Max II system and appropriately software (Biosystems XA, Buxco Electronics, Sharon, CT).

PV maneuvers were performed using controlled inflation (5.0 ml/min) of the lungs to TLC and passive deflation (expiration) of the lung to FRC, the point where Pao = 0. Lung volume (integrated from flow across the pneumotachograph) was plotted against corresponding Pao and the steepest portion of the expiratory PV curve was used to calculate quasi-static compliance (Cqst). FV maneuvers were performed by inflating the lungs to TLC and then connecting the airway opening to a large reservoir maintained at 50 cm H_2O sub-ambient pressure. Flow was measured via the pneumotachograph and volume was measured as a function of Pao. Deflation of the lungs continued to the point there was no longer flow. The volume of air removed from the lungs from Pao = 0 to maximum sub-ambient Pao was defined as expiratory reserve volume (ERV). This was subtracted from FRC to obtain residual volume (RV), the volume of remaining in the lungs at airway closure. The volume of air between RV and TLC was defined as forced vital capacity (FVC). Forced expiratory flow (FEF) at 50, 25 and 10 % of FVC (FEF_{50}, FEF_{25} and FEF_{10} respectively) were recorded. Forced expiratory volume (FEV) at 0.05, 0.1 and 0.2 seconds into the expiratory maneuver ($FEV_{0.05}$, $FEV_{0.1}$, and $FEV_{0.2}$ respectively) were recorded. Mid mean expiratory flow (MMEF) was recorded as the average flow between 75 and 25 % of FVC.

Single breath carbon monoxide diffusing capacity measurement (DLco) was made after elimination of voluntary breathing the animals by injection of gallamine triethodide (i.v. 2.0 mg/kg). Three separate determinations of DLco were made in each animal. Animals were transferred to a second, modified 1.5 L whole-body plethysmograph (PLYAN, Buxco Electronics, Sharon, CT) configured as a constant volume device. This plethysmograph did not have a valve assembly located at the breathing port to facilitate connection of the animal to a respirator (Pressure Controlled Ventilator, Kent Scientific, Litchfield, CT) to support ventilation between maneuvers. In this plethysmograph trans-thoracic pressure (Ptt) was measured as the difference between Pao and pressure inside the plethysmograph. For this measurement TLC was defined as a Ptt of 30 cmH_2O, which was measured with a differential pressure transducer (model DP15-22, Validyne Engineering, Northridge, CA) located within the plethysmograph.

Pressure differential (model DP 15 – 26, Validyne Engineering, Northridge, CA) between the inside and outside of the sealed plethysmograph was used to determine volume. The lungs were inflated with a volume of test gas (air containing 0.5 % CO and 0.5 % Ne, Matheson Gas Co. Twinsburg, OH) corresponding to inspiratory capacity (IC = TLC - FRC). After a 10 second breath hold the sample with air was withdrawn in approximately equal aliquots, defined as the initial and end expiratory samples (IES and EES respectively). Gas chromatography (thermoconductive, model GC-8A, Shimadzu Inc., Kyoto, Japan) was used to compare CO and Ne concentrations between the IES, EES, and the test gas. Relative Ne concentration in both IES and EES was used to calculate volume of relaxation (Vr), a gas dilution correlate of FRC. For calculation of DLco the IES was considered to contain air from the regions of the lung in which gas exchange does not occur, therefore the relative concentration of CO in the EES only was used to calculate DLco.

Transducer signals whether used for flow, pressure, or volume determinations were calibrated using appropriate flow, pressure or volume NIST traceable standards prior to each series of pfts.

Lung Free Cell Differential Counts

Bronchoalveolar lavage (BAL) was performed on 4 animals from each group on day 14 post exposure. The animals were anesthetized with ethyl carbamate and the trachea was exposed for insertion of an 18 ga. needle covered with polyethylene tubing (2.3 mm O.D.). Prior to intubation the animals were given an overdose sodium pentobarbital (i.v. - 120 mg/kg) and exsanguinated via transection of the abdominal aorta. 7 ml of phosphate buffered saline (PBS) was flushed in and out of the intact lungs 5 times with a syringe. The recovered BAL fluid (approximately 6 ml) was centrifuged (model GLC-1, Sorvall Inc., Newtown, CT) at 2500 rpm for 10 min to collect lung free cells. Excess fluid was decanted and 1 ml of PBS was used to resuspend the cells. The concentration of white and red blood cells in the suspension was counted in triplicate (model Ac•T Series Counter, Beckman/Coulter Corp., Miami, FL). 50 µl of the suspension was centrifuged on to glass slides (model Cytospin 3, Shandon Inc., Pittsburgh, PA). The cells were stained (Hema 3, Wright-Giemsa stain, Biochemical Sciences Inc., Swedesboro, NJ) for examination under a light microscope. The fraction of white blood cells

that were pulmonary alveolar macrophages (PAM), polymorphnuclear leukocytes (PMN), or lymphocytes was determined by actual count using light microscopy.

Histopathology

After the collection of pfts, the animals were euthanized by overdose of pentobarbital, the lungs excised intact, and then perfused through the trachea with buffered formalin/glutarldehyde fixative at a pressure of 30 cm H_2O for 24 hours. Nasal turbinates also were harvested for examination after decalcification and fixation in 10 % formalin. Fixed tissues were prepared for histopathologic examination by sectioning and paraffin imbedding. Tissue sections (5 μm) were mounted on glass slides and stained with hematoxylin-eosin for examination by light microscopy.

Statistical Analysis

Blood gas and CO-oximetry analyses were made once for each animal. Physiologic measurements made during spontaneous breathing animals were taken over a 10 minute period. The mean value of all complete breaths taken per min were recorded, thus the total number of breaths analyzed depended upon the animal's breathing frequency. Typically, the total number of breaths analyzed per animal ranged from 800 to 1200. All other pfts, which required manipulation of breathing, were made in triplicate in each animal. Total cell counts in BAL fluid also were made in triplicate and in some instances there was no difference between repeated counts given animals. Cell differential determinations were made counting a minimum of 300 cells per animal. The mean value of a repeated measurement in an animal was used to calculate group mean values. Unless other wise specified data are presented as group mean ± standard error of the mean (SEM). Comparisons among groups were made by analysis of variance with subsequent multiple t-tests (SigmaStat, Jandel Scientific, San Rapheal, CA). When comparisons between an experimental group and the control group means yielded a $p \leq 0.05$ for acceptance of the null hypothesis the difference in means was considered significant.

RESULTS

Combustion Conditions

Despite the presence of a spark source in the SwRI furnace, B2-ACM combustion did not enter the flaming mode and under the present conditions O_2 concentration in the smoke did not fall below 18 %. CO_2 to CO ratio in the smoke was an average 2.8:1 for all B2-ACM loads. Consequently the ISO/TC92/SC3 fire classification was non-flaming –1.b (Hartzell – personal communication). It was apparent from the steady increase in temperature during pyrolysis of the samples that combustion of B2-ACM releases heat. Thermogravimetric analysis (model TGA 7, Perkin Elmer Instruments, Norwalk, CT) showed that pyrolysis of B2-ACM in air began at approximately 350 C^o with complete loss of sample mass at 800 C^o (Figure 4). However, this proved somewhat misleading when larger and more intact samples were burned. Sustained combustion of 2 to 100 g of B2-ACM (intact or pulverized) at approximately 700 C^o resulted in 20 to 25 % mass loss, in the present investigation burning 10 to 100 g samples of pulverized material the average mass loss was 20.03 %. When un-pulverized samples were burned, the residue maintained the appearance of the woven fiber mesh, suggesting that mass loss is due to pyrolysis, primarily, of the epoxy matrix. Mass loss figures agreed well with published reports. Courson and colleagues (1996) found that pyrolysis of bismaleimide/carbon ACM at 650 to 700 C^o resulted in nearly 30 % mass loss. Soranthia and colleagues (1992) found a range of 6 to 28 % mass losses from ACMs with either carbon or glass fibers in a variety of different polymer matrices that were irradiated at 50-kW/m^2 in a cone calorimeter.

Smoke Concentration and Chemistry

Taking into account the total airflow into which the resultant smoke was entrained, pyrolysis of 10, 55 and 3 separate 100 g samples of B2-ACM produced nominal smoke mass concentrations of 3.2, 15.0, 27.8, 28.8 and 27.6 g/m^3 respectively (Table 1). The most abundant gas phase constituent of B2-ACM smoke was CO_2. Exposure chamber concentration of CO_2 increased steadily over the course of the exposures due to expiration of CO_2 by the animals (Figure 5). The highest, combined peak CO_2 concentration was 2.28% found at 2 hour in the 100g-2hr(1) group. The concentrations of CO_2 produced by pyrolysis of B2-ACM were from 0.18 to 0.72 % in the 10g-1hr and 100g-1hr groups, respectively. On average, the CO_2 produced

by B2-ACM accounted for 43.5 % of the smoke mass concentration (Table 2). CO concentration in the exposure chamber steadily decreased during each exposure (Figure 6). The rate of decrease of CO in the exposure chamber was greater than the approximate 10% that would be predicted for dilution due to sampling by Silver's equations (Silver, 1946). The accelerated rate of decrease in CO concentration was attributed uptake by the animals in the chamber. Peak concentrations of CO in the exposure atmospheres ranged from 630 to 3750 ppm. Based on peak concentration, determined in the first few minutes of exposure, CO accounted for an average of 20.5 % of the B2-ACM smoke (Table 2). Oxygen concentration in the exposure atmospheres was slightly depressed and relatively steady state, fluctuating between 19.5 and 20.5 % (Figure 7). The lowest O_2 concentration measured was 18.7 %. The air feed back into the exposure chamber, during sampling, to maintain pressure was not O_2 enriched therefore replenishment and animal O_2 consumption rates were in balance. The decrease in chamber NOx concentration was greater than predicted for 55 and 100g load exposure groups (Figure 8). Animal uptake of NOx was most likely the reason for this observation. There are several oxidation states for nitrogen oxide ranging from NO to N_2O_5 that, theoretically, can under go rapid interconversion, hence the term NOx. However, NO_2 appears to be the most stable atmospheric species (Gaston et al., 1994). In the present investigation NO was not found and the assumption was that the NOx was predominantly NO_2. NOx peak concentrations ranged from 20 to 201 ppm, and accounted for 1 to 1.8 % of the nominal mass concentration of the smoke (Table 2). Peak concentrations of SO_2 ranged from 33 to 202 ppm (Figure 9), accounting for 1 to 2 % of the smoke mass concentration (Table 2). The decrease in SO_2 concentration was much more rapid than that of the other gases measured. In addition to uptake by the animals, this rapid decrease was attributed to the reaction between water and SO_2 to form H_2SO_3. Hydrogen cyanide was found to be a constituent of the B2-ACM smoke (Figure 10). Peak concentration in the 10g-1hr exposure group was 52 ppm. However, concentration in the more concentrated smoke could not be determined reliably using our existing analytical methods. In a series of preliminary experiments the concentration of HCN in the smoke correlated well with the amount of B2-ACM using 2 and 40 g loads. However, the response of the analyzer was erratic when burning loads larger than 40 g. Sulfates, NOx, CO and CO_2 at concentrations in the smoke from burning 50 g and larger loads were found to interfere with the electrochemical HCN analysis method.

Peak aerosol concentrations in B2-ACM smoke ranged from 0.84 to 4.4 g/m^3 for the 10g-1hr and 100g-2hr(1) exposure groups, respectively (Figure 11). Similar the gaseous constituents of the smoke, aerosol concentration in the chamber decreased with time. An exponential expression was fitted (minimum $r^2 = 0.9997$) to the aerosol concentration data from each exposure and showed that, in all cases, the decrease in concentration had two components. The initial rapid phase was attributed to the evaporation of volatile constituents associated with the aerosol particle/droplets. Filter samples taken from the chamber during the first 5 to 7 minutes of smoke generation and weighed immediately lost from 22.4 to 31.3 % of their mass when weighed 24 hours later. Filter samples taken 30 min after smoke generation lost an average 2 % of their mass when weighed 24 hours later. Filter samples taken at 1 hour and beyond did not lose mass in 24 hours. Many of the approximately 90 individual and classes of organic compounds that have been identified as part of the aerosol fraction of ACM smoke are volatile (Lipscomb et al., 1997). The slow phase of aerosol concentration decrease was attributed to gravitational settling of aerosol particles and sampling procedures. Extrapolation of the aerosol concentration curves to time 0, was used to estimate the initial aerosol concentration in the smoke and it was determined that the combined solid and volatile components of the aerosol accounted, on average, for 20.5 % of the smoke nominal mass concentration (Table 2). The mass median aerodynamic diameters (MMAD) of the smoke aerosols were not substantially different among the various smoke concentrations, ranging from 1.5 to 1.8 μm; the geometric standard deviations (σg) of the aerosol size distributions were similar ranging from 1.7 to 1.9 (Figure 12). There were no differences between the MMADs or σgs of aerosol samples taken at the beginning and end of the exposures. Although loss of aerosol mass due to evaporation and early gravitational settling of larger particles would tend to reduce aerosol particle size, the data suggest that increase in particle size due to coagulation mitigated a change in aerosol size distribution. There was heterogeneity of aerosol particles in the smoke, which included small (≤ μm) diameter spheres, chain aggregates of these smaller particles, larger (several μm) irregularly shaped particles, and fibers (Figures 13, 15,17). Energy dispersive X-ray analysis of SEM samples (Figures 14, 16, 18) showed that the composition of the particle types differed. The major peak in the spectra of individual aerosol particles and droplets was carbon, with a few minor peaks contributed by metallic species. Aggregate spectra also had a large carbon peak accompanied by much stronger metallic peaks, particularly Cr. This indicated that the chain

aggregates were not strictly composed of smaller individual particles. The predominant peak in the spectra of fibers was silicon, not carbon. Size distribution analysis of the smoke aerosols indicated that a majority of the particles were respirable and could penetrate deep into the respiratory tree. Aerosol concentration and the length of exposure, not difference in particle size, were the predominant factors in determining differences among the exposure groups in theoretical deposition of particles in the pulmonary region of the lung (Kimmel et al., 1998; Carpenter and Kimmel, 1999 - Figure 19).

B2-ACM smoke was found, in this and previous studies, to be physicochemically complex. However, combined analysis of the aerosol fraction and a few common combustion gases accounted for an average of approximately 82 % of the calculated nominal mass concentration of the smoke. There was good agreement between calculated nominal mass concentration of the smoke, and the actual amount of B2-ACM that burned (Table 2).

Mortality and Morbidity

One hour exposure smoke generated from pyrolysis of 10, 55 or 100g loads of B2-ACM was not lethal. However, two hour exposure to smoke from 100g load of B2-ACM was lethal. Eleven of 28 animals in the 100g-2hr(1) exposure group died, whereas 13 of 28 animals in the 100g-2hr(2) exposure group died. All of the animals died during the exposures. There were differences in the nominal and chemical constituent concentrations between these exposures, but these were minor and the exposures were considered identical. Thus a 2 hour exposure to smoke generated from partial pyrolysis of 100g of B2-ACM was lethal to 42.8 % of the animals. Animals that survived this exposure and animals that were exposed to 100g of B2-ACM for 1 hr failed to gain weight at a normal rate (Figure 20).

Blood Chemistry

Blood gas analysis and CO-oximetry performed on animals immediately after exposure showed a dose-related suppression of oxygen carrying capacity and decrease in blood pH. Formation of carboxyhemoglobin (COHb) also was dose related (Figures 21, 22).

Lung Free Cell Population and Type

A significantly larger total number of leukocytes in the BAL fluids retrieved, on day 14, from the lungs of animals exposed to smoke from 10 and 55g loads of B2-ACM (Figure 23). Whereas, animals exposed to smoke from a 100g load for 2 hours had significantly fewer cells in their BAL fluid. The types of leukocytes present in the BAL varied as a function of exposure. The increase in total number of cells in the lungs of animals exposed to smoke from a 10 g load can be attributed elevations in the number of PAM and lymphocytes, neither of which were statistically significant. There was a significant increase in the number of lymphocytes in the lungs of animals exposed to smoke from 55g, 100g-1hr, and 100g-2hr loads. Lymphocytes accounted for 16.3 % of the leukocytes in the lungs of the 55g group animals, which was not significantly different than the 12.0 and 17.2 % found in the control and 10g group animals, respectively. However, lymphocytes accounted for a significantly larger fraction of total leukocytes in the lungs of 100g-1hr and 100g-2hr animals at 39.3 and 32.3%. Of the combined PAM and PMN leukocytes in the lungs of control, 10g, and 55g animals 97.2, 96.1 and 93.1 % were PAM. In the 100g-1hr and 100g-2hr animals the PAM accounted for a significantly lower 82.8 and 83.6 %. In these latter two groups eosinophils were found among the neutrophils in the PMN fraction. PAM from all animals exposed to smoke were laden with particles, most of which were opaque (Figure 24). However, a few PAM appeared to be laden with particles that were light refractory and appeared to be bundles of fibers.

Pulmonary Function

The only significant differences observed in ventilation of the exposed animals were an increase in Vt, on day 2, in animals exposed to smoke from a 100g load for 2 hours that resulted in a significant increase in Ve (Figures 25 & 26).

Mechanical properties of breathing were significantly effected by exposure to B2-ACM smoke. A significant decrease in both Cdyn and Cqst (Figures 27 & 28) were observed in animals exposed to smoke from a 100g load of B2-ACM for either 1 or 2 hours. The decrease in Cdyn persisted to day 14; however in the 100g-1hr animals Cqst was not significantly lower on day 7 and 14. No significant differences were observed in R_L among the groups (Figure 29).

Although there were no significant differences in TLC at any time among the groups, significant changes in subdivisions of lung volume were observed in smoke exposed animals (Figure 30). Significantly larger RV and FRC were found, on all post exposure observations, in

animals exposed to smoke from a 100g load for either 1 or 2 hours. Consequently, the fraction of TLC represented by FRC was elevated in these groups on all post exposure days (Figure 31). The fraction of FRC represented by RV also was elevated in these groups at these times and also was found to be elevated in the 55g group on day 14 as well (Figure 32).

Lung dynamic properties also were significantly altered by exposure to B2-ACM smoke. MMEF normalized to FVC (MMEF/FVC) was significantly elevated on day 2 in animals exposed to smoke from 55 and 100g loads. An elevated MMEF/FVC also was observed in 100g-2hr group animals on day 7 (Figure 33). Forced expiratory flow at 50 % of FVC, normalized to FVC, (FEF50/FVC) was elevated in both the 100g-1hr and 100g-2hr group animals on day 2, and remained elevated in 100g-2hr group animals on day 7 (Figure 34). Expiratory flow at the point where 75 % of the FVC had been expired, FEF25/FVC, was significantly elevated in 100g-1hr and 100g-2hr animals on both day 2 and 7, however only 100g-2hr animals had a significantly higher FEF25/FVC on day 14 (Figure 35). When 90% of FVC was expired, FEF10/FVC there were no differences in the flow volume relationship among the experimental groups (Figure 36). A significant increase was observed in the volume fraction of FVC expired in 0.05 sec., $FEV_{0.05}/FVC$, in animals exposed to smoke from 100g loads for either 1 or 2 hour on days 2 and 7. Only animals in the 100g-2hr group demonstrated a similar increase on day 14 (Figure 37). Both the 100g-1hr and 100g-2hr group animals showed a similar increase in $FEV_{0.1}/FVC$ on days 2 and 7 (Figure 38). There were no significant differences in $FEV_{0.1}/FVC$ among the groups on day 14.

Diminished gas exchange capacity was observed in animals exposed to 100g load smoke for either 1 or 2 hours as shown by significantly lower DLco on days 2 and 7 in these groups (Figure 39). At day 14 only the 100g-2hr group animals had a lower DLco.

Histopathology

Histopathological examination of tissue samples has not been completed. However initial observations on lung tissues harvested on day 2 indicate an early influx of neutrophils into the lung accompanied by moderate, diffuse thickening of alveolar septa in animals exposed for 1 or 2 hours to smoke from 100g loads of B2-ACM. Moderate histiocytosis reflective of focal hemorrhage also was evident in these animals' lungs. It remains to be seen if these observations persist beyond day 2.

DISCUSSION

Exposure atmosphere characterization data collected in this investigation suggest that collective analysis of a few chosen constituents in the atmosphere maybe used as indices of smoke mass concentration. Under fixed combustion conditions, nominal smoke concentration correlated well with the rate of B2-ACM pyrolysis. However, caution must be exercised before extrapolation of effects produced by the laboratory exposure atmospheres to combustion atmospheres as they occur in the field. Constraints related to the laboratory setting led to combustion conditions that only partially simulate fire conditions associated with an aircraft mishap. Sample procedures led to an artificial decrease in smoke concentration that need to be taken under consideration. Physiologically based deposition models exist that facilitate comparison of dose and potential toxicity between laboratory and field derived data once the latter are obtained (Kimmel, et al, 1998). Likewise, a decrease in aerosol concentration due sampling and gravitational settling is not likely to occur in larger scale fires. Fire related turbulence would keep particles suspended for longer periods of time and continuous generation of new particles and gases into the air stream from ongoing pyrolysis would keep the concentration of smoke components levels approaching steady state. Never the less, dose response relationships for lethality and inhalation injury to the lung can be formulated to serve as a basis for risk assessment.

Acute lethality caused by exposure for 2 hours to smoke from pyrolysis of 100g of bulk B2-ACM was most likely due to asphyxia since no deaths occurred after the animals were removed from the exposure environment. Decreased oxygen transport due to formation of COHb was not necessarily the sole factor since rats are capable of surviving even higher COHb levels for similar periods of time (Kimmel et al., 1999). Respiratory acidosis and with subsequent disruption of basal metabolism also was a contributing factor (Nattie, 1987;Cherniak, 1992). In addition to CO_2 other smoke constituents, particularly SO_2, were responsible for the acidification of the blood observed in surviving and assumed in dead animals.

The elevation in total lung leukocyte number in the 55g exposure group suggests that B2-ACM smoke elicits an inflammatory response in the lung (NRC 1989; Sibille and Reynolds, 1990). Despite the decrease in total leukocytes the lungs of animals exposed to B2-ACM smoke (100g load), the increase in proportion of PMNs and lymphocytes provided further evidence of

an inflammatory response (Henderson et al., 1988; Driscoll et al., 1990; Berman et al., 1990). The decrease in total leukocyte count by day 14 may have been due to a cytotoxic effect of phagocytosed particles on PAMs. The effect may have been the result of intrinsic particle cytotoxicity, the result of cytotoxins adsorbed onto the particles or a combination of both (McClellan, 1996; and Heinrich et al., 1994). An ongoing investigation in our laboratory of the cellular and molecular effects of B2-ACM smoke indicates within 2 to 4 days of exposure there is a substantial increase in the number of leukocytes found in the lungs of animals exposed at the 100g load level. PMNs (primarily neutrophils and eosinophils) accounted for approximately 50 % of the total leukocyte population in the lungs of these animals (unpublished observation).

Under most circumstances lesions resulting in obstructive or restrictive pulmonary dysfunction do not result in ventilatory changes due to the large dynamic reserves of the lung unless they are relatively severe (Costa et al., 1992). Normally, ventilatory pattern is controlled, centrally, to maintain normal blood gas values with a minimum of respiratory effort (Mead, 1961). Changes in ventilatory pattern and breath structure are used to evaluate immediate airway reactivity (AR) responses, such as bronchoconstriction, to irritants as well as airway hypersensitivity reactions (Kane and Alarie, 1977). These ventilatory changes occur during exposure and abate upon removal of the irritant. Blood and cerebrospinal fluid concentration of H^+ ion play an important role in the complex systems controlling ventilation (Cunningham, 1986). Elevated H^+ stimulates ventilation resulting first in a change in Vt and subsequently a change in *f* (Comroe, 1974). Research currently being conducted in our laboratory has shown that exposure to B2-ACM smoke at concentrations lower than those used in the present study elicit an AR response in guinea pigs (unpublished observation). However, the elevation of Vt, and subsequent elevation of Ve, observed at 2 days in the animals exposed to 100g load smoke for 2 hours in this study was attributed to a persistent elevation of blood H^+ in these animals. This may have resulted from either a residual metabolic acid-base imbalance or sustained release of acidic material from particles remaining in the lung.

Mechanical properties of breathing, Cdyn and R_L, which are direct measures of AR type responses, also are useful as functional indices of lung tissue lesions. Changes in Cdyn are thought to reflect peripheral airways and lung parenchymal abnormalities, while R_L is a measure of central airway integrity. Restrictive disorders which result in a "stiffening" of lung tissue are reflected by a reduction of Cdyn, as was observed in B2-ACM smoke exposed animals in this

investigation. Reduction of Cdyn, may be due to inflammation and interstitial edema, which may resolve with time depending on the extent of injury (Gross and White, 1985) or maybe permanent due to restructuring of the lung tissue as occurs in fibrosis (Raub et al., 1985). The techniques applied for determination of Cqst minimize the influence of inertial forces on the determination of lung "stiffness" and are more representative of essential tissue elastic recoil (West, 1979). The reductions of Cqst observed in animals exposed to B2-ACM smoke indicate an increase in lung elastic recoil (or resistance to stretching) from interstitial fluids or deposition of collagen tissue subsequent to repair of tissue damage (Mauderly, 1989). Increased R_L is pathognomonic of bronchoconstriction, but also is indicative of more persistent lesions that obstruct flow in the airways. Normal values post exposure values for R_L indicated that B2-ACM smoke exposure did not lead to large airway obstruction.

In humans, TLC is defined as the lung volume achieved at the maximum inspiratory pressure a subject can generate (McCarthy et al., 1980). However since laboratory animals cannot make voluntary maximal inspiratory efforts, TLC is defined as the volume at a fixed transpulmonary pressure (30 cm H2O in this instance). Maximal expansion of the lung is a combination of both filling of airspaces that normally are not in communication with the airways and, to a lesser degree, the actual expansion of individual airspaces. Lung disorders that completely block flow to distal airspaces and, to a certain extent, severe lesions that restrict expansion of lung tissues tend to reduce TLC. The moderate, but statistically insignificant, reduction of TLC observed in animals exposed to smoke from the pyrolysis of 100g loads of B2-ACM indicate that there was not occlusion of the smaller airways nor severe restriction of tissue elasticity. FRC is the volume of air remaining in the lung at the end of a tidal expiration, when transpulmonary pressure is at its resting minimum. The small airways are held open by the framework of adjacent airways and by the tethering effect of lung parenchyma. Loss of alveolar tissue can lead to loss of supporting structure and premature closure of airways, which traps air and elevates FRC. With tissue loss there is also loss of elastic recoil (elevation of compliance) less resistance to inflation (increased TLC) as well. Likewise, reduction of small airway caliber by thickening of the airway walls can lead to premature closure of the airways, at low transpulmonary pressure, with the same effect (Raub et al., 1982, Harkema et al., 1982). In very severe restrictive disorders, where small airway architecture is remodeled by deposition of connective tissue, the airways may be held open with a resultant decrease in FRC (Snider et al.,

1978). RV, which is the volume of air remaining in the lung at the end of a maximal expiration, is subject to similar changes from lesions in the distal airways. However, RV is less influenced by breathing frequency, inspiratory muscle tone or glottal breaking (Vinegar et al., 1978). Multiple factors contribute to setting TLC, consequently there may be greater variability in its measure among subjects. Consequently determination of the proportion of TLC constituted by FRC has been adopted as a measure to evaluate lung dysfunction. For similar reasons the proportion that RV constitutes of FRC is often calculated. The increases in FRC, and the portions ratios FRC/TLC and RV/FRC found in animals exposed to B2-ACM smoke indicate lesions to distal airspaces that foster premature closure of small airways, but without a corresponding loss of resistance to inflation at higher transpulmonary pressures.

Macklem and Mead (1967) demonstrated that distal airways account for less than 10% of R_L, therefore disorders, which affect distal airway function, may not be reflected in measures of R_L. Maximum expiratory flow volume curve measurements were developed to examine the dynamic properties of small airway function. Airflow during a forced expiratory effort after full inflation of the lungs to TLC may be limited through compression of the airways by transmural (inside and outside the airway) pressures (Diamond and O'Donnell, 1977). Additional expiratory effort does not increase expiratory flow once this compression occurs, and flow is said to be effort independent. This flow limitation is thought to occur more readily across the smaller, less rigid, airway walls. The prime determinants of effort independent flow are elastic recoil of the pulmonary tissues and airway resistance at the point of equal transmural pressures, located in the smaller airways. Points of restriction occur first in the larger airways and then move peripherally as lung volume decreases. Thus changes in flow at progressively lower lung volumes are indicative of changes in progressively smaller airways. Decreased airflow can be due to premature compression of the airways caused by tissue loss or increased flaccidity of the smaller airways (Damon et al., 1982). Conversely, disorders that increase the turgidity of the tissues, reducing elastic recoil (lowering compliance), may help maintain airway patency which increases resistance to transmural compression of the airways. In the absence of overt obstruction of the small airways, flows may be higher than normal. Measures of dynamic properties, FEF and FEV often are normalized to FVC to minimize the effect lung volume differences, the effect of which is to enhance the detection of minimal dysfunction (Costa, 1985). Likewise it is common to express calculate the fraction of FVC that is expired in a given time.

The higher than normal flows at various lung volumes and the higher than normal fractions of FVC expired in a given time encountered in animals exposed to smoke from pyrolysis of 100g loads of B2-ACM indicate an effect on the smaller airways. The nature of this effect appears to have been an increase in the resistance of the airway walls to collapse without significant obstruction of the airways. Interstitial edema, particularly peribroncheolar edema, could have had this effect.

The ultimate purpose of pulmonary function is to facilitate alveolar – capillary gas exchange. Gas transfer is passive and dependant upon diffusion across the membrane, which is driven by gas partial pressure difference across the membrane. The area of membrane that is brought into contact with the gases and the thickness of the diffusion pathway can limit the rate of gas exchange. Hence both loss of alveolar tissue and thickening of the diffusion pathway by fluid accumulation, cellular infiltration, and deposition of fibrin reduce gas exchange rate. Reduction DLco, is a non-specific indicator for any given type of lung lesion, primarily because of the various factors governing gas exchange. However, the magnitude and persistence of DLco decrement are indicative of the severity of the lesion and may provide insight to the nature of the disorder. Resolution of fluid accumulation in the interstitial space may lead to a return to normal DLco over time.

The pattern of pulmonary dysfunction observed in this study suggests that B2-ACM smoke elicits an inflammatory response leading to diffuse interstitial edema. The reduction of total cells in BAL fluids at later times indicates that smoke particulates may be cytotoxic. However, it is not certain if this apparent cytotoxicity stems from the chemical properties of the particles or if phagocytes are succumbing to a "lung overload" phenomenon (Morrow, 1992; Lehnert et al., 1994; Oberdorster 1996; Warheit et al., 1996). Generally dysfunction reflects underlying structural damage, however it also has been shown that pulmonary dysfunction may be evident prior to overt pathology particularly with when the pathology is diffuse in nature (Drazen 1984; O'Neil and Raub; 1984; Mauderly 1989; Costa et al, 1985,1992). Many of the functional parameters return to normal within the 14 day observation period, whereas others continue to be abnormal. Preliminary findings from histopathological examination of lung and nasal tissues do not indicate extensive or severe damage to lung tissue over the course of the investigation. The results concur with the functional assessment of diffuse interstitial disease. The preliminary histopathological evidence and the fact that some of indices of dysfunction

return to normal, with time, indicate that tissue injury from inhalation of B2-ACM smoke, like other inhaled toxins, may resolve depending on the extent of the tissue damage (Gross and White 1985). However, the persistence of other measures of dysfunction, primarily in animals exposed for 2 hours to smoke from 100g of B2-ACM, suggest that dysfunction may be sustained. Although functional measurements are often used to monitor progress of lung impairment, correlation between dysfunction and tissue damage may be most clear cut in end stage disease (Macklem and Permutt, 1979). The possibility that initial damage may evolve into more severe damage cannot be discounted. Severe respiratory disorders such as acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are progressive, and are thought to stem from an unregulated inflammatory response and interstitial disease (Kimmel and Still, 1999). Furthermore, ALI and ARDS have been attributed to acute smoke exposure, and may be delayed in onset.

The present investigation suggests the existence of a dose response relationship between short-term pulmonary dysfunction and acute exposure to B2-ACM smoke. If so, exposure to higher concentrations of smoke or similar concentrations for longer periods of time would be expected to lead to more substantial lung damage and dysfunction. The acute pulmonary effects of B2-ACM smoke cannot be attributed to any single smoke constituent or combination of constituents, and given the chemical complexity of smoke it is not likely that such a relationship can be drawn. Just because there was some evidence of resolution of B2-ACM smoke induced lung dysfunction in the present investigation it should not be construed that there are no long-term sequelae of these exposures (Crapo et al., 1992). In fact, the presence of fibers in the B2-ACM smoke atmosphere has raised concern over the possibility of chronic, long-term effects from acute exposure (Formisano, 1989; Thomson, 1989; Siebert, 1990; Warheit et al.,1991; Everitt et al., 1999). The present investigation does not provide adequate information to address this health risk issue.

CONCLUSION

The results of the present investigation indicate that acute exposure, for 2 hours, to smoke generated from burning B2-ACM at a rate of approximately 2.6 g/min can be lethal. Survivors of this exposure and victims exposed for 1 hour to B2-ACM smoke generated at a rate as low as 2.15 g/min suffer from pulmonary dysfunction indicative of an early inflammatory response and

diffuse pulmonary edema often associated with smoke exposure (Prien et al, 1987; Laffon et al., 1999). It is not known, in this instance, whether these lesions will progress into a more severe, and lethal, lung disease such as Acute Lung Injury and Acute Respiratory Distress Syndrome (ALI/ARDS). Smoke inhalation has long been known to be a cause of ALI/ARDS (Clark, 1990). This highly probable result of B2-ACM smoke exposure is in need of experimental verification. Furthermore, ongoing studies to be reported subsequently, clearly show that in an animal model of occupational asthma, exposure to concentrations of B2-ACM smoke to low to be visible elicit an airway reactivity response severe enough to lead to convulsions. (Kimmel - unpublished observation). This observation and what is known of structure activity relationships of some of the constituents identified in B2-ACM smoke suggest that exposure may lead to a non-specific airway hypersensitization in some individuals. This distinct possibility also needs further investigation in order to more fully comprehend the potential health risks associated with the use of ACM structural components.

REFERENCES

Baltimore Sun. 1997. September 15, 1997.

Berman JS, Beer DJ, Theodore AC, Korlfeld H, Bernardo J and Center DM. 1990. State of the Art: Lymphocyte recruitment to the lung. *Am. Rev. Respir. Dis.* 142:238-257.

Carpenter RL and Kimmel EC. 1999. Aerosol deposition modeling using ACSL. Drug *and Chem. Toxicol.* 22(1):73-90.

Cherniak RM. 1992. Gas transport and acid-base balance. In: *Pulmonary Function Testing, 2nd Edition*, pp. 89-106. WB Saunders Co. Philadelphia, PA.

Clark WR. 1990. Smoke inhalation: Models for research. In: *Respiratory Injury: Smoke Inhalation and Burns*. Eds Hopnik EF, Munster AM. pp347-399. McGraw-Hill, Inc. New York, NY.

Comroe JH. 1974. *Physiology of Respiration.* Year Book Medical Publishers. Chicago, IL.

Costa DL, Tepper JS and Raub JA. 1992. Interpretations and limitations of pulmonary function testing in small laboratory animals. In: *Comparative Biology of the Normal Lung*. pp 367-399. Parent RA (Ed.). CRC Press. Boca Raton, FL.

Costa DL. 1985. Interpretation of new techniques used in the determination of pulmonary function in rodents. *Fundam. Appl. Toxicol.* 5:423-434.

Courson DL, Flemming CD, Kuhlmann KJ, Lane JW, Grabau JH, Miller CR, Cline JM, Larcom BJ and Lipscomb JC. 1996. Smoke Production and Thermal Decomposition Products from Advanced Composite Materials. US Air Force, Armstrong Laboratory, *AL/OE – TR-1996-0124.*

Crapo J, Miller FJ, Mossman B, Pryor WA and Kiley JP. 1992. Environmental Lung Diseases: Relationship between acute inflammatory response to air pollutants and chronic lung disease. *Am. Rev. Respir. Dis.* 145:1506-1512.

Cunningham DJC, Robbins PA and Wolff CB. 1986. Integration of respiratory responses to changes in alveolar partial pressures of CO2 and O2 and Ph. In: *Handbook of Physiology, Section 3: the Respiratory System, Volume II: Control of Breathing.* pp 475-528. Fishman AP, Cherniack NS, Widdicombe DG, Geiger SR. (Eds.). American Physiological Society. Bethesda, MD.

Damon EG, Mauderly JL and Jones RK. 1982. Early effects of intertracheal insillation of elastase on mortality, respiratory function and pulmonary morphometry of F-344 rats. *Toxicol. Appl. Pharmacol.* 64:465-475.

Diamond L and O'Donnell M. 1977. Pulmonary mechanics in normal rats. *J. Appl. Physiol.* 43(6):942-948.

Drazen JM. 1984. Physiological basis and interpretation of indices of pulmonary mechanics. *Environ. Health Perspect.* 56:3-9.

Driscoll KE, Maurer JK, Lindenschmidt RC, Romberger D, Rennard SI and Crosby L. 1990. Respiratory tract responses to dust: Relationships between dust burden, lung injury, alveolar macrophage fibronectin release and the development of pulmonary fibrosis. *Toxicol. Appl. Pharmacol.* 106:88-101.

DuBois AB, Botelho SY, Bedell GN, Marshall R, and Comroe JH. 1956. A rapid plethysmographic method for measuring thoracic gas volume: A comparison with nitrogen washout method for measuring functional residual capacity in normal subjects. *J. Clin. Invest.* 35:322-326.

Everitt JI, Bermudez E, Mangum JB, Martin JT, Wong BA, Moss OR and Janszen DB. 1999. Fiber-induced pleural disease in rodent models. *CIIT Activities* 19(7):2-7.

FAA. 1997. Handbook: Manufacturing Advance Composite Components for Airframes, Federal Aviation Administration, Department of Transportation. *DOT/FAA/AR-96/75.*

Flight International. 1991. Composites menace crash teams, pp. 17, 24 July 1991.

Formisano JA. 1989. Composite fiber field study: Evaluation of potential personnel exposures to carbon fibers during investigation of a military aircraft crash site. *Appl. Indust. Hyg.* 12:54-56.

Gad SC and Anderson RC. 1990. *Combustion Toxicology.* CRC Press. Inc., Boca Raton, FL.

Gaston B, Drazen JM, Loscalzo J and Stamler JS. 1994. State of the Art: The biology of nitrogen oxides in the airways. *Am. J. Respir. Crit. Care Med.* 149:538-551.

Gideon FG. 2000. A message from the Chief of Safety. *Flying Safety.* 56(1 & @2):3., AFSC/SEMM. Kirtland AFB, NM.

Gross KG and White HJ. 1985. Functional and pathological consequences of a 52-week exposure to 0.5 ppm ozone followed by a clean air recovery period. *Am. Rev. Respir. Dis.* 131(2):183-195.

Harkema JR, Mauderly JL and Hahn FF. 1982. The effects of emphysema on oxygen toxicity in rats. Am. Rev. Respir. Dis. 126:1058-1065.

Heinrich U, Peters L, Creutzenberg O, Dasenbech C and Hoymann HG. 1996. Inhalation exposure of rats to tar/pitch condensation aerosol on carbon black alone or in combination with irritant gases. In: *Toxic and Carcinogenic Effects o Solid Particles in the Respiratory Tract.* pp 433-441. Mohr U, Dungworth DL, Mauderly JL, Oberdorster G. (Eds.). ILSI Press. Washington, DC.

Henderson RF, Pickrell JA, Jones RK, Sun JD, Benson JM, Mauderly JL and McClellan RO. 1988. Response of rodents to inhaled diluted diesel exhaust: Biochemical and cytological changes in the bronchoalveolar lavage fluid and in lung tissue. *Fundam. Appl. Toxicol.* 11:546-567.

JDW. 1991. Danger toxic aircraft, *Janes Defence Weekly*, pp. 711, 19 October1991.

Kane LE and Alarie Y. 1977. Sensory irritation to formaldehyde and acrolein during single and repeated exposures in mice. *AIHAJ.* 38:509-522.

Keskinen J, Pietarinen K and Lehtimaki M. 1992. Electrical low pressure impactor. *J. Aerosol Sci.* 23(4):353-360.

Kimmel EC and Diamond L. 1984. The role of nicotine in the pathogenesis of pulmonary emphysema. *Am Rev. Respir. Dis.* 129:112-117.

Kimmel EC and Reboulet JE. 1998. Calculation of exposure chamber leak rate with thermal correction: A measure of chamber integrity. *AIHAJ* 59:779-784.

Kimmel EC and Still KR. 1999. Acute lung injury, acute respiratory distress syndrome and inhalation injury: An overview. *Drug and Chem.Toxicol.* 22(1):91-128.

Kimmel EC, Carpenter RL, Reboulet JE and Still KR. 1999. A physiological model for predicting carboxyhemoglobin formation from exposure to carbon monoxide in rats. J. *Appl. Physiol.* 86(6): 1977-1983.

Kimmel EC, Carpenter RL, Smith EA, Reboulet JE and Black BH. 1998. Physiologic models for comparison of inhalation dose between laboratory and field-generated atmospheres of a dry powder fire suppressant. *Inhal. Toxicol.* 10:905-922.

Kimmel EC, Winsett DW and Diamond L. 1985. The augmentation of elastase-induced emphysema by cigarette smoke: Description of a model and review of possible mechanisms. *Am. Rev. Respir. Dis.* 13:885-894.

Kimmel EC, Yerkes KL and Carpenter RL. 1997. Performance, fluid mechanics, and design of a small-animal, whole-body inhalation exposure chamber. *Inhal. Toxicol.* 9:287-315.

Kymalainen M, Janka K, Keskinen J, Moisio M, Backman R and Hupa M. 1996. Measurement of time-dependent fume release rate during black liquor pyrolysis. *J. Pulp and Paper Sci.* 22(1):17-23.

Laffon M, Pettit J-F, Modelska K, Matthay MA, and Young DM. 1999. Interleukin-I mediates injury from smoke inhalation to both the lung endothelial and the alveolar epithelial barriers in rabbits. *Am. J. Respir. Crit. Care Med.* 160:1443-1449.

Lehnert BE, Sebring RJ and Oberdorster G. 1994. Pulmonary macropages; Phenomena associate with the "particle overload: condition. In: *Toxic and Carcinogenic Effects o Solid Particles in the Respiratory Tract.* pp 159-176. Mohr U, Dungworth DL, Mauderly JL, Oberdorster G. (Eds.). ILSI Press. Washington, DC.

Lipscomb JC, Kuhlmann KJ, Cline JM, Larcom RD, Peterson RD and Courson DL. 1997. Combustion products from advanced composite materials. *Drug and Chem. Toxicol.* 20:281-292.

Macklem PT and Mead J. 1967. Resistance of central and peripheral airways measured by retrograde catheter. *J. Appl. Physiol.* 22:395-401.

Macklem PT and Permutt S. *The Lung in Transition between Health and Disease.* Marcel Dekker,Inc. New York, NY.

Mauderly JL. 1989. Effect of inhaled toxicants on pulmonary function. In: *Concepts in Inhalation Toxicology.* pp 347-401. McClellan RO, Henderson RF (Eds.). Hemisphere Publishing Corp. New York, NY.

McCarthy DS, Ostrow DN and Hershfield ES. 1980. Chronic obstructive pulmonary disease following idiopathic pulmonary fibrosis. *Chest* 77:473-477.

McClellan RO. 1994. Lung cancer in rats from prolonged exposure to high concentrations of carbonaceous particles: Implications for human risk assessment. *Inhal. Toxicol.* 8(suppl):193-226.

Mead J. 1961. Mechanical properties of the lung. *Physiol. Rev.* 41:281-330.

Morrow PE. 1992. Dust overloading of the lungs: Update and Appraisal. *Toxicol. Appl. Pharmacol.* 113:1-12.

Nattie EE. 1987. Gas exchange in acid-base disturbances. In: *Handbook of Physiology, Section 3; The Respiratory System, Vol. IV. Gas Exchange.* pp 421-438. Fishman AP, Farhi LE, Tenney SM, Geiger SR. (Eds.). American Physiological Society. Bethesda, MD.

NRC. 1989. Markers of inflammatory and immune response. In: *Biologic Markers in Pulmonary Toxicology.* pp 91-103. Henderson RF (Ed). Subcommittee on Pulmonary Toxicology, Committee on Biologic Markers, National Research Council. National Academy Press, Washington, DC.

O'Neil JJ and Raub. 1984. Pulmonary function testing in small laboratory animals. *Environ. Health Perspect.* 56:11-22.

Oberdorster G. 1996. Significance of particle parameters in the evaluation of exposure-dose-response relationships of inhaled particles. *Inhal. Toxicol.* 8(suppl):73-89.

Palecek F. 1969. Measurement of ventilatory mechanics in the rat. *J. Appl. Physiol.* 27(1):149-156.

Prien T, Traber LD, Herndon DN, Stothert JC, Jr., Lubbesmeyer HJ, and Traber DL. 1987. Pulmonary edema with smoke inhalation undetected by indicator-dilution technique. *J. Appl.* Physiol. 63(3):907-911.

Raub JA, Hatch GE, Mercer RR, Grady M and Hu PC. 1985. Inhalation studies of Mt. St. Helens volcanic ash in animals: II. Lung function, biochemistry and histology. *Environ. Res.* 37:72-83.

Raub JA, Mercer RR, Miller FJ, Graham JA and O'Neil JJ. 1982. Dose response of elastase induced emphysema in hamsters. *Am. Rev. Respir. Dis.* 125:432-435.

Reuters News Service. 1997. September 15, 1997.

Sabo J, Kimmel EC and Diamond L. 1984. The effects of the clara cell toxin 4-ipomeanol on pulmonary function in rats. *J. Appl. Physiol.* 54:337-344.

Seibert, JF. 1990. Composite Fiber Hazards. Air Force Occupational and Environmental Health Laboratory (AFOEHL). Brooks Air Force Base, Texas. *AFOEHL Report 90-226E100178MGA.*

Sibille Y and Reynolds HY. 1990. State of the Art: Macrophages and polymorphonuclear neutrophils in lung defense and injury. Am. Rev. Respir. Dis. 141:471-501.

Silver SD. 1946. Constant flow gassing chambers: Principles influencing design and operation. *J. Lab . Clin. Invest.* 31:1153-1161.

Snider GL, Celli BR, Goldstein RH, O'Brien JJ, and Lucey EC. 1978. Chronic interstitial fibrosis produced in hamsters by endotracheal bleomycin. *Am. Rev. Respir Dis.* 117:289-297.

Soranthia UT, Rollhauser CM and Hughes WA. 1992. Improved fire safety of composites for naval applications. *Fire and Materials.* 16:119-125.

Thomson SA. 1989. Toxicology of carbon fibers. Appl. Indust. Hyg. 12:29-33.

Vinegar A, Sinnett EE and Leith DE. 1978. Dynamic mechanisms determine functional residual capacity in mice, *Mus. musculus. J. Appl Physiol.* 46:867-871.

Warheit DB, Hansen JF, Carakostas MC and Hartsky MA. 1991. Inhalation or respirable size carbon fibers produces transient pulmonary inflammatory effects. Am Rev. Respir Dis. 143: A699.

Warheit DB, Hansen JF, Yuen IS, Snajdr SI and Hartsky MA. 1996. Prolonged cellular proliferation and pulmonary inflammation are produced in rats inhaling high concentrations of low-toxicity insoluble particulates: Comparisons with cytotoxic dusts. *Inhal. Toxicol.* 8(suppl):155-167.

West JB. 1979. *Pulmonary Physiology – The Essentials.* Williams and Wilkins, Inc. Baltimore, MD.

Whitehead GS, Kimmel EC, Reboulet JE and Still KR. 1999. Pulmonary function in normal rats. Naval Health Research Center Detachment (Toxicology). TOXDET 99-5. pp 1-22. Wright-Patterson AFB, OH.

TABLE 1. B2-ACM NOMINAL EXPOSURE CONCENTRATIONS.

Group	Mass Loaded (g)	Mass Burned (g)	Time Burned (min)	Burn Rate (g/min)	Flow (L/min)	Volume (m^3)*	Nominal Concentration (g/m^3)
Control	0	0	0	0	0	0.69	0
10g-1hr	10	2.21	2.73	0.81	90.62	0.69	3.19
55g-1hr	55	10.35	4.81	2.15	90.62	0.69	15.00
100g-1hr	100	19.10	7.33	2.61	84.15	0.69	27.68
100g-2hr #1	100	19.89	7.42	2.68	89.71	0.69	28.85
100g-2hr #2	100	19.03	7.63	2.49	90.92	0.694	27.59

* The total volume is the exposure chamber volume (690 L) unless the flow·time product is greater.

TABLE 2. EXPOSURE MASS BALANCE

Load	Particles solid	Particles volatile	Particles total	CO_2	CO	NO_2	SO_2	Total
10g - 1hr	15.2	4.4 *22.4%*	19.6	44.3	15.3 *2.9*	1.0	1.0	78.5
55g - 1hr	15.1	6.9 *31.3%*	22	44.5	15.9 *2.8*	1.2	1.6	85.2
100g - 1hr	13.9	5.7 *29%*	19.6	44.9	15.6 *2.9*	1.3	1.8	83.2
100g - 2hr (1)	15.7	5.1 *24.5%*	20.8	40.9	14.7 *2.8*	1.2	1.7	79.4
100g - 2hr (2)	14.6	6.1 *29.5%*	20.7	43.3	15.6 *2.8*	1.5	2	83.1
Mean	**14.9**	**5.6** ***27.3%***	**20.5**	**43.5**	**15.4** ***2.8***	**1.2**	**1.6**	**81.8**

Values are % of nominal concentration represented by the initial concentration of each species. Numbers in *Italics* are % of total particle mass represented by volatile compounds and ratio of CO_2 to CO.

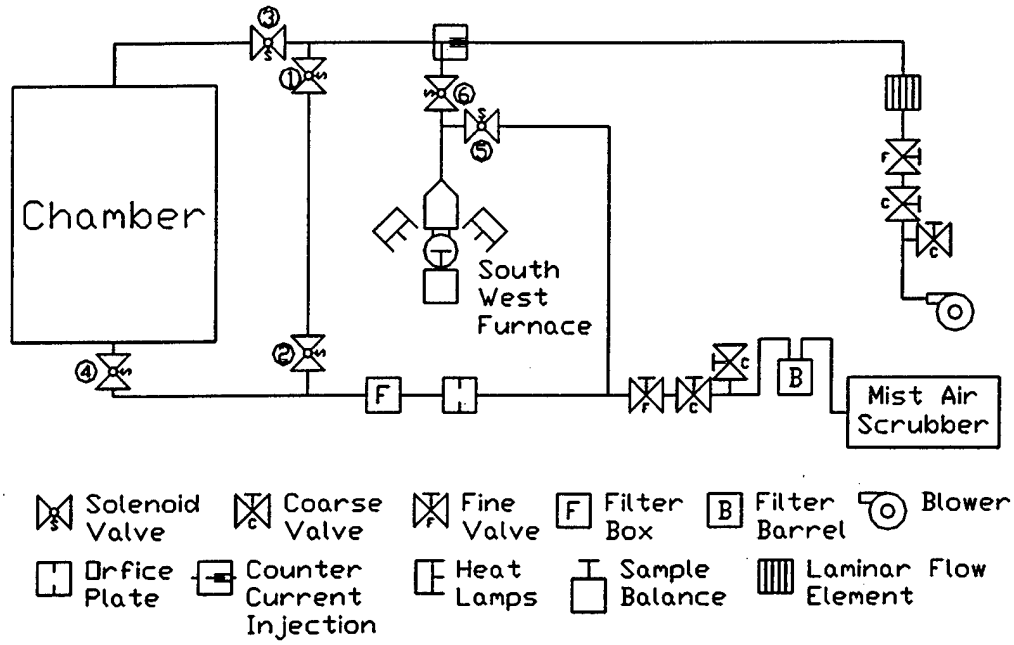


Figure 1. Schematic Diagram of the Exposure System.

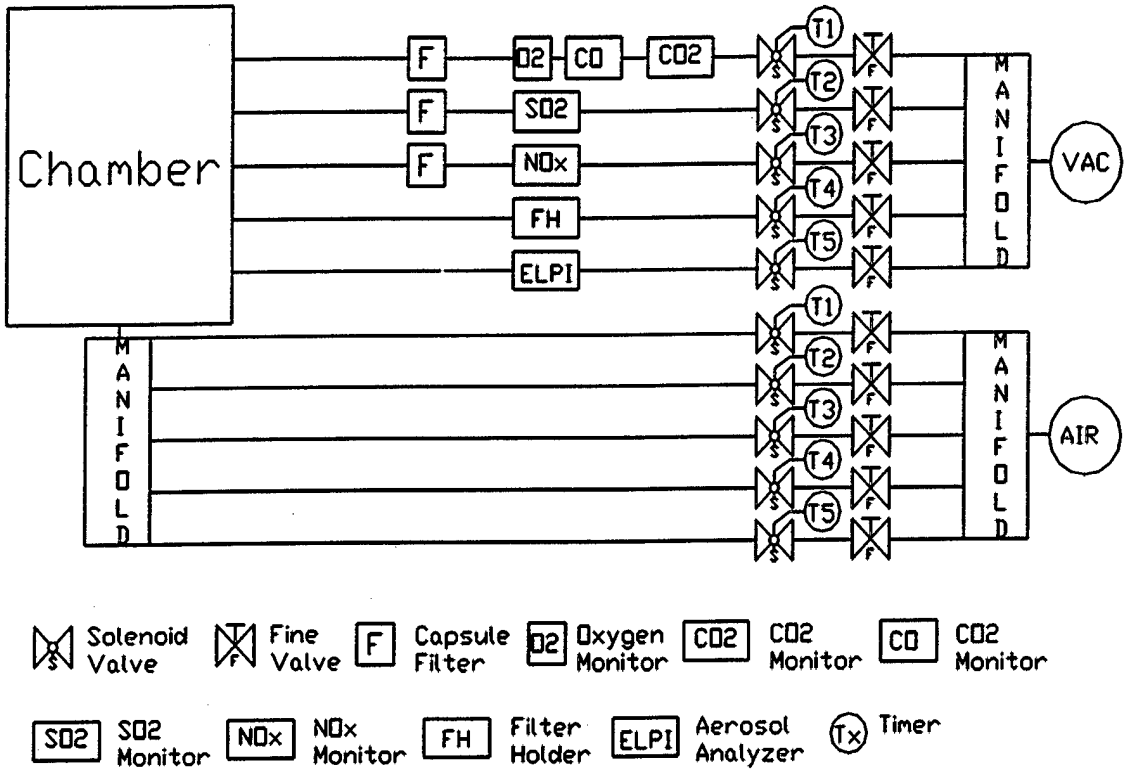


Figure 2. Schematic Diagram of the Sampling System.

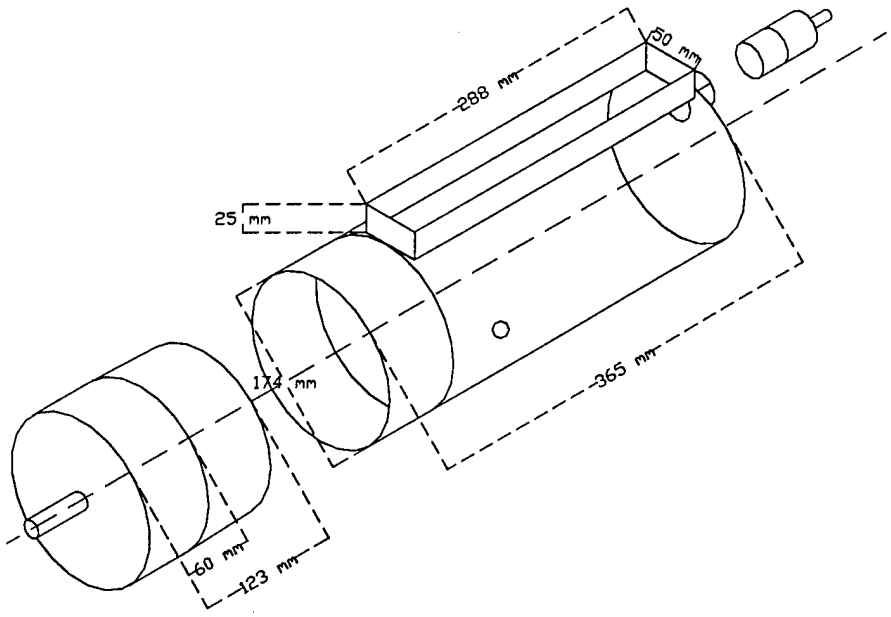


Figure 3. Schematic Diagram of the SwRI Furnace.

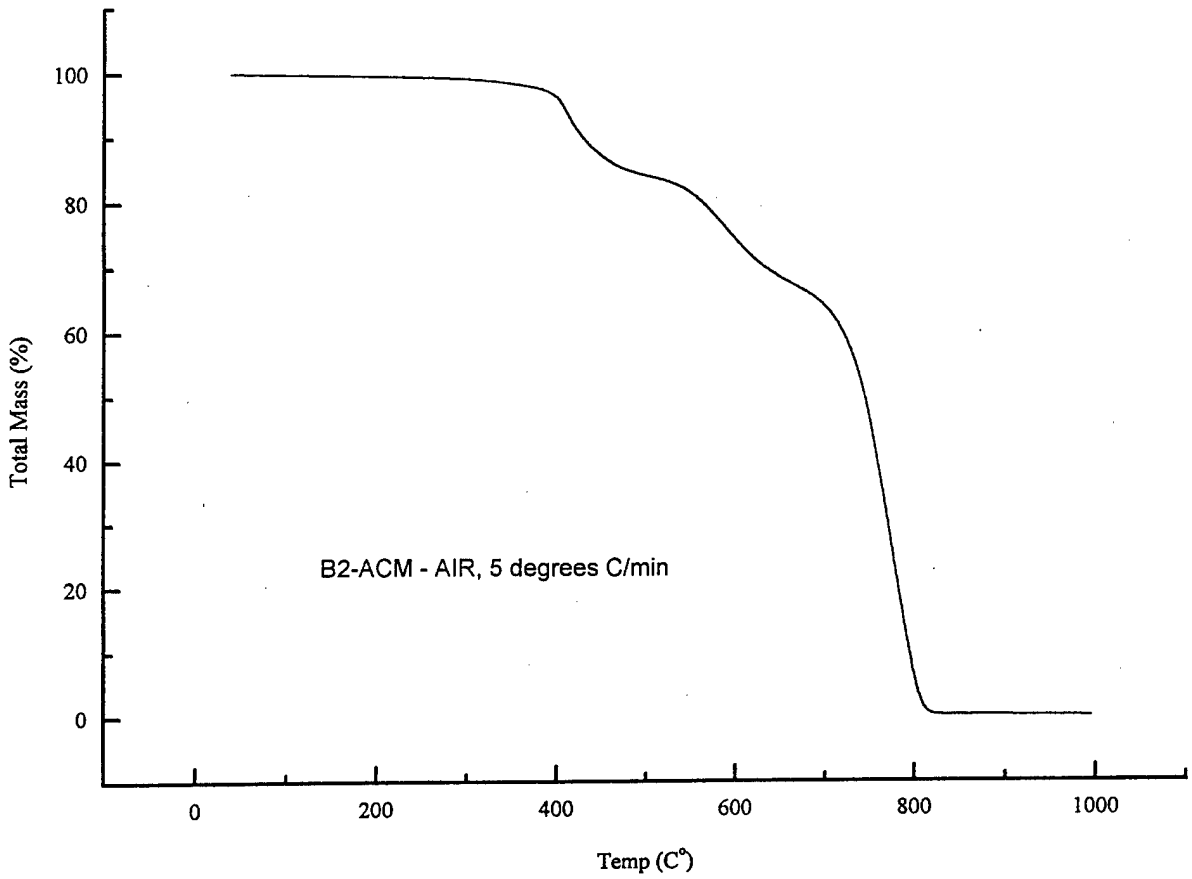


Figure 4. Thermal Gravimetric Spectrum of a Small (1 mg), B2-ACM Sample.

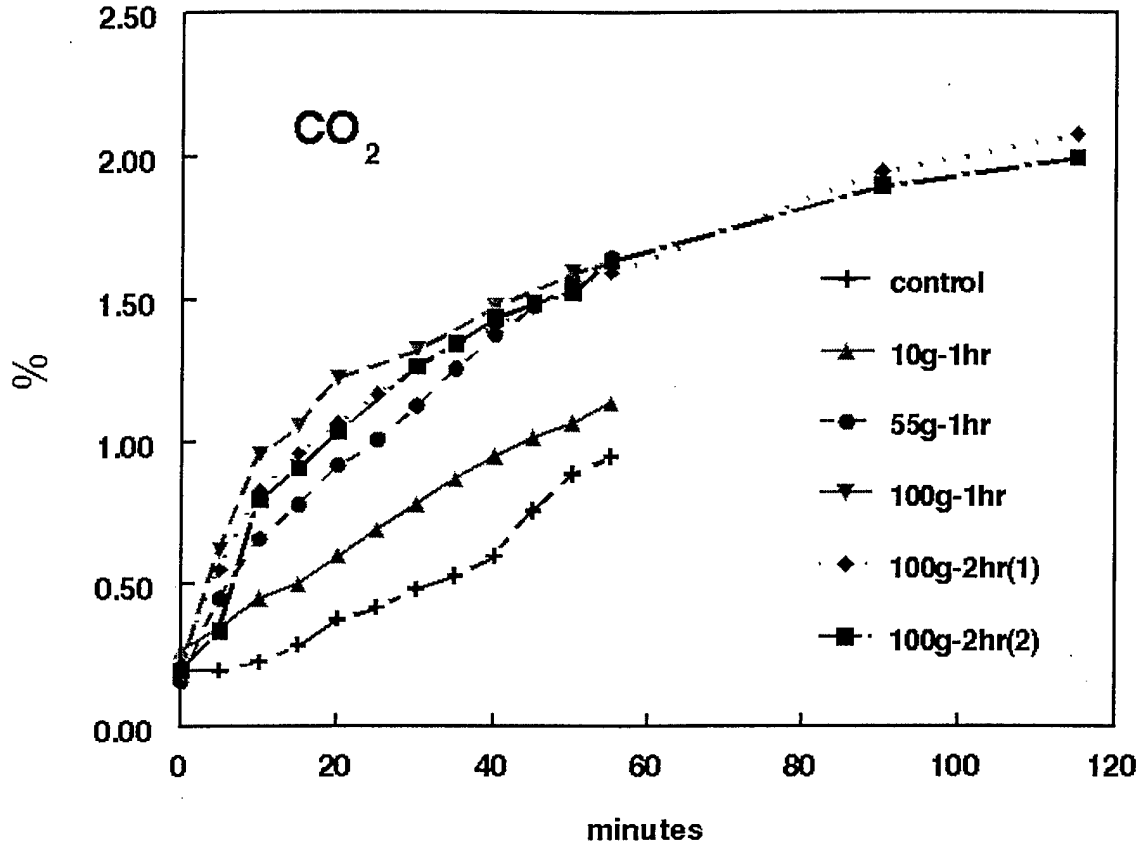


Figure 5. Carbon Dioxide Concentration in B2-ACM Smoke Exposures.

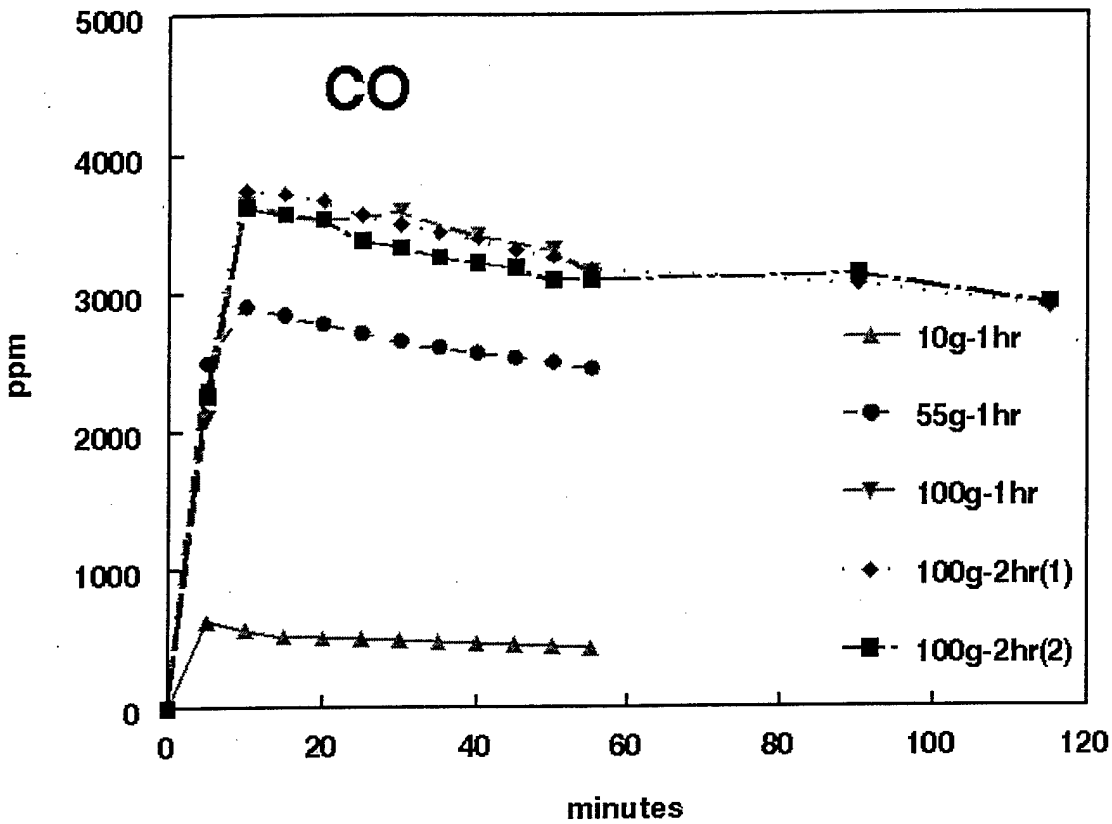


Figure 6. Carbon Monoxide Concentration in B2-ACM Smoke Exposures.

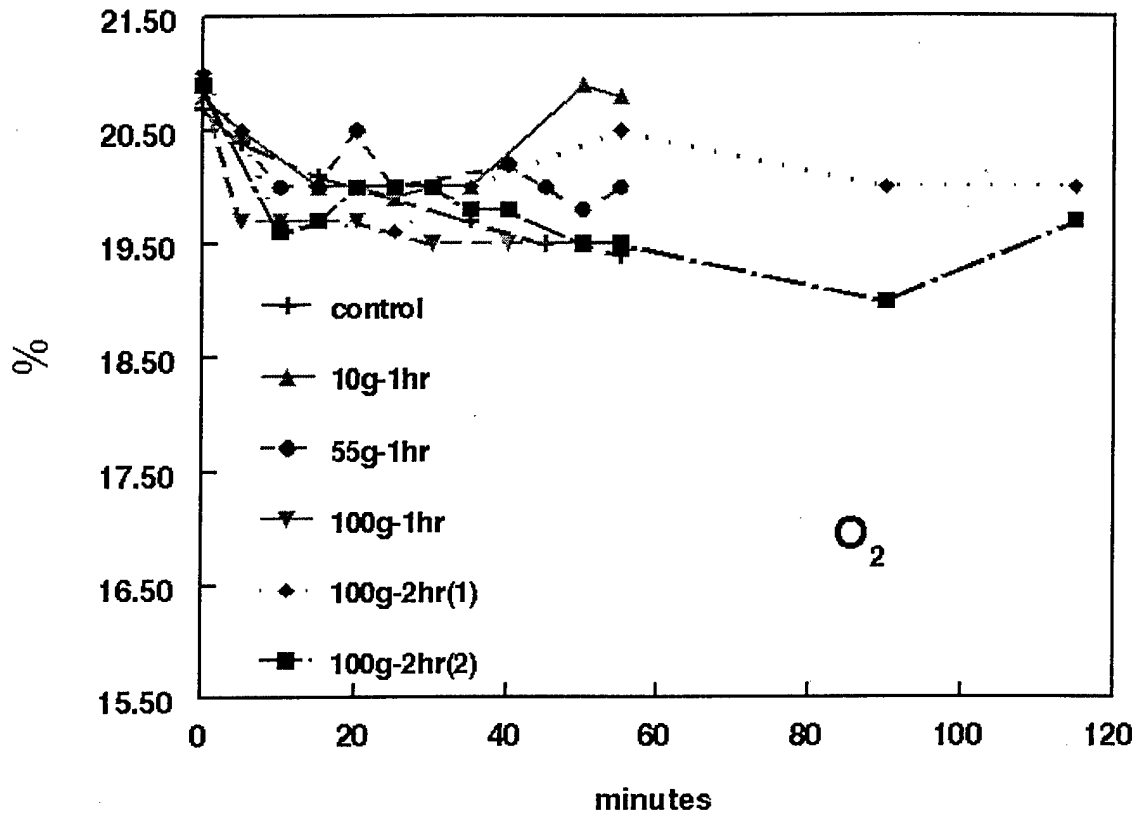


Figure 7. Oxygen Concentration in B2-ACM Smoke Exposures.

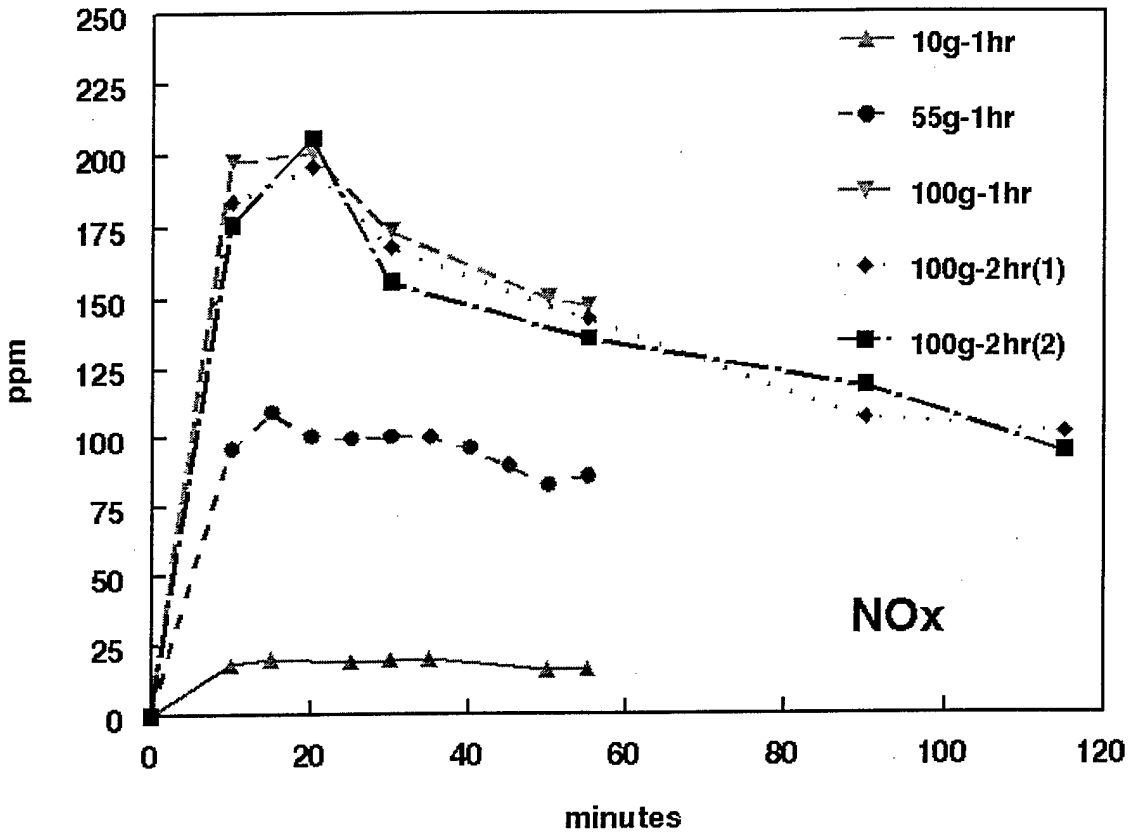


Figure 8. Nitrogen Oxide(s) Concentration in B2-ACM Smoke Exposures.

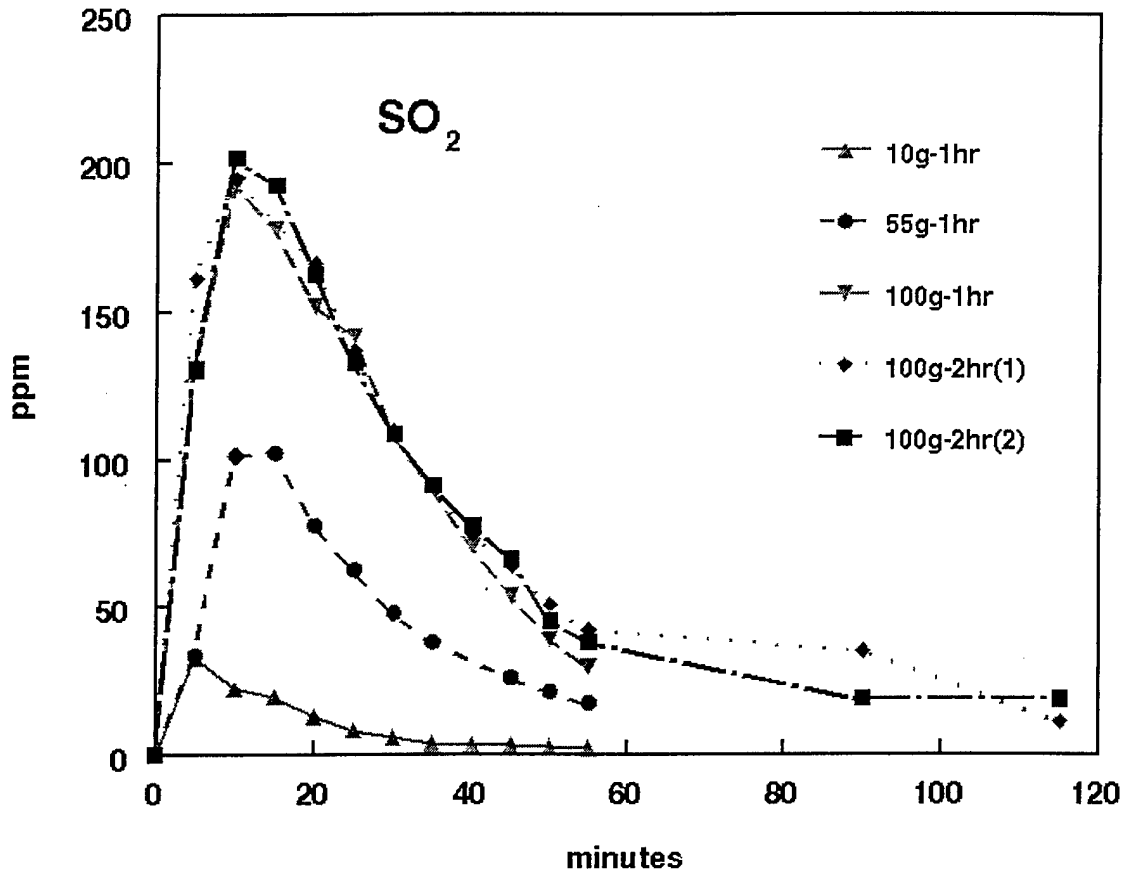


Figure 9. Sulfur Dioxide Concentration in B2-ACM Smoke Exposures.

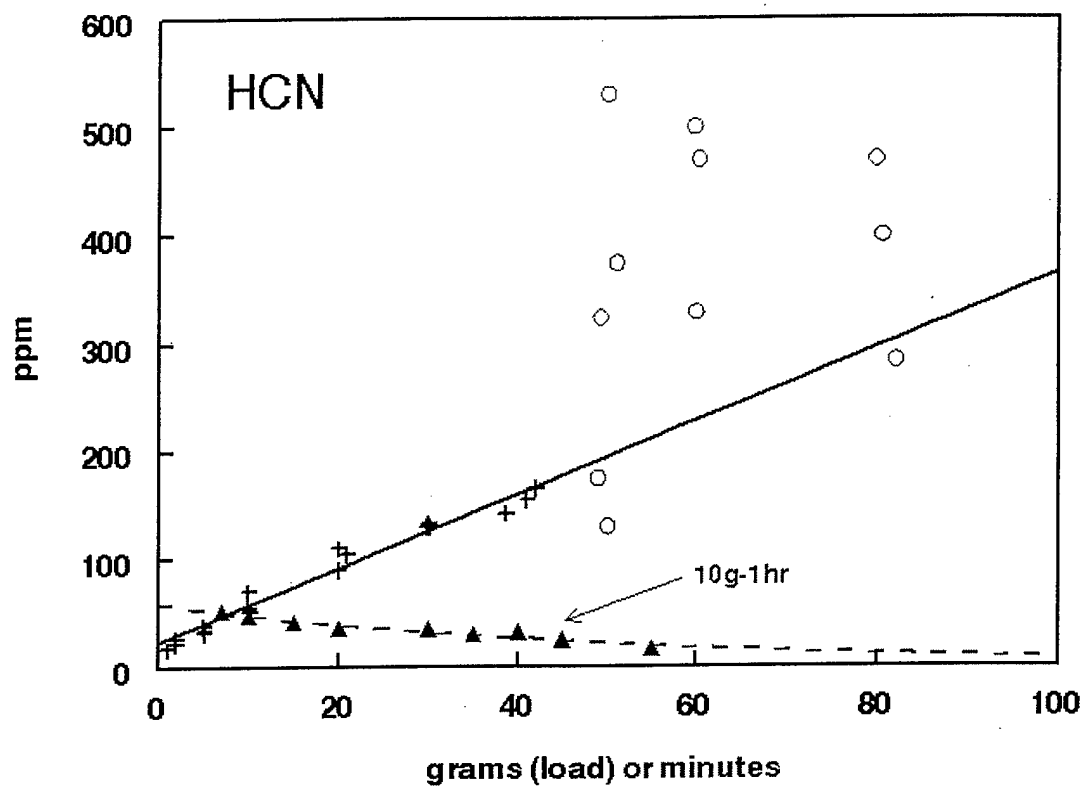


Figure 10. Hydrogen Cyanide Concentration in B2-ACM Smoke Exposures.

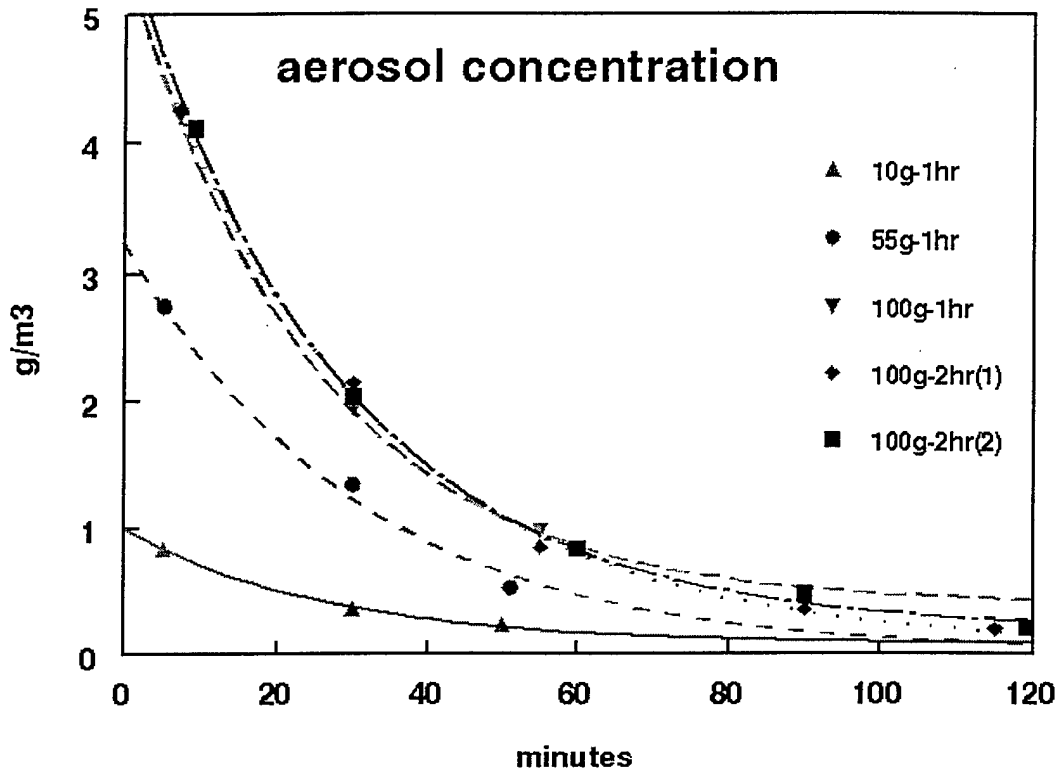


Figure 11. Aerosol Concentration in B2-ACM Smoke Exposures.

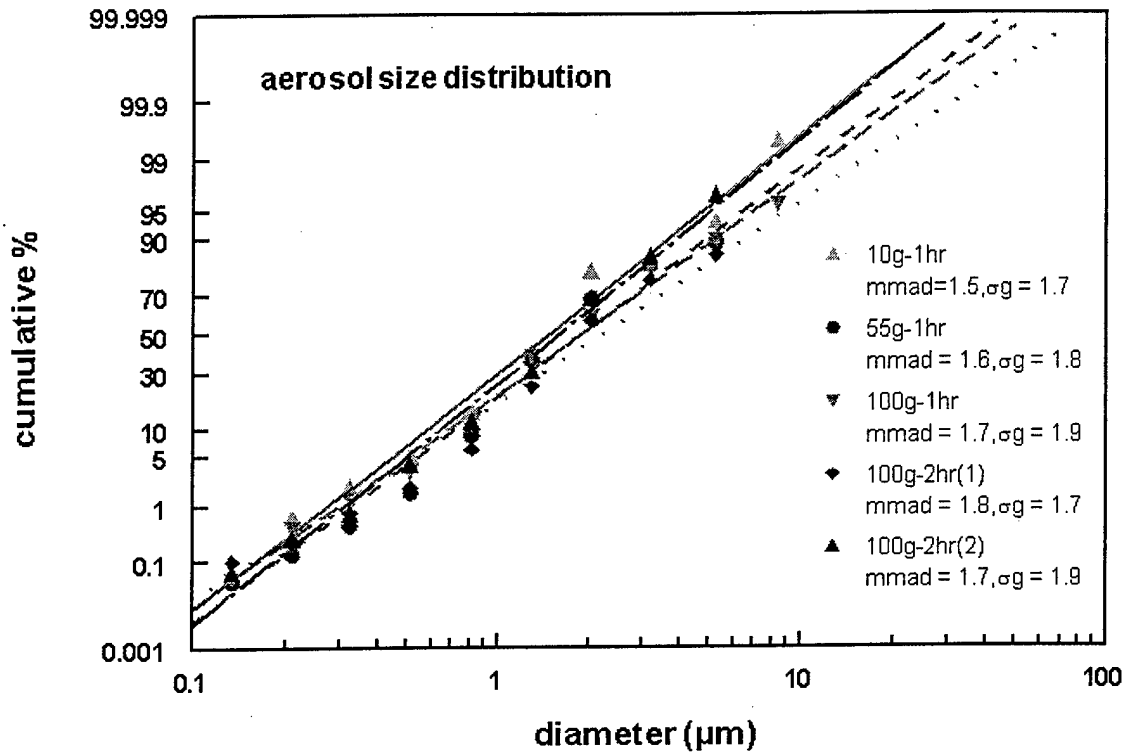


Figure 12. Aerosol Size Distribution in B2-ACM Smoke Exposures.

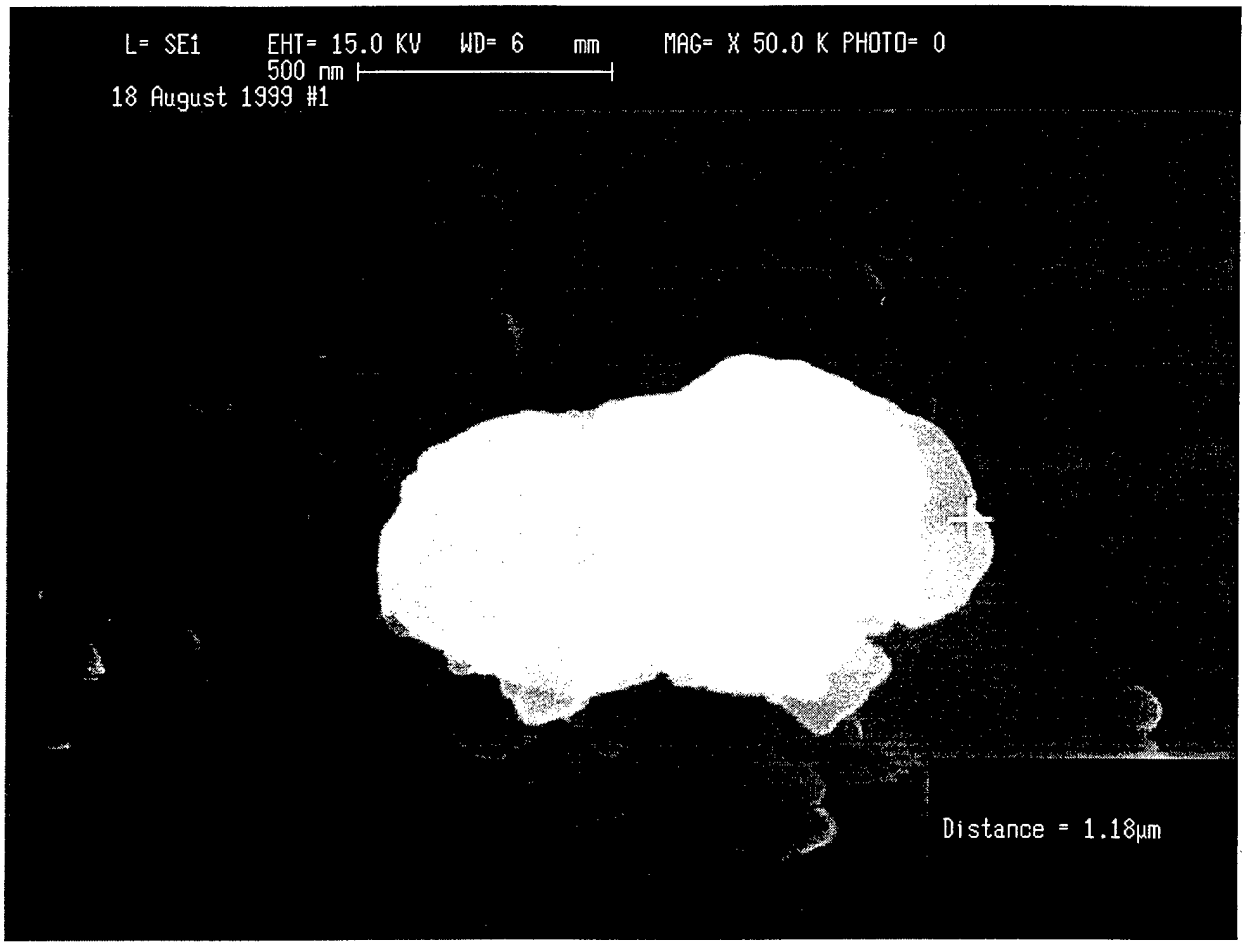


Figure 13. Typical B2-ACM Smoke Aerosol Particle.

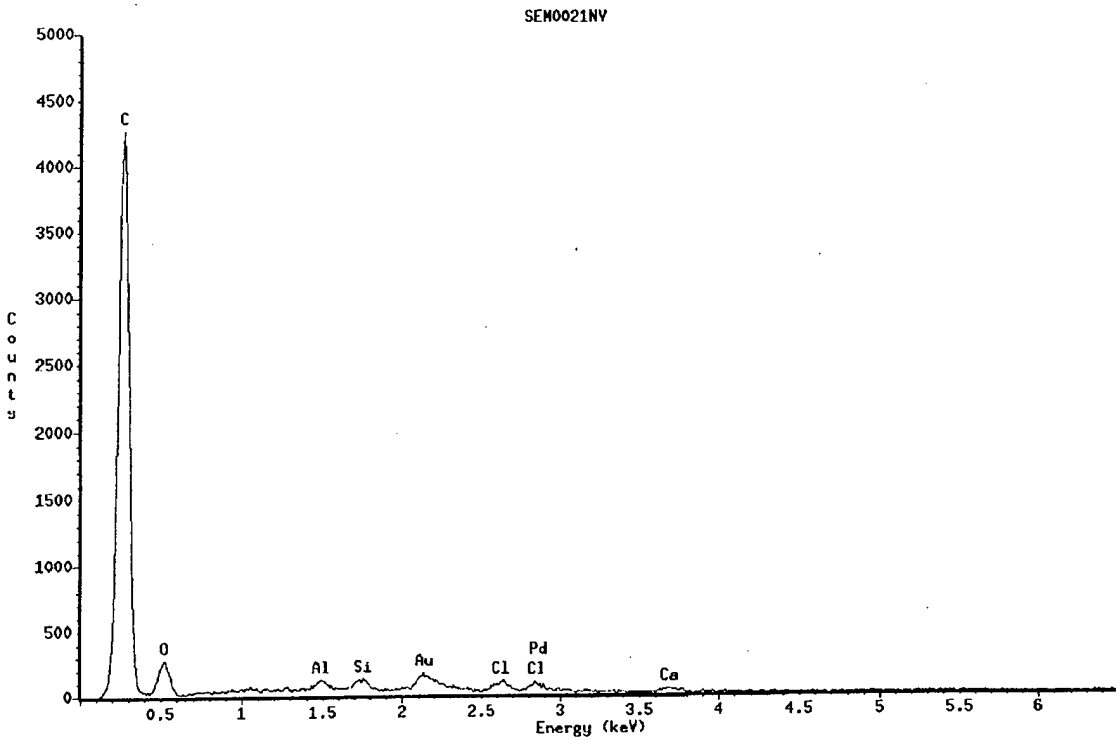


Figure 14. Energy Dispersive X-Ray Spectrum from a Single Aerosol Particle.

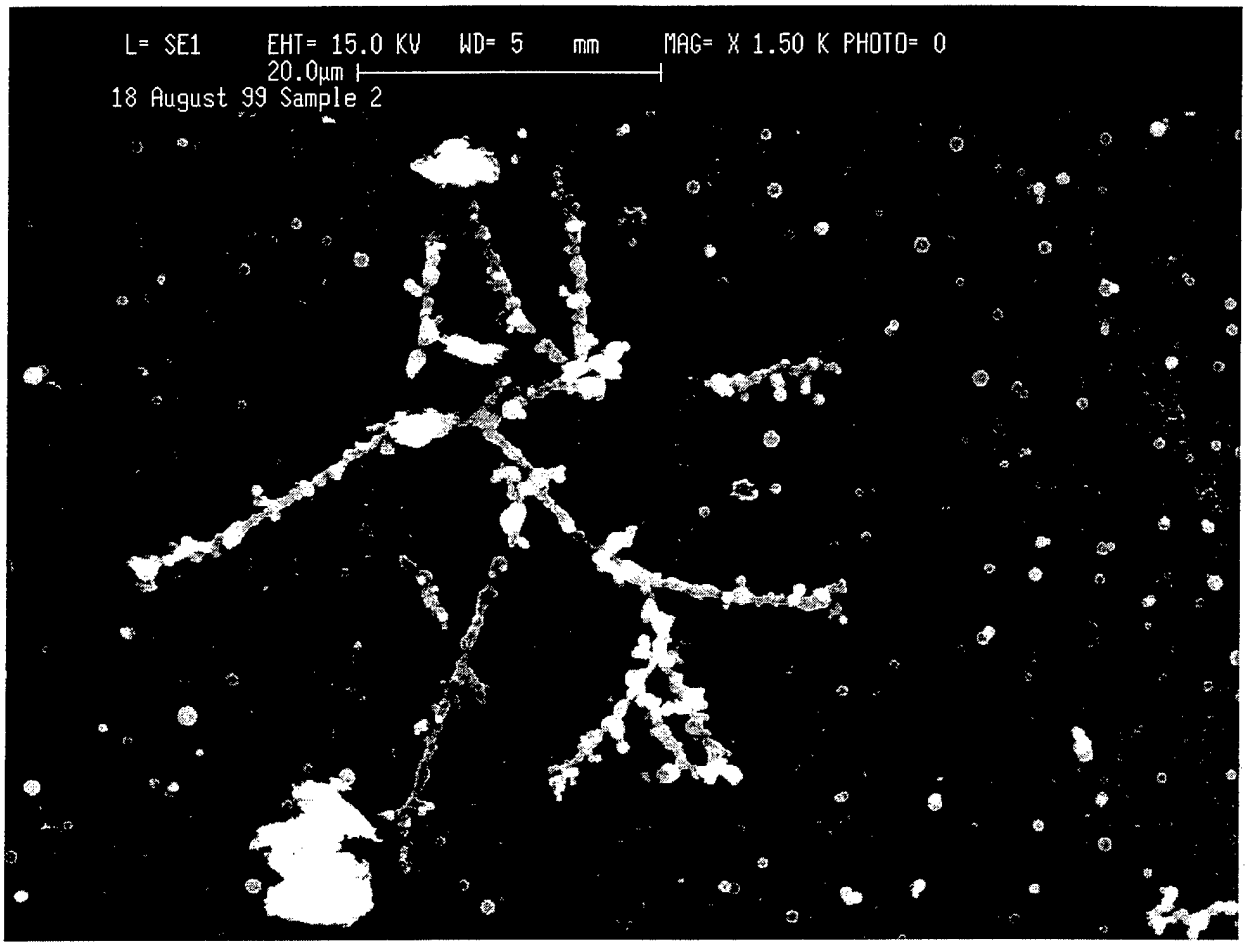


Figure 15. Cluster of B2-ACM Smoke Aerosol, Chain Aggregate Particles.

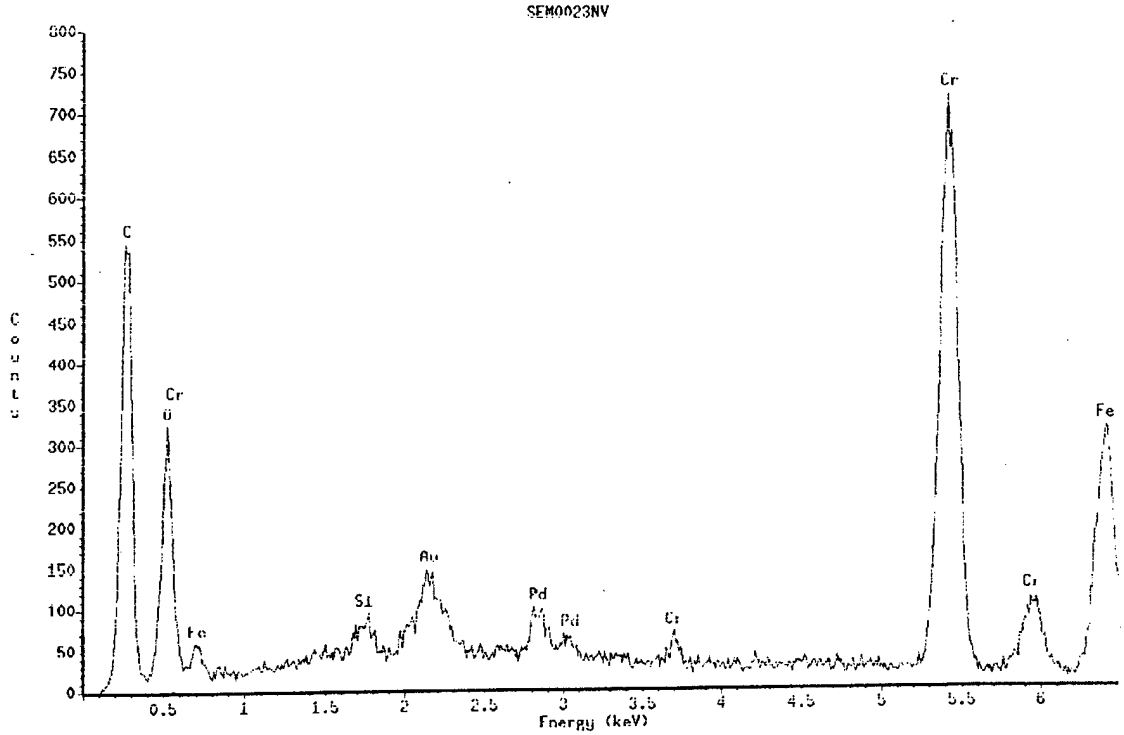


Figure 16. Energy Dispersive X-Ray Spectrum from a Chain Aggregate Aerosol Particle.

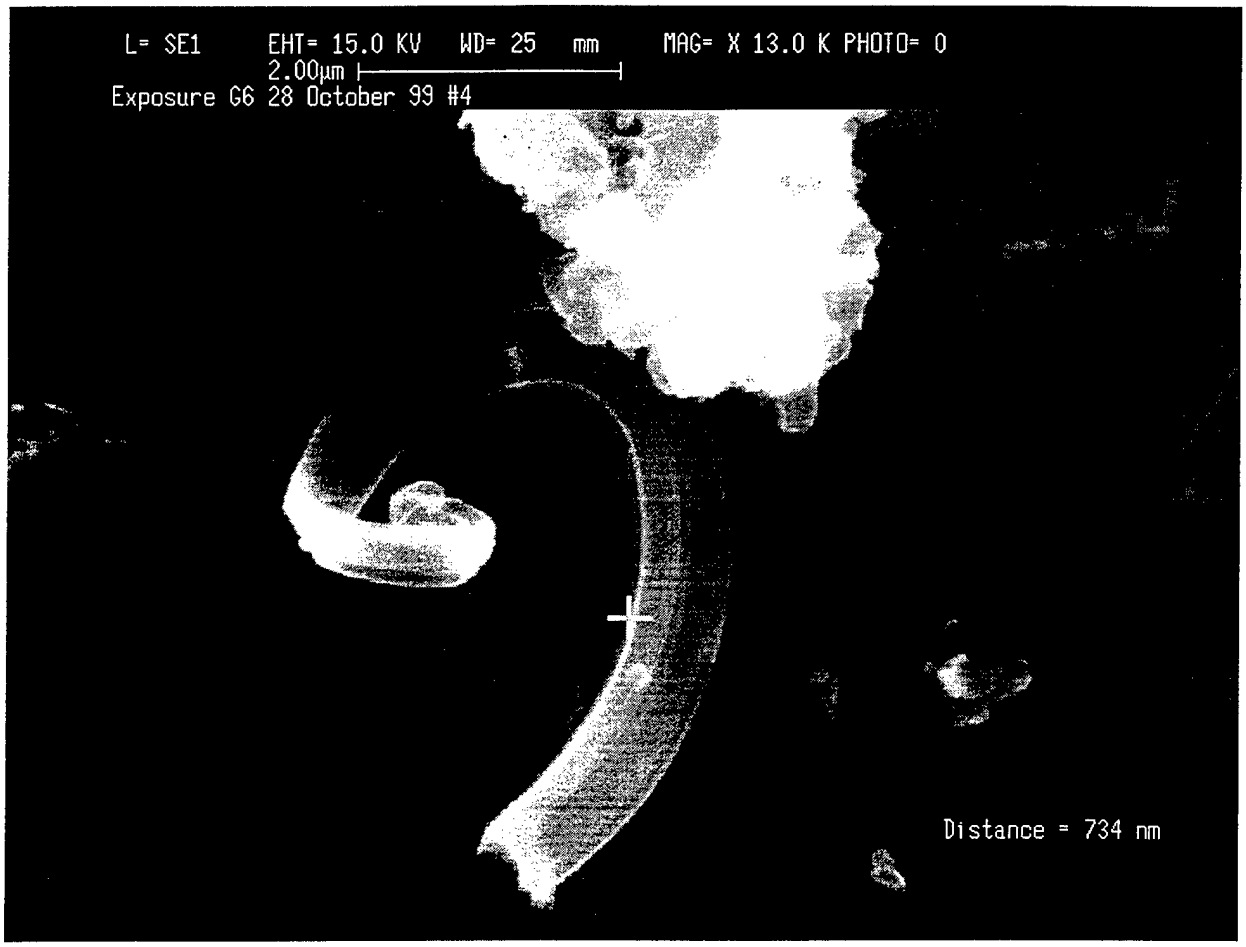


Figure 17. Typical B2-ACM Smoke Aerosol Fiber.

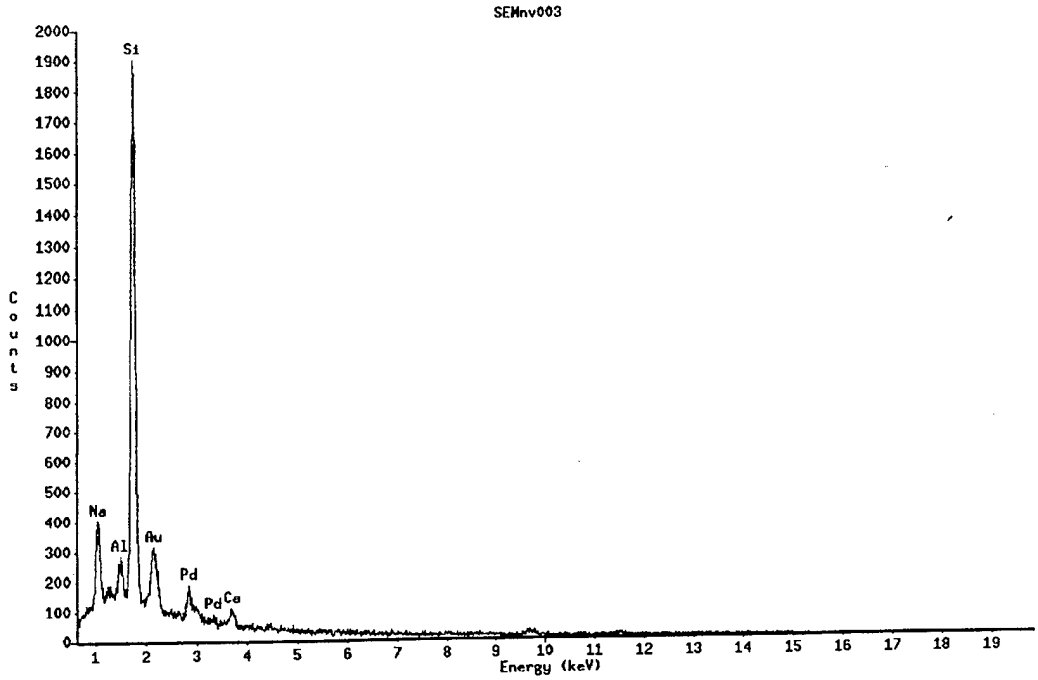


Figure 18. Energy Dispersive X-Ray Spectrum from a Smoke Aerosol Fiber.

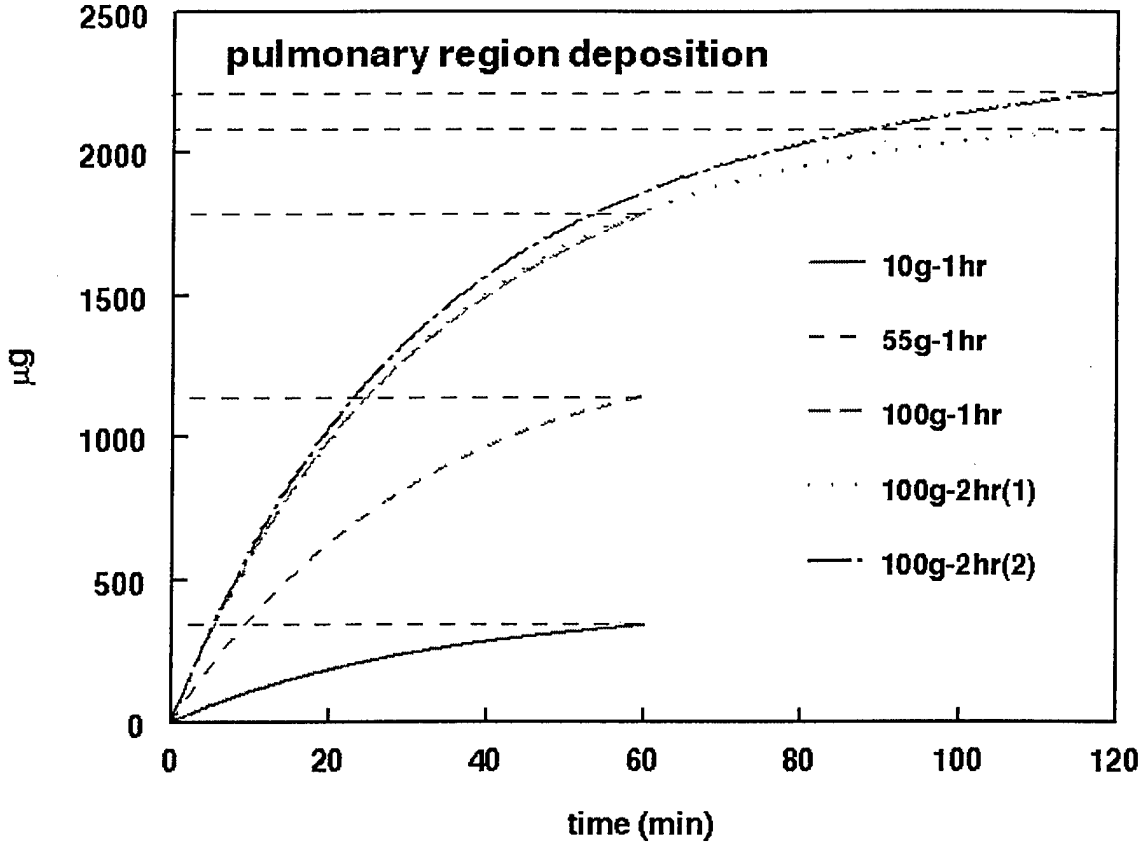


Figure 19. Theoretical Aerosol Deposition in the Pulmonary Region of the Lung.

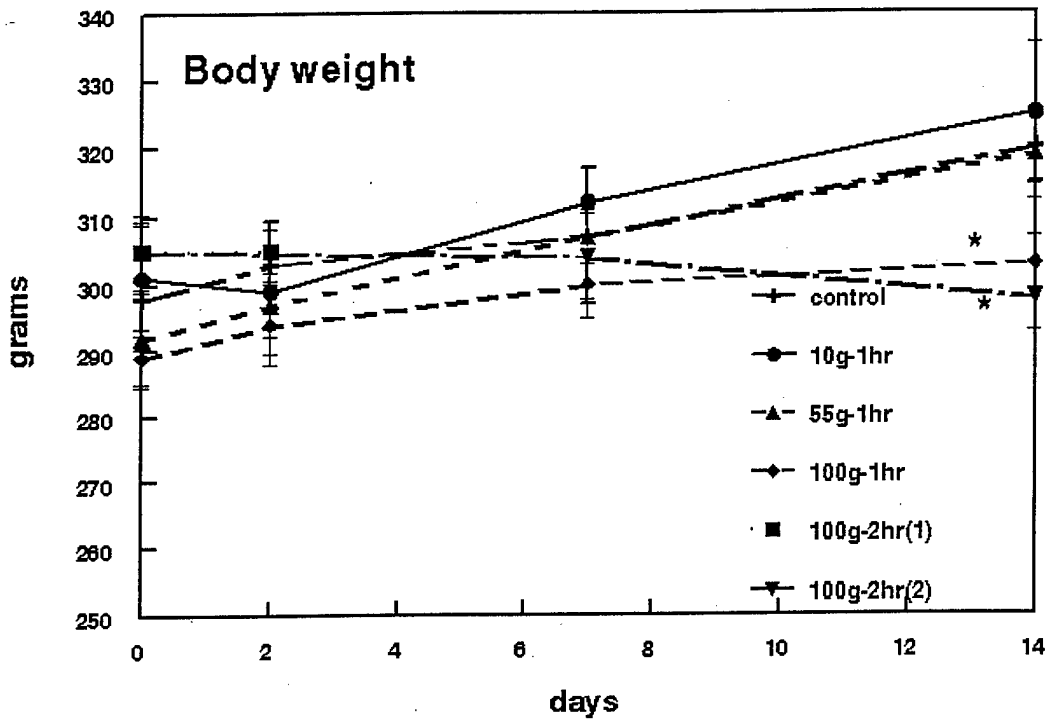


Figure 20. Animal Body Weight to 14 Days Post Exposure.
Error bars are ± standard error of the mean. Asterisk denotes significant difference, $p \leq 0.05$.

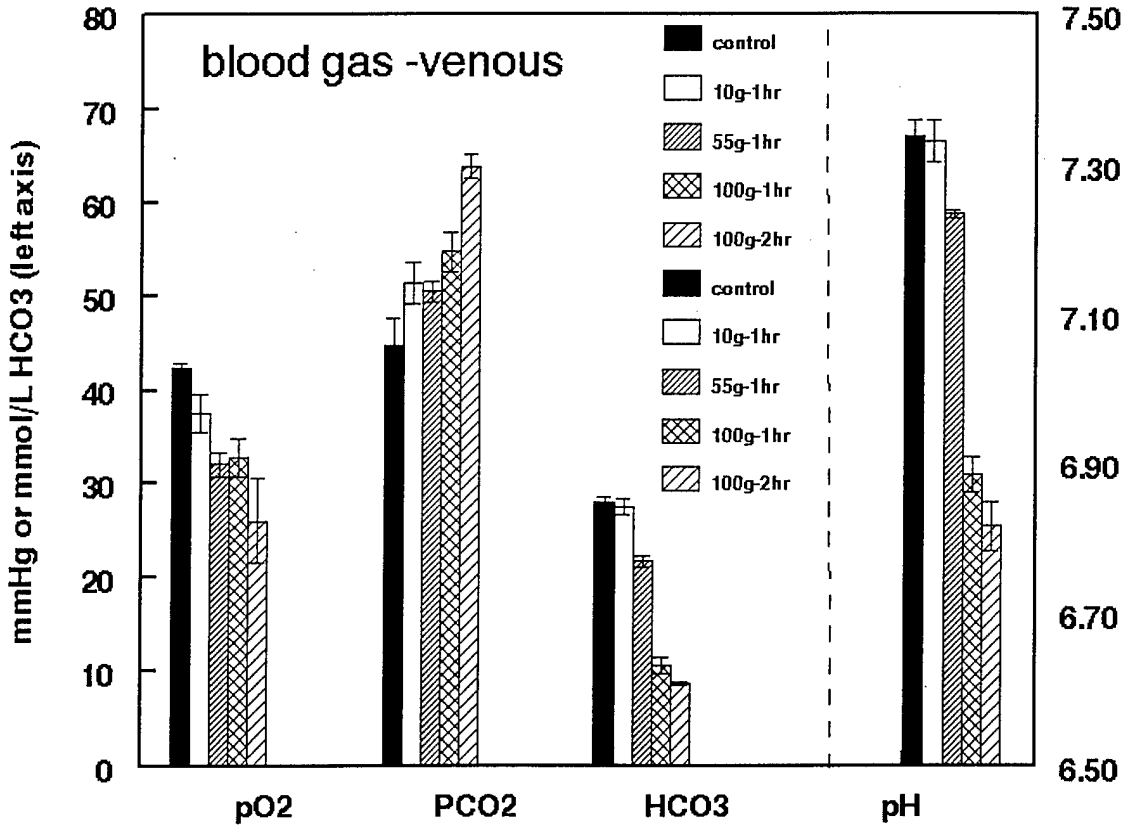


Figure 21. Venous Blood Gas, Carbonate, and pH values.
Error bars are ± standard error of the mean.

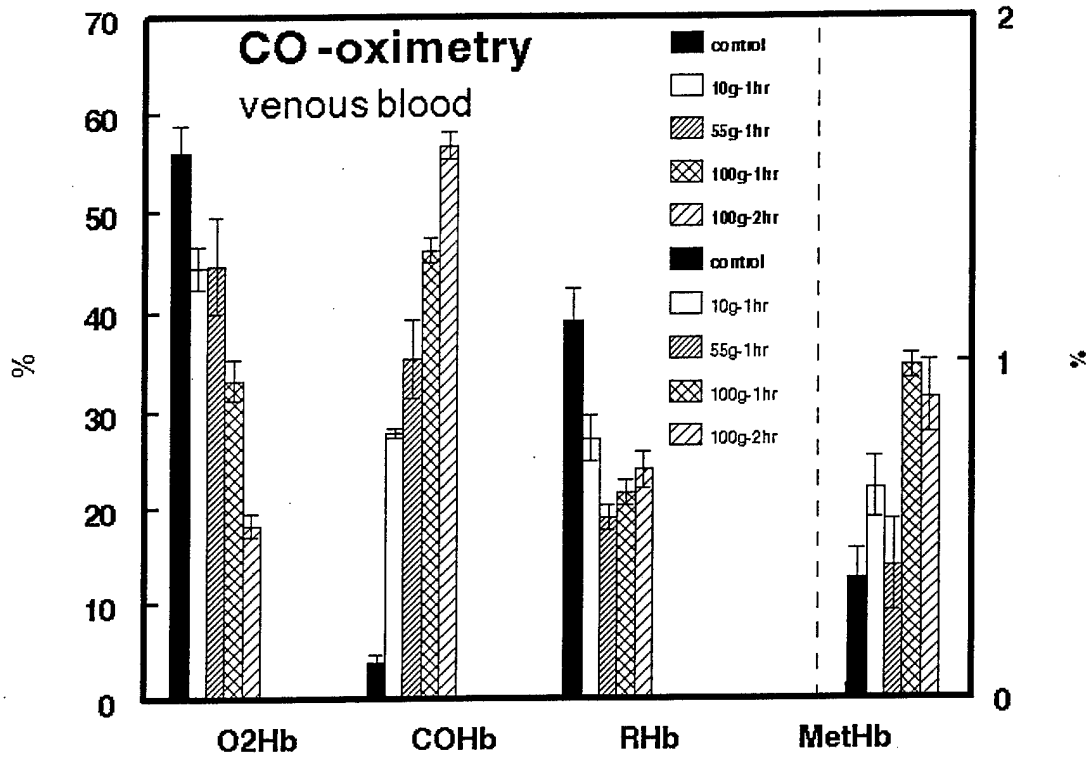


Figure 22. Venous Blood Hemoglobins.
Error bars are ± standard error of the mean.

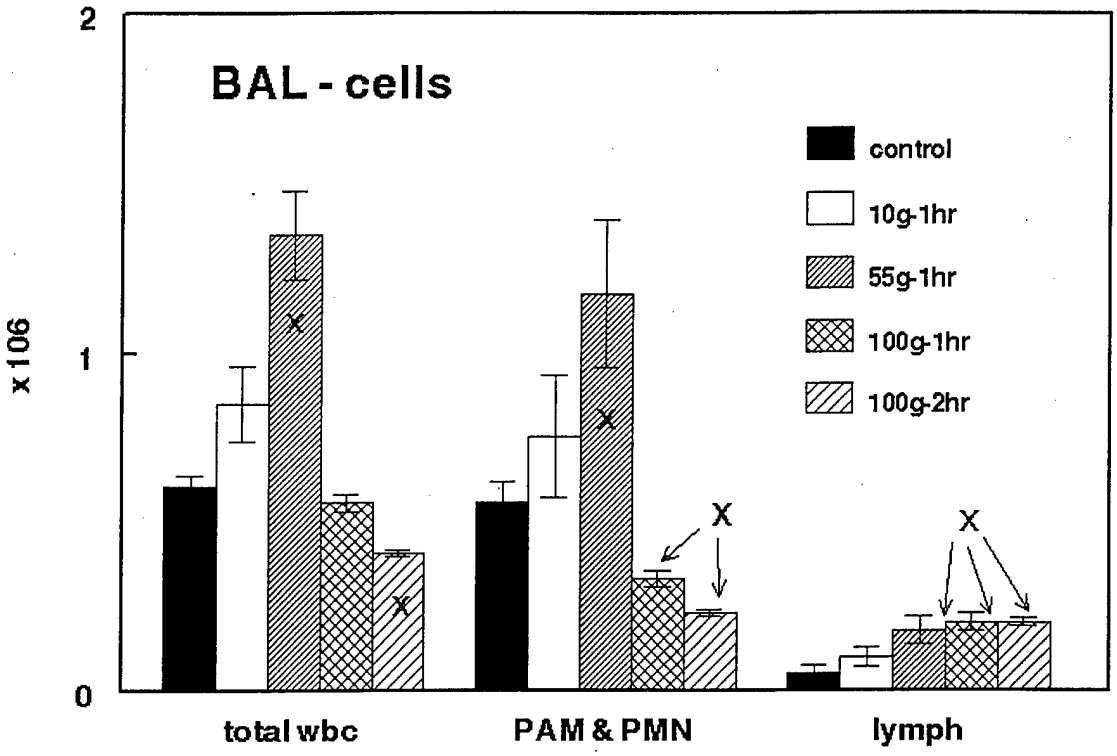


Figure 23. Bronchoalveolar Lavage Fluid Mobile Cell Population and Type.
Error bars are ± standard error of the mean. X denotes significant difference, $p \leq 0.05$.

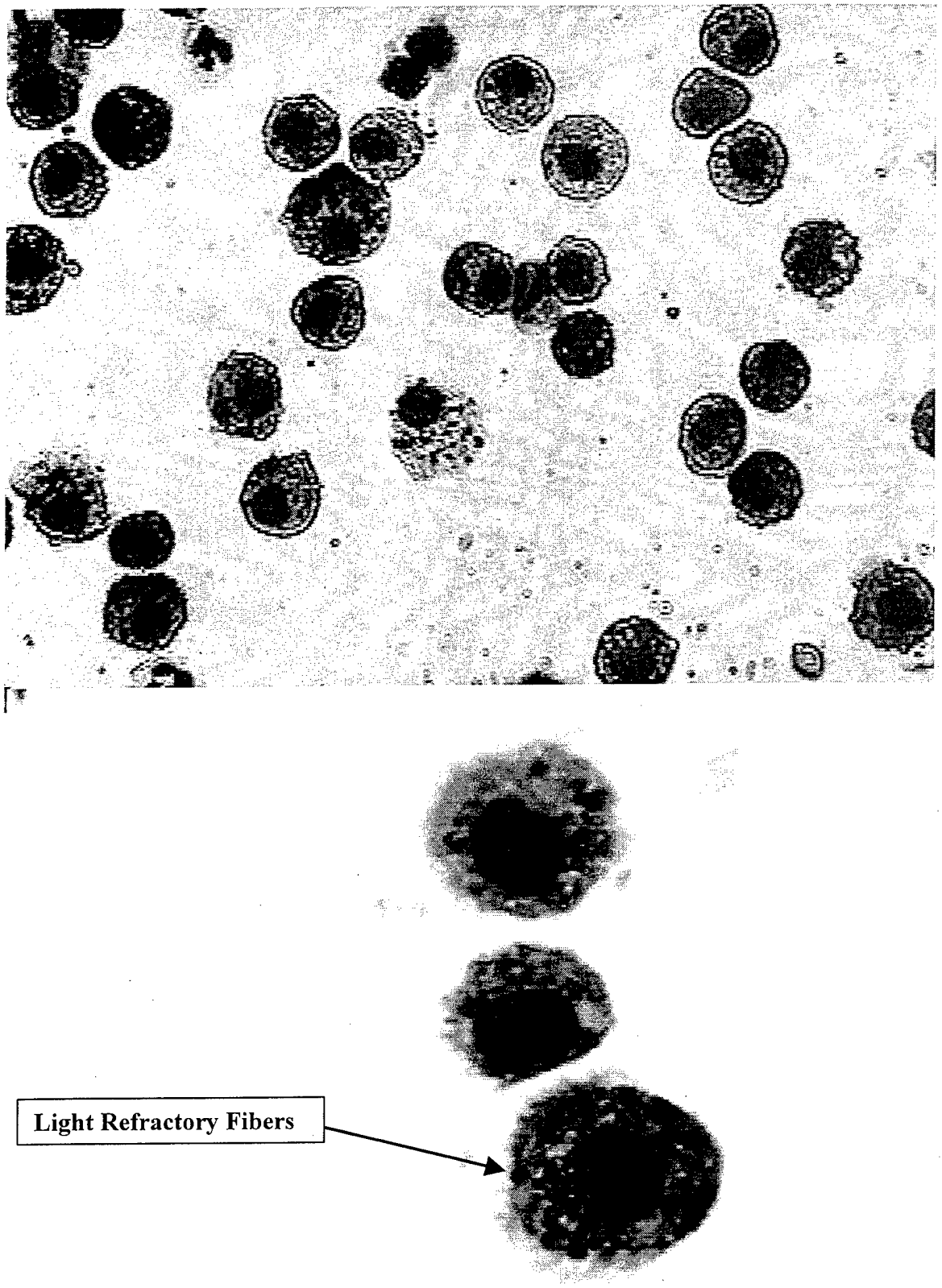


Figure 24. Particle Laden Pulmonary Alveolar Macrophages – 100g-2hr Group.

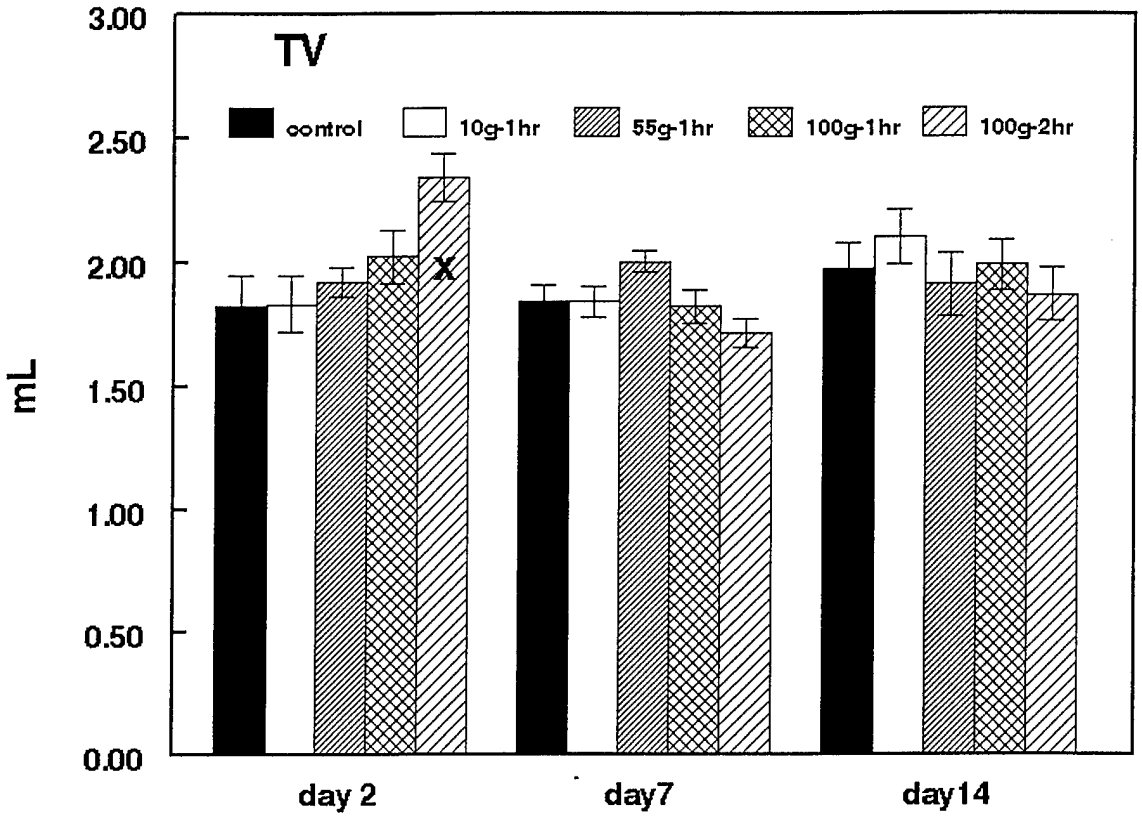


Figure 25. Tidal Volume.

Error bars are ± standard error of the mean. X denotes significant difference, $p \leq 0.05$.

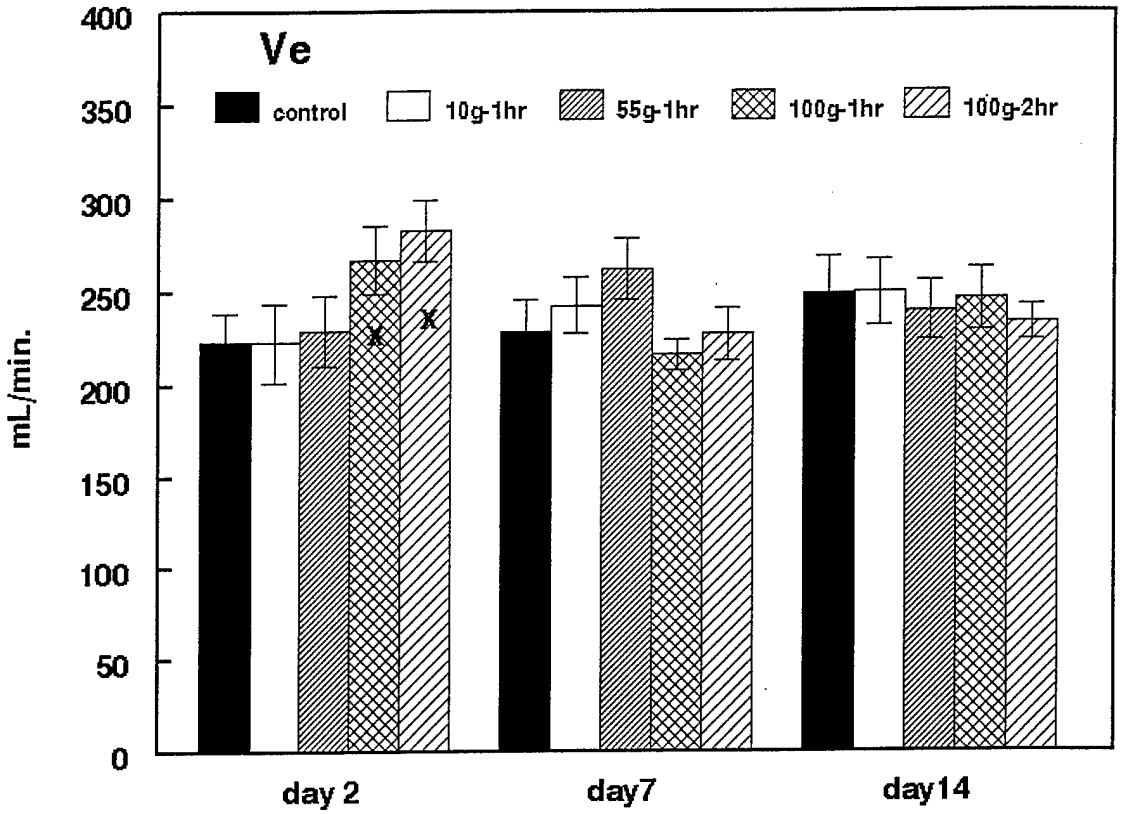


Figure 26. Minute Ventilation.
Error bars are ± standard error of the mean. X denotes significant difference, $p \leq 0.05$.

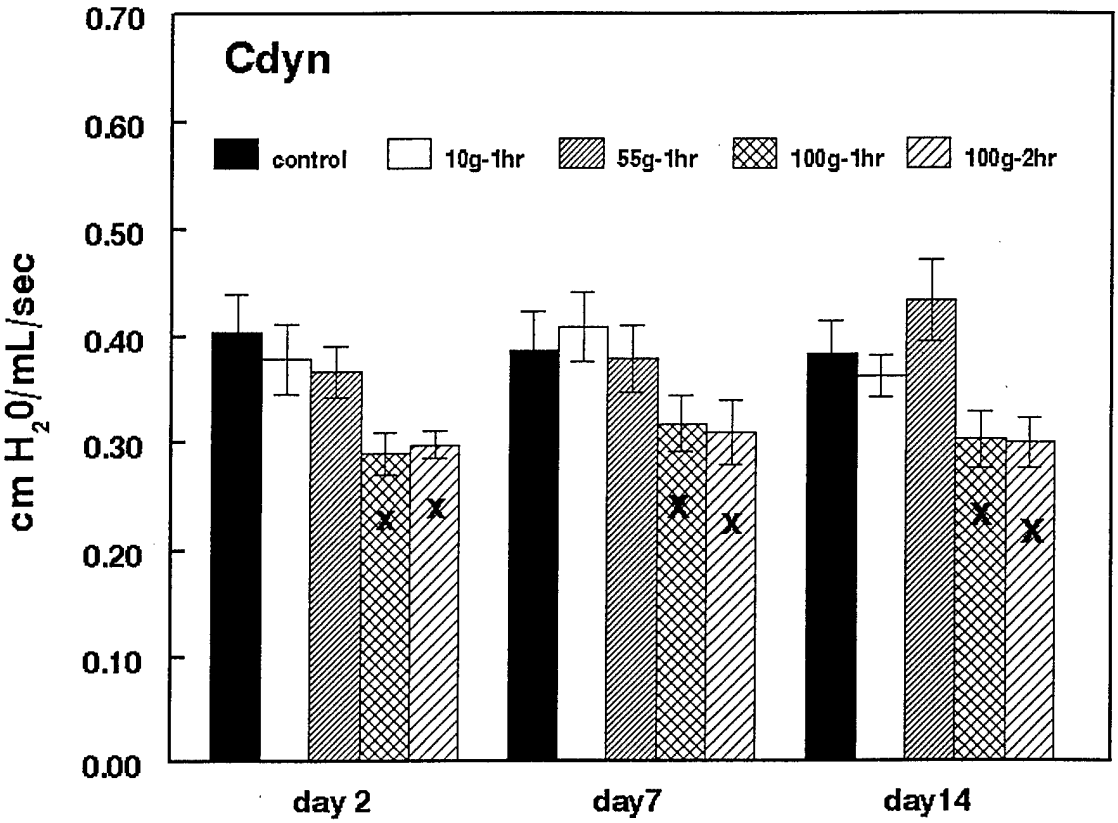


Figure 27. Dynamic Compliance.
Error bars are ± standard error of the mean. X denotes significant difference, $p \leq 0.05$.

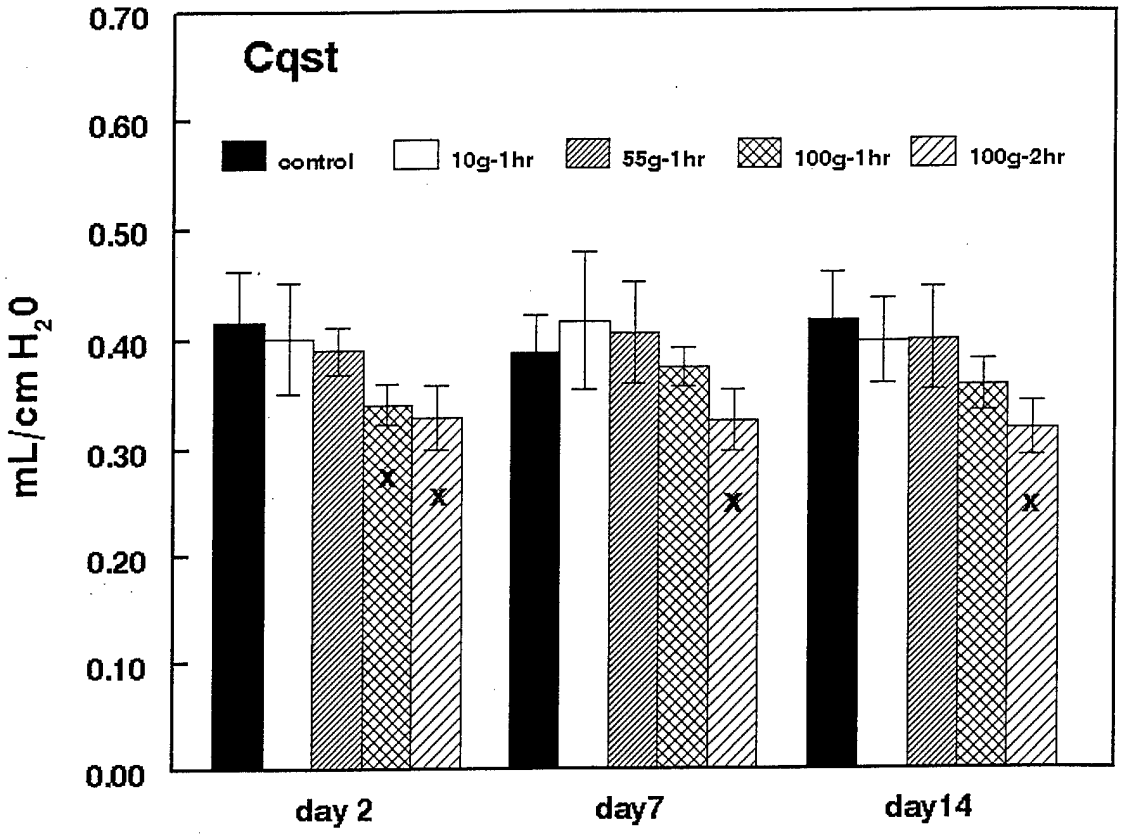


Figure 28. Quasistatic Compliance.
Error bars are ± standard error of the mean. X denotes significant difference, $p \leq 0.05$.

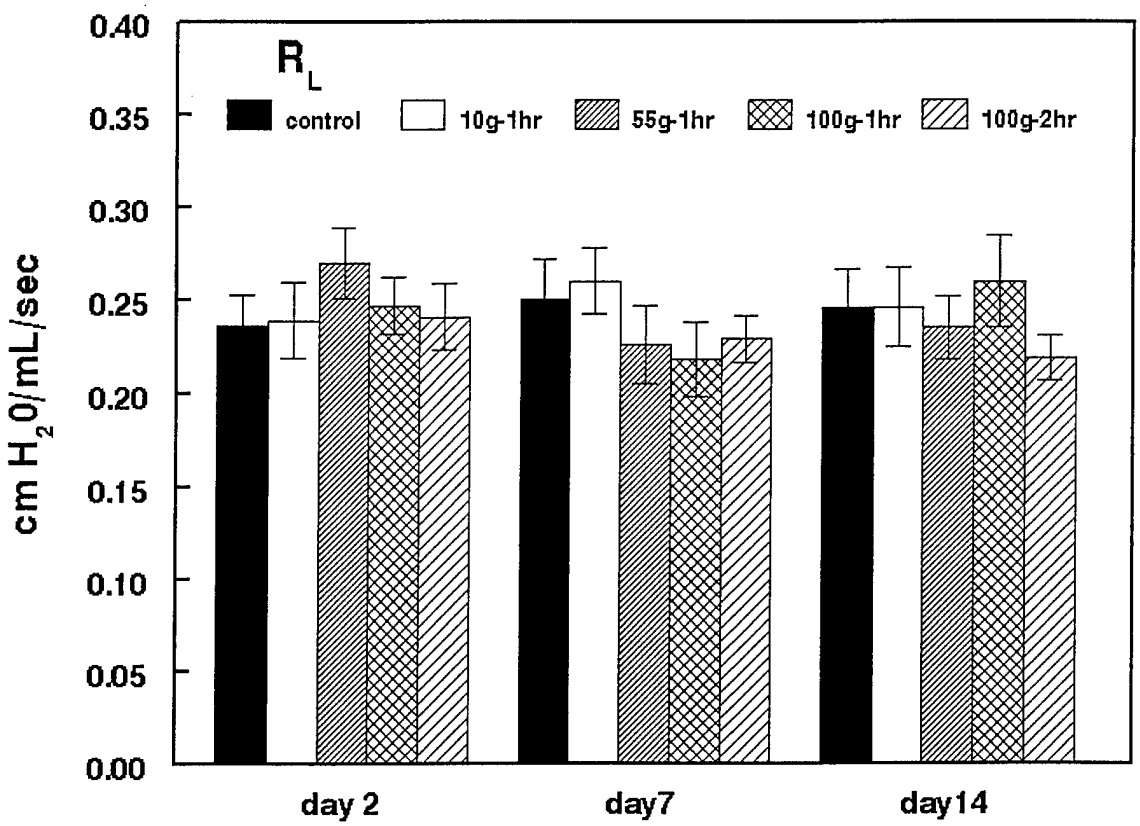


Figure 29. Lung Resistance.
Error bars are ± standard error of the mean. X denotes significant difference, $p \leq 0.05$.

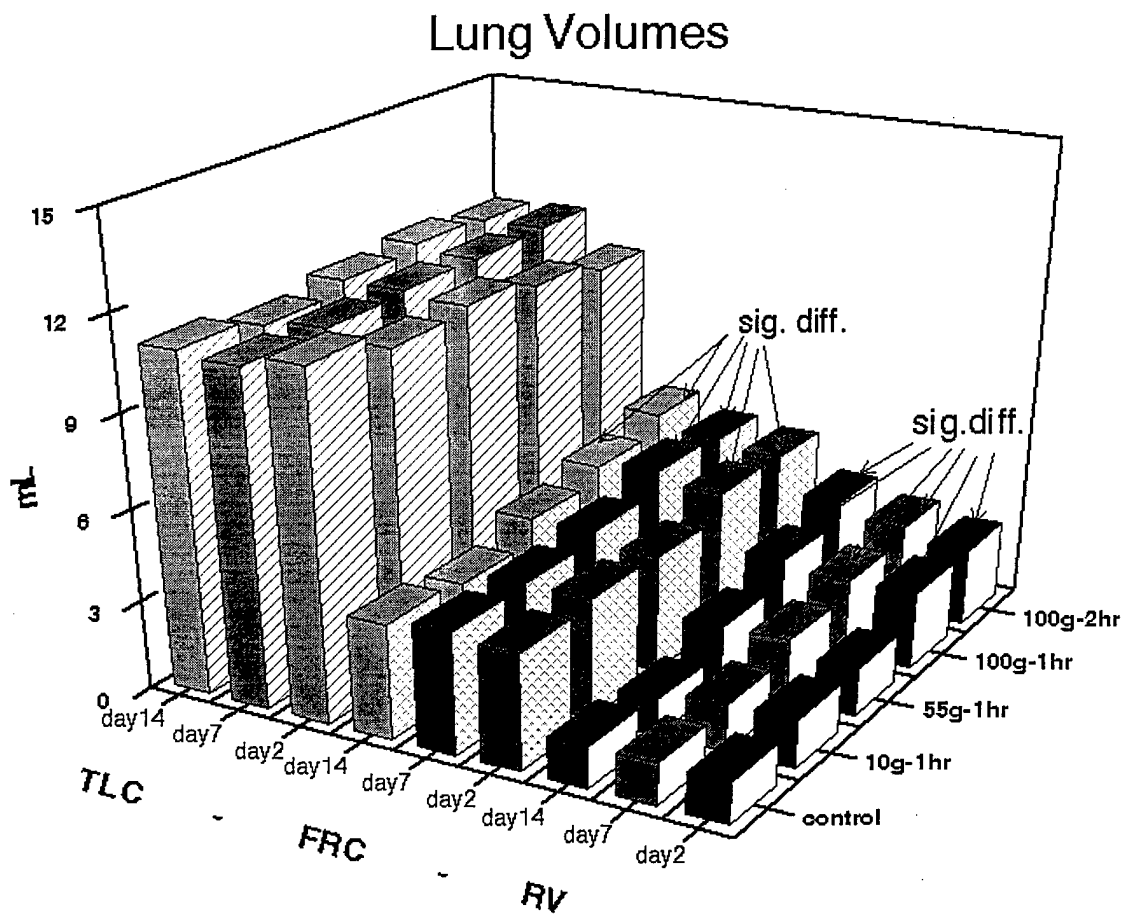


Figure 30. Lung Volumes.

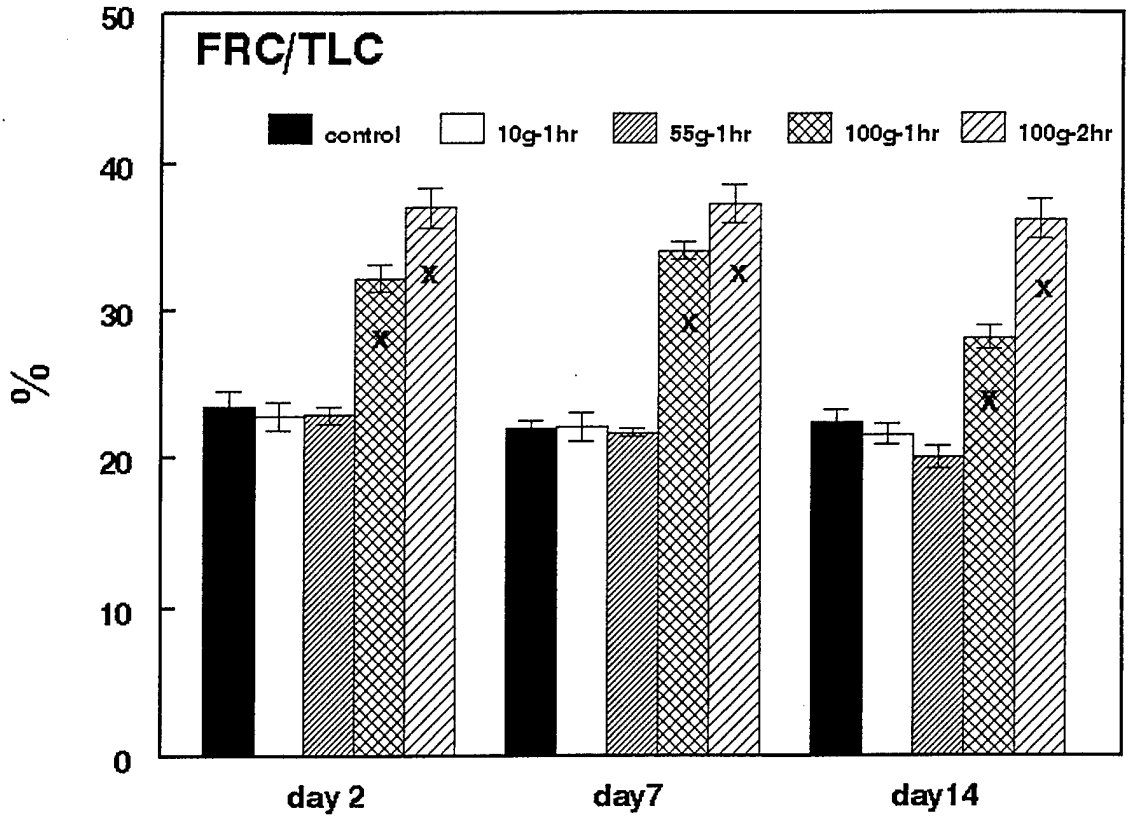


Figure 31. Percent of Total Lung Capacity Represented by Functional Residual Capacity.
Error bars are ± standard error of the mean. X denotes significant difference, $p \leq 0.05$.

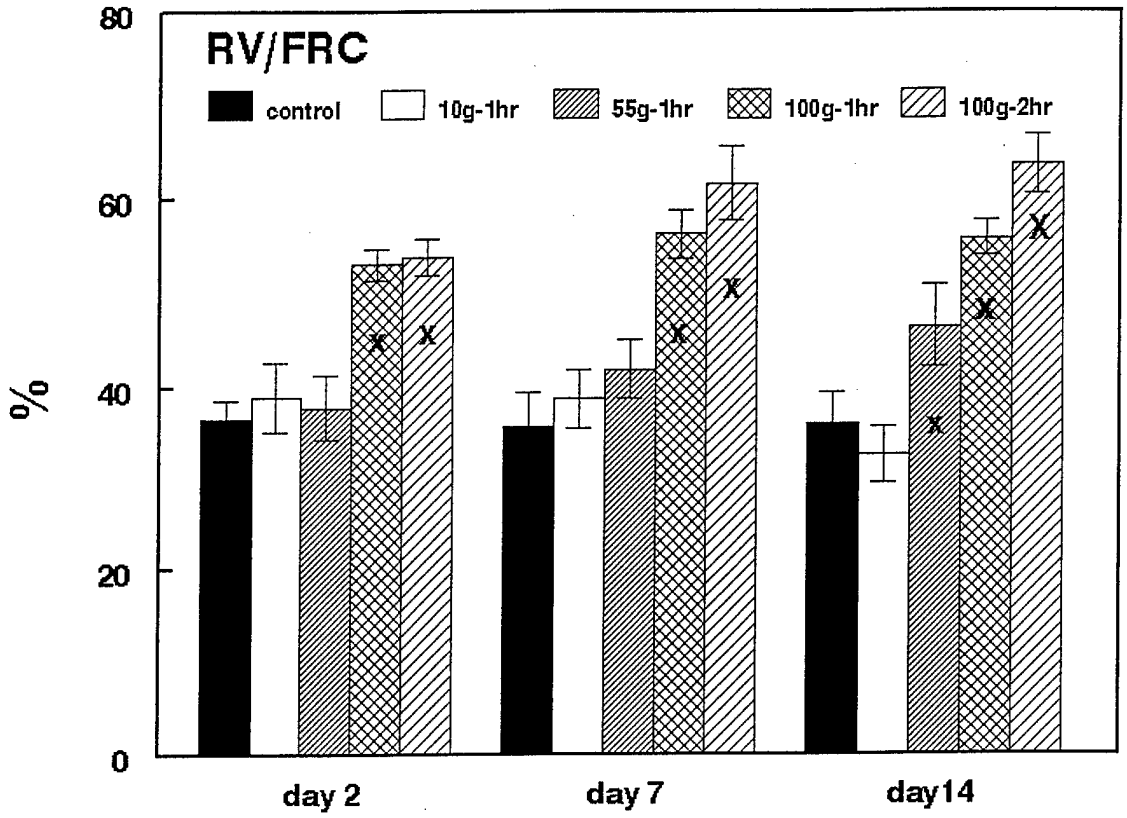


Figure 32. Percent of Functional Residual Capacity Represented by Residual Volume.
Error bars are ± standard error of the mean. X denotes significant difference, $p \leq 0.05$.

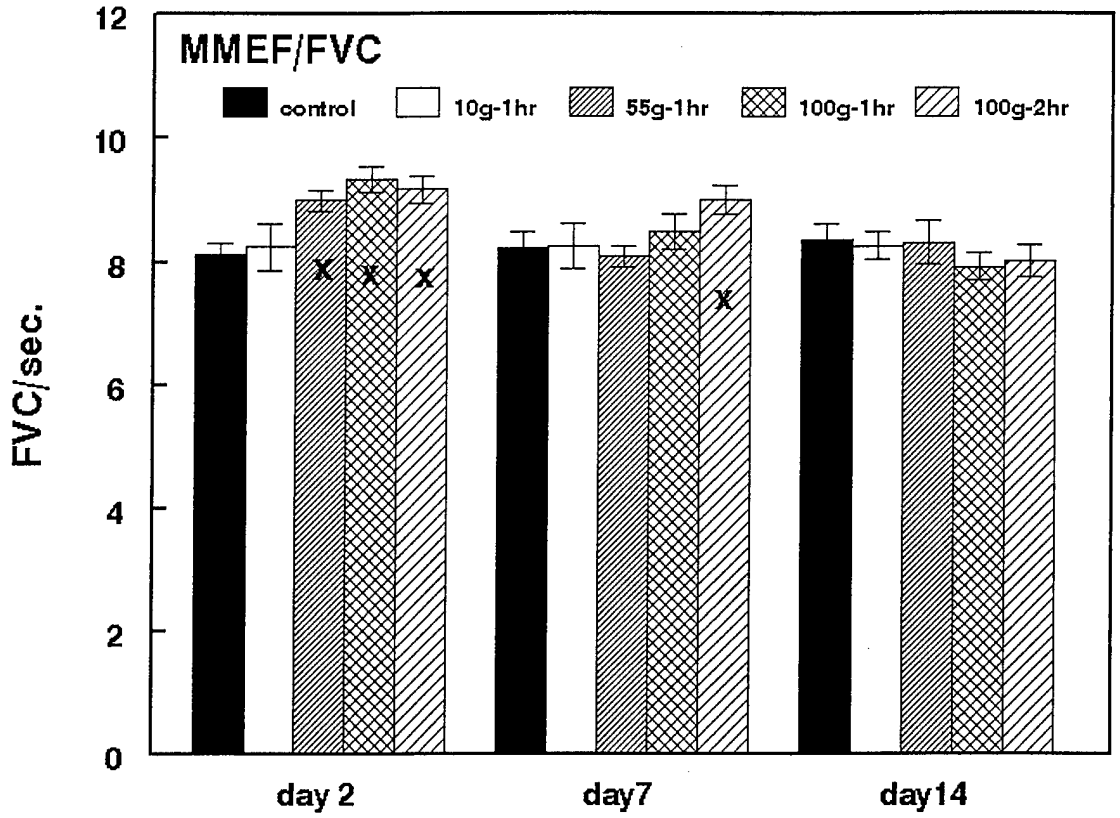


Figure 33. Mid-mean Expiratory Flow Normalized to Forced Vital Capacity.
Error bars are ± standard error of the mean. X denotes significant difference, $p \leq 0.05$.

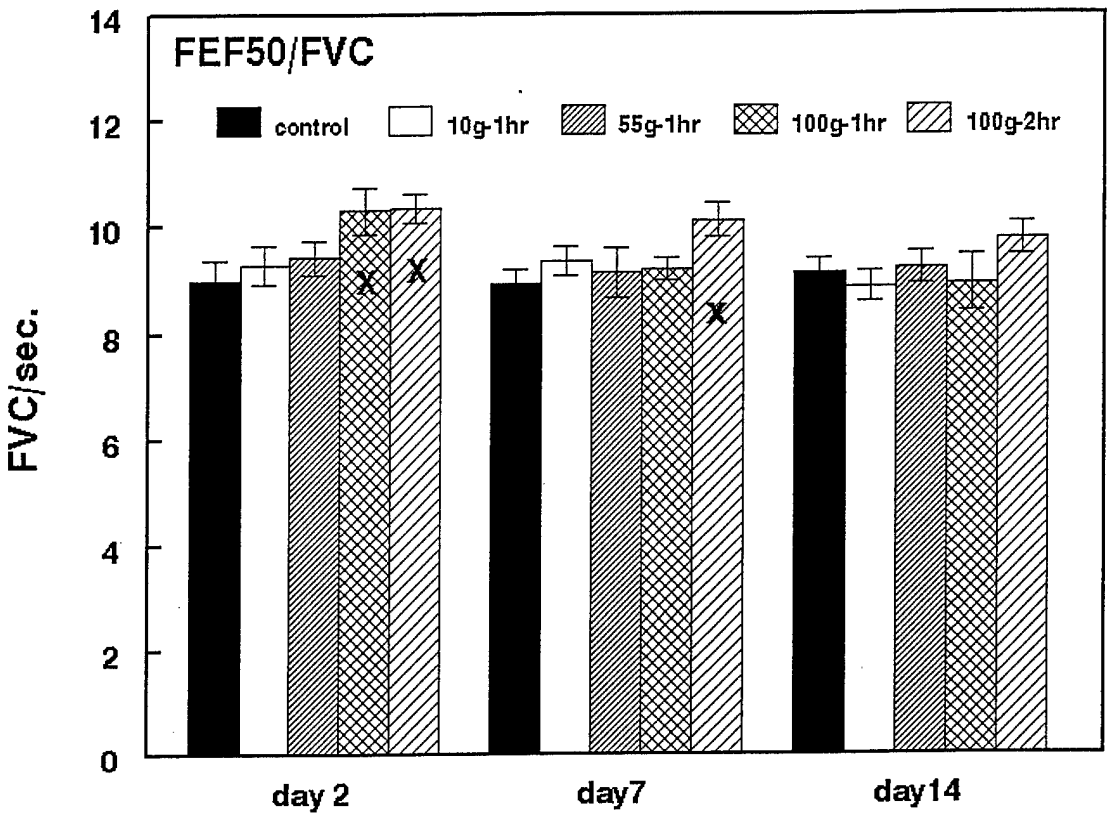


Figure 34. Forced Expiratory Flow at 50% Forced Vital Capacity Normalized to Forced Vital Capacity.

Error bars are ± standard error of the mean. X denotes significant difference, $p \leq 0.05$.

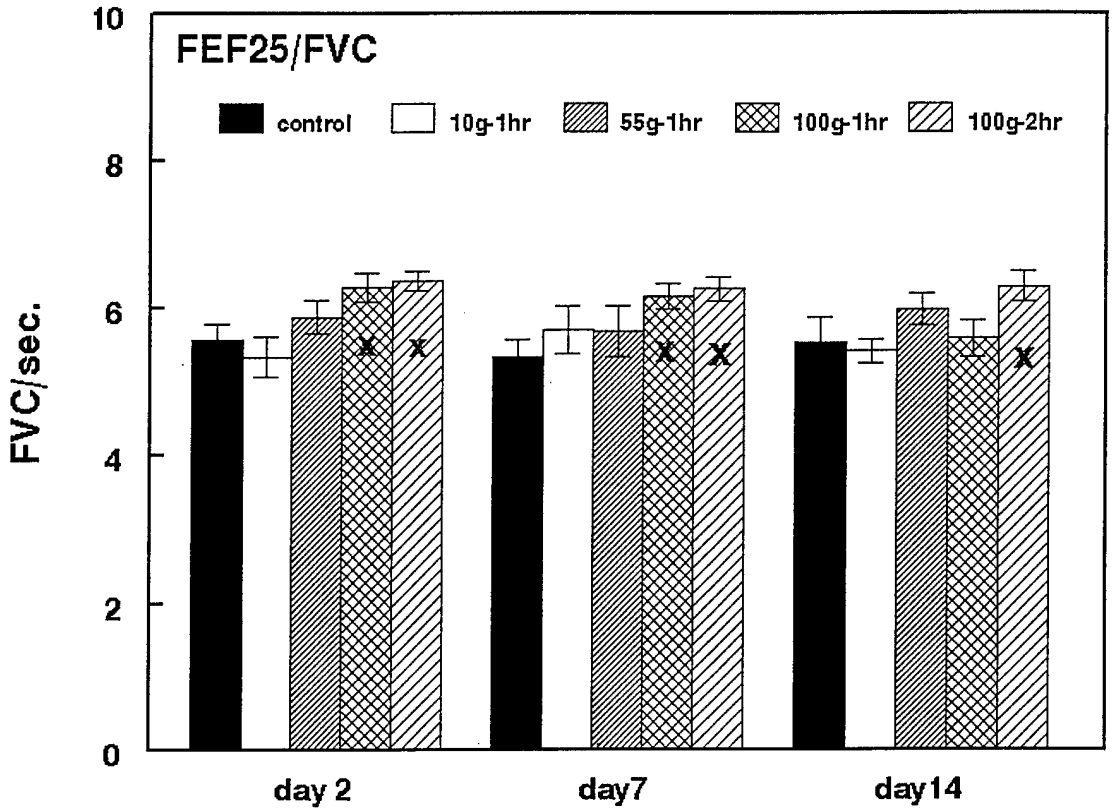


Figure 35. Force Expiratory Flow at 25% of Forced Vital Capacity Normalized to Forced Vital Capacity.

Error bars are ± standard error of the mean. X denotes significant difference, $p \leq 0.05$.

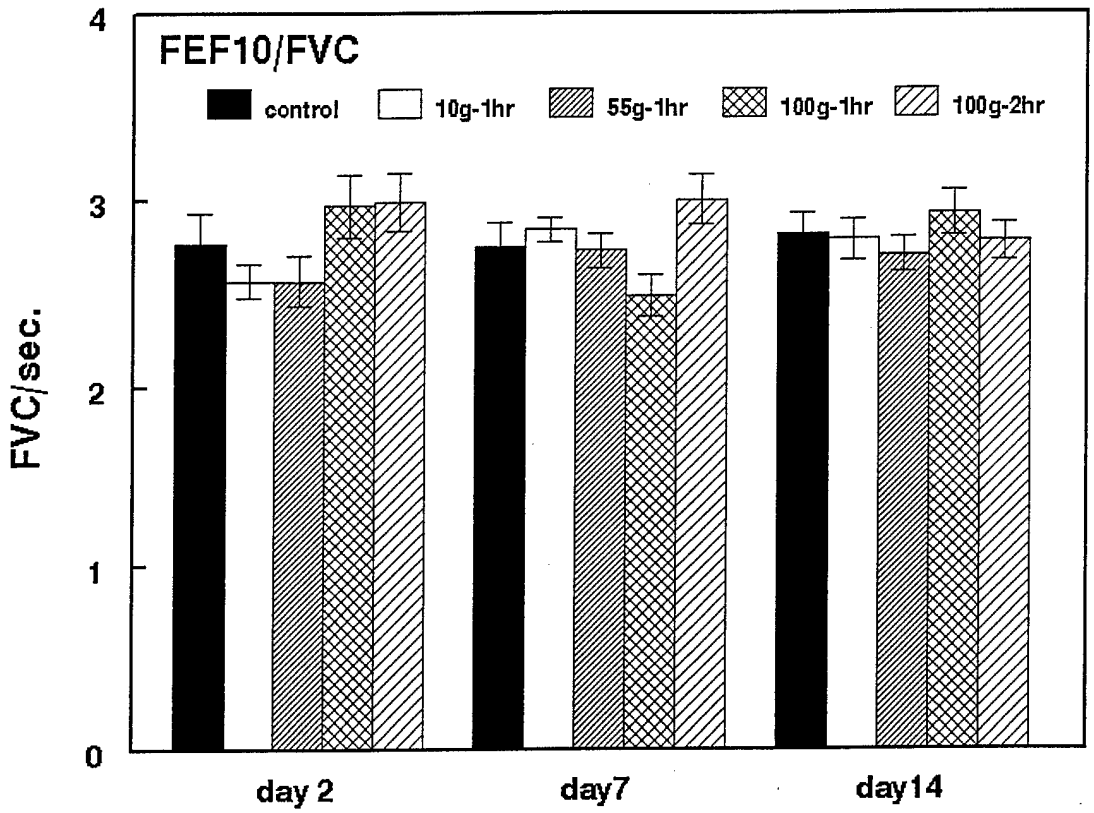


Figure 36. Force Expiratory Flow at 10% of Forced Vital Capacity Normalized to Forced Vital Capacity.

Error bars are $\pm$ standard error of the mean. X denotes significant difference, $p \leq 0.05$.

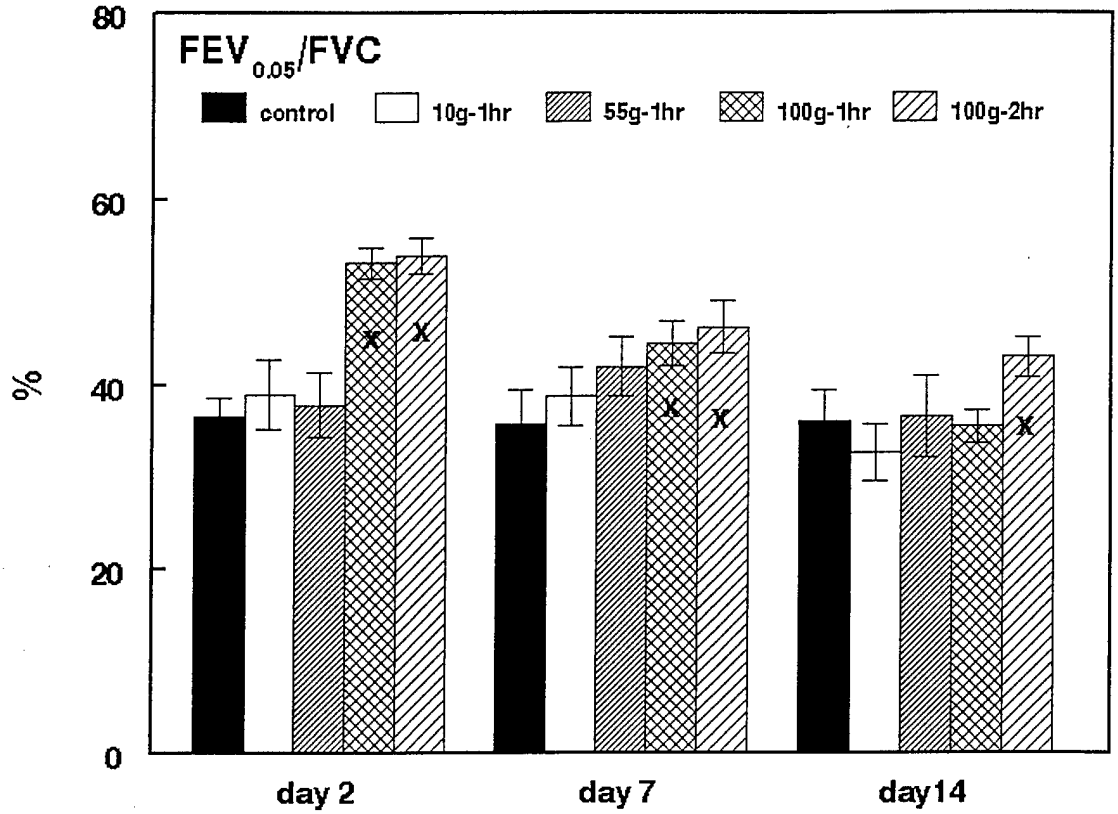


Figure 37. Forced Expiratory Volume at 0.05 Second.
Error bars are ± standard error of the mean. X denotes significant difference, $p \leq 0.05$.

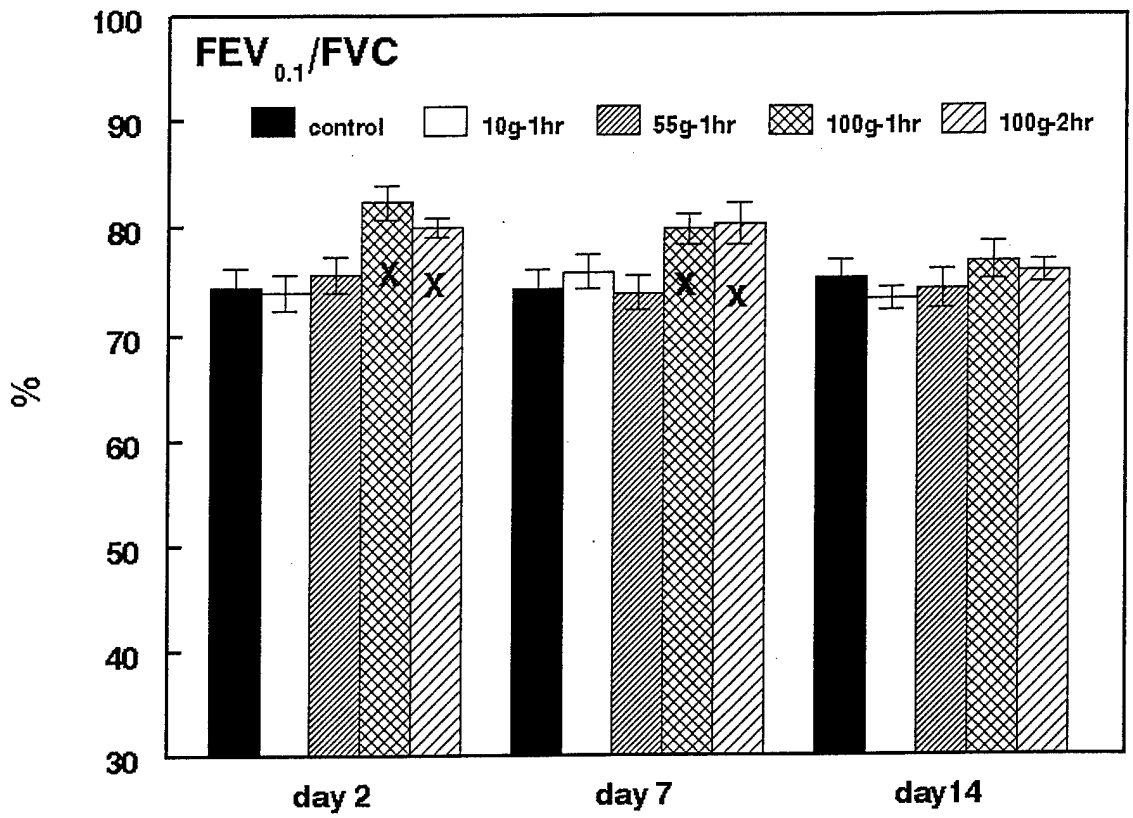


Figure 38. Forced Expiratory Volume at 0.1 Second.
Error bars are ± standard error of the mean. X denotes significant difference, $p \leq 0.05$.

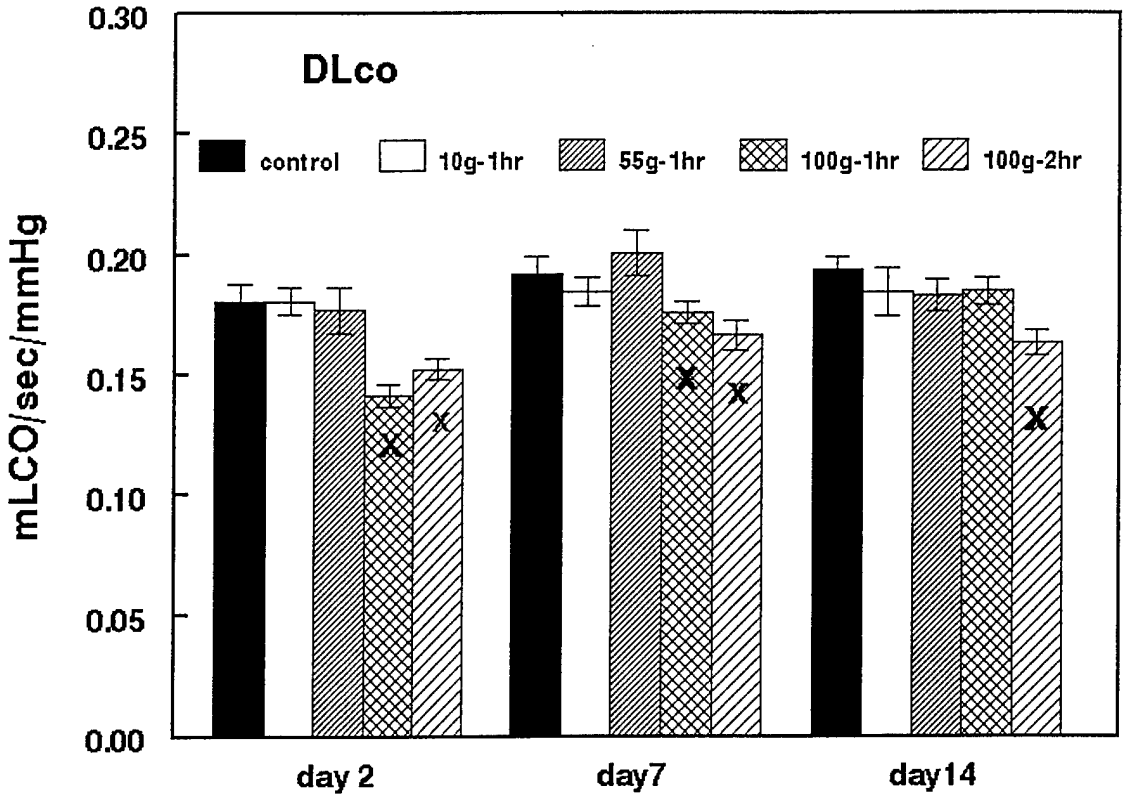


Figure 39. Single Breath Carbon Monoxide Diffusing Capacity.
Error bars are ± standard error of the mean. X denotes significant difference, $p \leq 0.05$.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services. Directorate for information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.
PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.

1. **REPORT DATE** *(DD-MM-YYYY)* 03-03-2000	2. **REPORT TYPE** March 2000	3. **DATES COVERED** *(From - To)* March 2000
4. **TITLE AND SUBTITLE** Acute Respiratory Toxicitiy of Advanced Composite Material (ACM) Combustion Atmospheres: B2-ACM		5a. **CONTRACT NUMBER** 5b. **GRANT NUMBER** 5c. **PROGRAM ELEMENT NUMBER**
6. **AUTHOR(S)** Edgar C. Kimmel, David L. Courson, James E. Reboulet, Gregory S. Whitehead, Kimberly A. Rice, William K. Alexander, Kirk A. Phillips, Robert L. Carpenter, and Kenneth R. Still		5d. **PROJECT NUMBER** 5e. **TASK NUMBER** 5f. **WORK UNIT NUMER** 63706N-M00095.004.1714
7. **PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)** Naval Health Research Center Detachment Toxicology NHRC/TD 2612 Fifth Street, Building 433 Area B Wright-Patterson AFB, OH 45433-7903		8. **PERFORMING ORGANIZATION REPORT NUMBER** TOXDET-00-01
9. **SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)** Naval Health Research Center Detachment Toxicology NHRC/TD 2612 Fifth Street, Building 433 Area B Wright-Patterson AFB, OH 45433-7903		10. **SPONSOR/MONITOR'S ACRONYM(S)** 11. **SPONSOR/MONITOR'S REPORT NUMBER(S)**

12. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. **ABSTRACT** *(Maximum 200 words)* Exposure for 2 hr to smoke generated from pyrolysis of 100 g of B2-ACM was lethal to experimental animals. Surviving animals showed elevated carboxyhemogobin levels, severe respiratory acidosis, and diminished oxygen transport in the blood, the combination of which was deemed the cause of death. These animals also showed signs of pulmonary inflammatory response. Two days post exposure, animals exhibited pulmonary pathophysiology indicative of restrictive lung disease. The pattern of pulmonary dysfunction at 14 days post exposure was similar, with some measures of lung function returning to normal while others remained significantly different from normal values. Although no deaths occurred, similar patterns of pulmonary dysfunction were evident in animals exposed for 1 hr to smoke from pyrolysis of 55 or 100 g of B2-ACM. These animals also had elevated carboxyhemogobin levels, decreased blood pH, and diminished oxygen capacity immediately post exposure however these symptoms were not as severe as those in animals exposed for 2 hr to smoke generated from pyrolysis of 100 g of B2-ACM. Quantitative analysis of a few selected smoke gases (CO, CO_2, NOx, SO_2) and aerosol particulate consistently accounted for over 80 % of the amount of B2-ACM that actually burned. Calculation of the nominal concentration of the smoke correlated well with the chemical analysis, the bulk amount of B2-ACM used to generate the smoke, and the pyrolysis rate. Consequently these estimates of smoke concentration could be used for dose-response determinations which, in turn, provide a suitable basis for risk assessment.

15. **SUBJECT TERMS**
B2-ACM smoke, Pulmonary pathophysiology, Inhalation Toxicity

16. SECURITY CLASSIFI-CATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT UNCL.	b. ABSTRACT UNCL.	c. THIS PAGE UNCL.	UL	85	CAPT K. R. Still 19b. TELEPHONE NUMBER *(Include area code)* (937) 255-6058

Standard Form 298 (Rev. 8/98)
Prescribed by ANSI Std. Z39.18

INSTRUCTIONS FOR COMPLETING SF 298

1. REPORT DATE. Full publication date including day, month, and year, if available (e.g. 1 Jan 88). Must cite at least the year and be Year 2000 compliant, e.g. 30-06-1998; XX-06-1998; xx-xx-1998..

2. REPORT TYPE. State the type of report, such as final, technical, interim, memorandum, masterís theses, progress, quarterly, research, special, group study, etc.

3. DATES COVERED. Indicate the time during which the work was performed and the report was written, e.g., Jun 1997 - Jun 1998; 1-10 June 1996; May - November 1998; Nov 1998.

4. TITLE. Enter titles and subtitle with volume number and part number, if applicable. On classified documents, enter the title classification in parentheses

5a. CONTRACT NUMBER. Enter all contract numbers as they appear in the report, e.g. F33615-86-C-5 169.

5b. GRANT NUMBER. Enter all grant numbers as they appear in the report, e.g. AFOSR-82-1234.

5c. PROGRAM ELEMENT NUMBER. Enter all project numbers as they appear in the report, e.g. 61101 A.

5d. PROJECT NUMBER. Enter all project numbers as they appear in the report, e.g. 05; RF0330201; T4112.

5e. TASK NUMBER. Enter all task numbers as they appear in the report, e.g. 001; AFAPL30480105.

5f. WORK UNIT NUMBER. Enter all work unit numbers as they appear in the report, e.g. 001; AFAPL30480105.

6. AUTHOR(S). Enter name(s) of person(s) responsible for writing the report, performing the research, or credited with the content of the report. The form of entry is the last name, first name, middle initial, and additional qualifiers separated by commas, e.g. Smith, Richard, J, Jr.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES). Self-explanatory.

8. PERFORMEING ORGANIZATION REPORT NUMBER. Enter all unique alphanumeric report numbers assigned by the performing organization, e.g. BRL-1234; AFWL-TR-85-4017-Vol-21 PT-2.

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES). Enter the name and address of the organization(s) financially responsible for and monitoring the work.

10. SPONSOR/MONITORíS ACRONYM(S). Enter, if available, e.g. BRL, ARDEC, NADC.

11. SPONSOR/MONITORíS REPORT NUMBER(S). Enter report number as assigned by the sponsoring/monitoring agency, if available, e.g. BRL-TR-829; -215.

12. DISTRIBUTION/AVAILABILITY STATEMENT. Use agency-mandated availability statements to indicate the public availability or distribution limitations of the report. If additional limitations/ restrictions or special markings are indicated, follow agency authorization procedures, e.g. RD/FRD, PROPIN, ITAR, etc. Include copyright information.

13. SUPPLEMENTARY NOTES. Enter information not included elsewhere such as: prepared in cooperation with; translation of; report supersedes: old edition number, etc.

14. ABSTRACT. A brief (approximately 200 words) factual *summary* of the most significant information.

15. SUBJECT TERMS. Key words or phrases identifying major concepts in the report.

16. SECURITY CLASSIFCATION. Enter security classification in accordance with security classification regulations, e.g. U, C, S, etc. If this form contains classified information, stamp classification level on the top and bottom.

17. LIMITATION OF ABSTRACT. THis block must be completed to assign a distribution limitation to the abstract. Enter UU (Unclassified Unlimited) or SAR (Same as Report). An entry in this block is necessary if the abstract is to be limited.

Standard Form 298 Back (Rev. 2-89)