United States
Department of
Agriculture

Animal and
Plant Health
Inspection
Service

Animal Damage
Control

Denver Wildlife Research Center
Building 16, P.O. Box 25266
Denver Federal Center
Denver, CO 80225-0266

(1)

BEHAVIORAL-PHYSIOLOGICAL EFFECTS OF RED PHOSPHOROUS SMOKE INHALATION ON TWO WILDLIFE SPECIES

TASK I REPORT

(Inhalation Equipment Development/Ambient CO Evaluation/Aerosol
Distribution and Air Quality Study)

R. T. Sterner
S. A. Shumake
B. E. Johns
R. D. Thompson

December 1987

U. S. DEPARTMENT OF AGRICULTURE
Animal and Plant Health Inspection Service
Denver Wildlife Research Center
Denver Federal Center
Denver, Colorado 80225-0266

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, MD 21701-5012

Project Order No. 85PP5847

COR: Henry S. Gardner, Jr.

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

APHIS—Protecting American Agriculture

Unclassified
SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION Unclassified	**1b. RESTRICTIVE MARKINGS**
2a. SECURITY CLASSIFICATION AUTHORITY	**3. DISTRIBUTION/AVAILABILITY OF REPORT** Approved for public release; distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	**5. MONITORING ORGANIZATION REPORT NUMBER(S)**
6a. NAME OF PERFORMING ORGANIZATION U.S. Department of Agriculture Animal & Plant Health	**6b. OFFICE SYMBOL** *(If applicable)*
7a. NAME OF MONITORING ORGANIZATION	
6c. ADDRESS *(City, State, and ZIP Code)* Inspection Service Denver Wildlife Research Center Denver Federal Center, Denver, CO 80225	**7b. ADDRESS** *(City, State, and ZIP Code)*
8a. NAME OF FUNDING/SPONSORING ORGANIZATION U.S. Army Medical Research & Development Command	**8b. OFFICE SYMBOL** *(If applicable)*
9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER Project Order 85PP5847	
8c. ADDRESS *(City, State, and ZIP Code)* Fort Detrick Frederick, MD 21701-5012	**10. SOURCE OF FUNDING NUMBERS**

PROGRAM ELEMENT NO.	PROJECT NO. 3E1	TASK NO.	WORK UNIT ACCESSION NO.
62720A	62720A835	AA	003

11. TITLE *(Include Security Classification)*
(U) Behavioral-Physiological Effects of Red Phosphorous Smoke Inhalation on Two Wildlife Species

12. PERSONAL AUTHOR(S)
R.T. Sterner, S.A. Shumake, B.E. Johns, and R.D. Thompson

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final, Task 1	FROM Mar. '85 TO Dec.'86	1987 December	75

16. SUPPLEMENTARY NOTATION

17. COSATI CODES

FIELD	GROUP	SUB-GROUP
06	06	
06	11	

18. SUBJECT TERMS *(Continue on reverse if necessary and identify by block number)*
Red phosphorous - butyl rubber
Aerosol
Inhalation toxicology
Spatial uniformity
Temporal uniformity
Combustion products
Air quality

19. ABSTRACT *(Continue on reverse if necessary and identify by block number)* Cu m

Tests to evaluate the spatial and temporal uniformity of red phosphorous - butyl rubber smoke produced in a commercial 1-m^3 inhalation chamber are described. Several modifications to the inhalation exposure system aimed at improving air filtration, relative humidity, and temperature control for the conduct of animal studies are also presented. Smoke generation involved the use of a system for the continuous generation of phosphoric acid aerosols developed by staff of the Analytical Chemistry Division, Oak Ridge National Laboratory. Assessments of spatial and temporal uniformity of smoke were based upon measurements of aerosol mass concentration (gravimetric analysis), phosphoric acid deposition (titration analysis), aerosol opacity (infrared sensor), and particle size (cascade impactor); assessments of air quality and combustion products within the chamber involved checks for oxygen, carbon dioxide, phosphine, hexane, and carbon monoxide using either gas chromatograph or industrial-hygiene-analyzer tubes. Results for aerosol mass, phosphoric acid, and particle size showed that the within-chamber smoke was highly uniform among burns. (Continued)

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS	**21. ABSTRACT SECURITY CLASSIFICATION** Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL Mary Frances Bostian	**22b. TELEPHONE** *(Include Area Code)* 301/663-7325 — **22c. OFFICE SYMBOL** SGRD-RMI-S

DD Form 1473, JUN 86 *Previous editions are obsolete.*

SECURITY CLASSIFICATION OF THIS PAGE
Unclassified

BLOCK 19: ABSTRACT (concluded)

Although a number of statistically significant effects were obtained, further inspection showed these to be limited to specific sampling locations and within *a priori* criteria established to define acceptable uniformity. Specific findings of note include: (a) Cage Site 12 adjacent to the sampling port used with industrial-hygiene-analyzer tubes yielded 10 percent lower aerosol values than other sites, (b) an "overall dilution effect" occurred for mean aerosol mass and phosphoric acid titration values collected from the Bottom Shelf of the chamber (i.e., about -3 percent), and (c) a slight gradient of larger aerosol particle sizes occurred from the Top to Bottom Shelf of the chamber -- 0.52 to 0.55 µm, respectively. Regarding air quality and combustion contaminants, oxygen and carbon dioxide levels within the chamber were always at sufficient levels for normal respiratory functions, and phosphine and hexane contaminants were rare and occurred in only negligible quantities. Carbon monoxide was found to occur in measurable amounts during practically all burns, with average readings of from 8.8 to 35.5 ppm between the slower and faster red phosphorous - butyl rubber extrusion rates; avoidance of excessive carbon monoxide will involve lower extrusion rates in future studies. In conclusion, the results confirmed that conditions within the exposure chamber were uniform and satisfactory for the exposure of animals to controlled doses of red phosphorous - butyl rubber smoke in future experiments.

Keywords: Toxicology, Toxicity; Prairie dogs; rock doves. (RW)

BEHAVIORAL-PHYSIOLOGICAL EFFECTS OF RED PHOSPHOROUS SMOKE INHALATION ON TWO WILDLIFE SPECIES

TASK 1 REPORT

(Inhalation Equipment Development/Ambient CO Evaluation/Aerosol
Distribution and Air Quality Study)

R. T. Sterner
S. A. Shumake
B. E. Johns
R. D. Thompson

December 1987

U. S. DEPARTMENT OF AGRICULTURE
Animal and Plant Health Inspection Service
Denver Wildlife Research Center
Denver Federal Center
Denver, Colorado 80225-0266

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, MD 21701-5012

Project Order No. 85PP5847

COR: Henry S. Gardner, Jr.

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

EXECUTIVE SUMMARY

The objective of this project is to determine certain effects of red phosphorous/butyl rubber (RP/BR) smoke on two wildlife species -- prairie dogs and rock doves. Data will provide information about acute behavioral and physiological effects associated with inhalation of this obscurant. To aid accomplishment of the objective, the research is divided into three tasks: Task 1--Inhalation Equipment Development/Ambient Carbon Monoxide (CO) Evaluation/Aerosol Distribution and Air Quality Study, Task 2 -- Toxicity Rangefinding and Basal Physiological Studies of RP/BR Smoke Exposure with Prairie Dogs and Rock Doves, and Task 3 -- RP/BR Effects upon Spontaneous Activity, Startle Response, Pulmonary Function, and Blood Chemistry of Prairie Dogs and Rock Doves.

This report describes Task 1 of the project. Briefly, an inhalation chamber and aerosol generation system was designed and installed by staff of the Analytical Chemistry Division, Oak Ridge National Laboratory (ORNL). Staff of the Denver Wildlife Research Center (DWRC) then made several modifications to the system which improved air filtration, temperature regulation and humidity control. Next, an evaluation of ambient CO levels was undertaken; numerous CO readings were collected at selected sites within and near the Laboratory during a 16-day period. Finally, a study was conducted to characterize the homogeneity/heterogeneity of the chamber atmosphere during RP/BR smoke generation. This involved a total of 64 RP/BR burns at target concentrations of 0.4, 1.5, and 3.0 mg/l and 3.0, 4.5, and 6.0 mg/l with air-flow rates of 500 and 250 l/min, respectively. Each burn lasted approximately 1 h and 45 min. Spatial uniformity of RP/BR concentration was assessed by sampling aerosol from 12 animal cage sites and the center of chamber. Temporal uniformity was determined by comparing three 10-min collections of aerosol during each burn. Homogeneity/heterogeneity of smoke was assessed based upon measurements of aerosol mass, phosphoric acid (H_3PO_4), and particle size; air quality was determined by routine checks for phosphine (PH_3), hexane (C_6H_{14}), oxygen (O_2), carbon dioxide (CO_2), and CO.

Throughout Task 1, within-chamber temperature and humidity conditions were maintained within acceptable limits. Temperature ranged between 19.5 and 24°C. Relative humidity typically averaged between 39 and 49 percent across the various RP/BR concentrations produced.

Results of the ambient CO evaluation showed that no detectable levels of CO were found within the Laboratory.

Data from the aerosol homogeneity/heterogeneity study confirmed that the aerosol generation system performed well. Although some minor heterogeneities in aerosol distribution were found, aerosol distribution and air quality proved satisfactory for the conduct of animal studies. Key findings were:

Aerosol Mass.-- Analysis of variance showed that aerosol mass collections (gravimetric analysis) were significantly less when sampled from cage sites located on the bottom shelf of the chamber, particularly when drawn from Cage Site 12 (i.e., adjacent to the port used for collecting air quality samples). However, the mean mass collections from bottom shelf cages were only 4 and

1 percent lower than measurements obtained for the top and middle shelves, respectively. Cage Site 12, on the other hand, yielded 10 percent lower average readings than other cages. Because of the Cage Site 12 result, we elected to exclude the use of this cage during exposures of animals to RP/BR smoke in Tasks 2 and 3.

No temporal variation in aerosol mass was detected among 10-min filter collections at 20-30, 35-45, and 50-60 min of each burn.

Phosphoric Acid (H_3PO_4) Titration.-- Titration recoveries of H_3PO_4 generally yielded results similar to those described for aerosol mass; however, actual mg recoveries of acid were only about three-fourths of the aerosol mass weights. Evidence of a dilution effect of RP/BR aerosol for the Bottom Shelf of the chamber was also observed for these data.

Unlike results for aerosol mass, a temporal difference in H_3PO_4 recoveries was found between the 20-30 min filter collection and the 35-45 and 50-60 min collections. Actual mgs of H_3PO_4 for the three 10-min samplings were 22.7, 22.2, and 22.2 mg, respectively. The simplest explanation for this result is that filling of the chamber with aerosol involved a slightly elevated RP/BR concentration for a short period before the metering pump was able to stabilize the aerosol level within the chamber.

Aerosol Opacity.-- Plots of the infrared sensor data used to describe aerosol densities throughout each burn demonstrated that (1) irregularities in smoke opacity characterized most burns and (2) completion of "chamber fill" with RP/BR aerosol typically required 15-25 min.

Particle Size.-- Cascade impactor measurements showed that mass median aerodynamic diameter (MMAD) increased for higher target concentrations under the 500 l/min air flow condition, but were larger and constant at concentrations under the 250 l/min air flow condition. Mean MMAD sizes of aerosol particles ranged between 0.2, and 0.7 µm with 500 l/min air flow; whereas, mean MMAD sizes were 0.7 µm with 250 l/min air flow. Analysis of variance for these data revealed that MMAD values were slightly larger on the bottom shelf. Nevertheless, all MMAD values were greater than 0.1 µm -- the upper threshold of particle size generally considered to be nonexpirable and associated with tracheo-bronchial distress.

Respiratory Gases.-- Mean sample readings for O_2 and CO_2 were always at sufficient levels; these ranged from 20 to 22 percent and from 0.5 to 0.7 percent, respectively.

Contaminant Gases.-- Detections of PH_3 and C_6H_{14} were found in only 18 and 5 percent of samples analyzed, with all detections indicating negligible quantities (i.e., $\leq$ 54 ppb PH_3 and $\leq$ 9 ppm C_6H_{14}) of these gases. Measurable amounts of CO were detected during practically all burns; however, 85 percent of these detections were below the 35 ppm standard established as a 1-h Short-term Threshold Limit Value for human industrial exposures.

FOREWORD

This report is prepared for the Health Effects Research Division, U.S. Army Biomedical Research and Development Laboratory (USABRDL) by staff of the Sections of Bird Damage Control and Predator Studies, Denver Wildlife Research Center (DWRC), Animal Plant and Health Inspection Service, U.S. Department of Agriculture in compliance with Project Order 85PP5847.

The Task 1 Report (Inhalation Equipment Development/Ambient CO Evaluation/Aerosol Distribution and Air Quality Study) is the first in a series of reports (i.e., Tasks 1-3 plus Final Report) required to fulfill the stated Project Order. Contents describe: (1) the set up of an inhalation chamber system for the standardized exposure of small animals to red phosphorous/butyl rubber (RP/BR) smoke, (2) subsequent assessments to monitor the ambient carbon monoxide present in the test area, and (3) determinations of the homogeneous/heterogeneous distribution of RP/BR smoke within the chamber.

Any use of trade names and trademarks within this publication is for descriptive purposes only and does not constitute an endorsement by the Federal Government.

Authors' address: USDA/APHIS/ADC, Denver Wildife Research Center, Building 16, Federal Center, Denver, Colorado 80225-0266.

ACKNOWLEDGEMENTS

We gratefully acknowledge the assistance of the following individuals during the conduct of Task 1:

Jack Moneyhun and Tom Gayle of ORNL provided invaluable technical support during setup of the inhalation chamber system; their advice, coupled with recommendations by Roger Jenkins (ORNL) and Robert Holmberg (ORNL), regarding aerosol generation and analysis was instrumental to the successful completion of the homogeneity/heterogeneity study.

Catherine Aranyi of IIT Research Institute (Chicago, IL) furnished backup RP/BR extruder equipment and the cascade impactor used to collect particle size measurements. Stan Vana and Alan Snelson of this Institute also provided useful technical information concerning aerosol monitoring and aerosol chemistry.

Ken Crane and Stan Gaddis performed most of the modifications needed to improve the humidity, temperature and air-filtration control for the chamber system. Additionally, Ken Crane built the Aerosol Sampling System and was responsible for the operation of the RP/BR generator during the aerosol homogeneity/heterogeneity study. Stan Gaddis also performed some of these RP/BR burns and most of the titration analyses used to measure phosphoric acid deposition on filter disks.

John Gillis performed the gas chromatography analyses for phosphine and hexane contaminates; whereas, Don Elias collected the measurements involved in the Ambient Carbon Monoxide Evaluation.

Jerry Rosencranz (Full Spectrum, Lakewood, CO) provided the technical illustrations of the inhalation and aerosol-sampling equipment (i.e., Figures 2, 3, 5, 8, and 9); and Jeff Homan provided the graphs of aerosol opacity and certain aerosol effects (i.e., Figures 11, 12, 13, 14, and 15).

Statistical consultation and data entry provided by Rick Engeman and Paige Groninger is greatly appreciated.

We wish to also thank Lela Reibe, Ann Grove and Ed Thurston for their help with a variety of administrative and procurement duties that facilitated the start and completion of this work.

Special appreciation is extended to Jean Alleman for handling a myriad of budgetary details associated with the project and painstaking typing of the manuscript.

CONTENTS

CONTENTS (continued)

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

I. INTRODUCTION

Red phosphorous butyl rubber (RP/BR) is the active ingredient in a smoke grenade used by the U.S. Army to conceal troop movements (Burton, Clark, Miller and Schirmer 1982). A major responsibility of the Health Effects Research Division, U.S. Army Biomedical Research and Development Laboratory (USABRDL) is to determine potential health and environmental risks associated with munitions and munitions by-products (i.e., including smokes and obscurants). Data concerning environmental/health impacts of obscurants are crucial to effective Command Decisions affecting the use of military lands and chemicals.

The objective of Project Order 85PP5847 is to delineate potential behavioral and physiological effects of RP/BR smoke upon two wildlife species (see Fig. 1) -- black-tailed prairie dogs (Cynomys ludovicianus) and rock doves (Columba livia). The research entails three tasks: Task 1 -- Inhalation Equipment Development/Ambient CO Evaluation/Aerosol Distribution and Air Quality Study, Task 2 -- Toxicity Rangefinding and Basal Physiological Tests and Task 3 -- Behavioral and Physiological Studies of RP/BR Smoke Exposure.

This report describes the activities and results associated with Task 1. Briefly, contents describe: (1) the setup of an inhalation chamber system for the standardized exposure of black-tailed prairie dogs and rock doves to RP/BR smoke, (2) a brief check of ambient levels of CO within and nearby the building used to house the inhalation chamber, and (3) a study to determine the distribution of RP/BR aerosol and air quality within the chamber.

II. INSTALLATION AND MODIFICATION OF THE RP/BR EXTRUDER AND INHALATION CHAMBER SYSTEM.

Upon authorization of Interagency Agreement (IAG) 14-16-0009-85-965 in September 1985, staff of the Bio/Organic Analysis Section, Analytical Chemistry Division, ORNL began the purchase and assembly of equipment needed to construct a RP/BR Extruder and Inhalation Chamber System. Delivery of 200 RP/BR billets with appropriate chemical analyses was included as part of this IAG. Actual assembly and delivery of the RP/BR System took about four months, with shipment of most components to DWRC occurring by about 7 February 1986. Installation of the System at DWRC was accomplished during the week of 3-7 March 1986.

Following installation of the RP/BR System, approximately three months were devoted to conducting pilot burns. This effort proved invaluable in that a number of System deficiencies were discovered. These included: (1) inadequate air filtration of chamber air supply, (2) inadequate in-chamber humidity regulation, (3) inadequate in-chamber temperature regulation, and (4) acid-caused deterioration of polyethylene (PE), air-intake tubing.

A. Original System And Equipment/Environmental Deficiencies

1. Original System

Figure 2 is a technical illustration of the Original RP/BR Extruder and Inhalation Chamber System installed by ORNL Staff. This System was virtually the same as that designed by Holmberg, Moneyhun, and Gayle (1985) and used by Aranyi (1983a and 1983b).

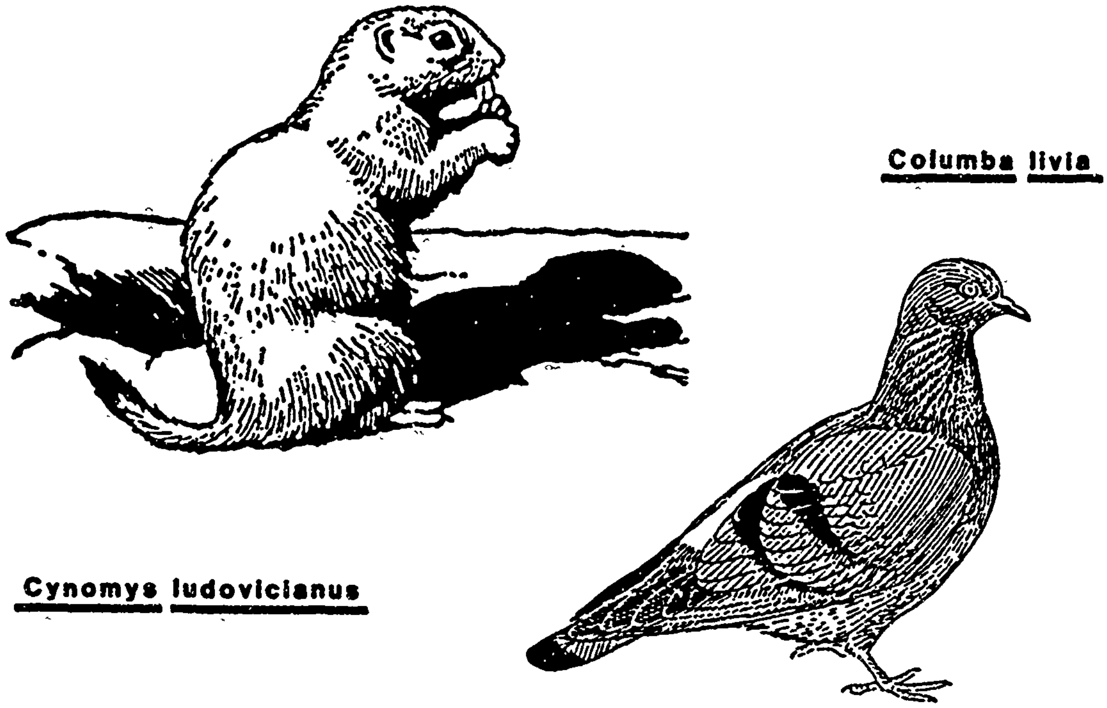


Figure 1. Drawings of a black-tailed prairie dog (Cyanomys ludovicianus) and rock dove (Columba livia) -- the representative wildlife species to be studied for behavioral-physiological effects of RP/BR aerosol under Project Order 85PP5847.

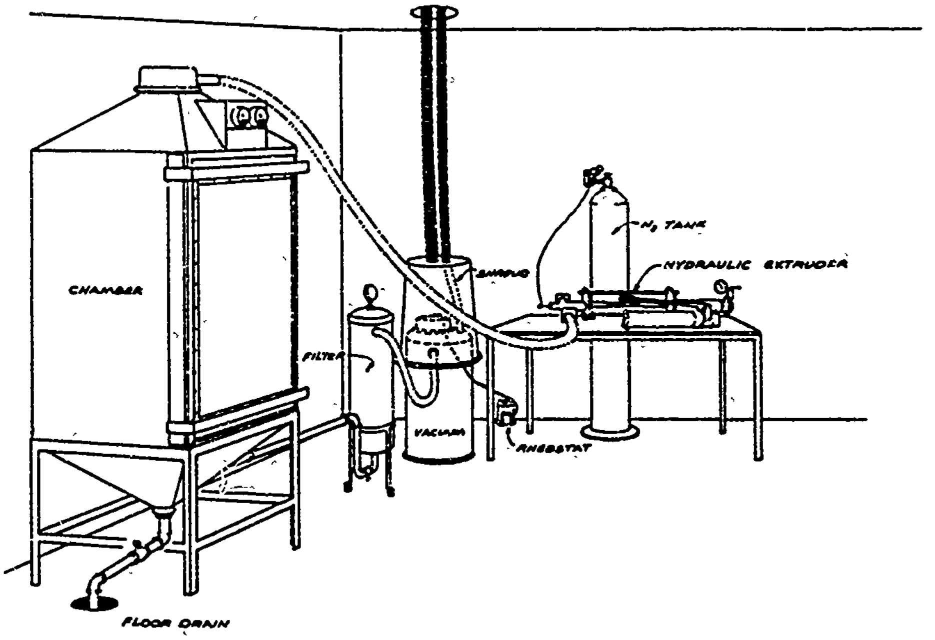


Figure 2. Technical illustration of the RP/BR Extruder and Inhalation Chamber System as installed by ORNL Staff. (Note.-- Components of the System are scaled relative to the perspective, i.e., 2.54 cm equals 0.6 m, but the locations of some components have been drawn to improve the visual display.).

Briefly, the Original System can be viewed as comprised of three subsystems: RP/BR extruder/generator, inhalation chamber, and air-flow/exhaust-filter subsystems. Pliable RP/BR is extruded under hydraulic pressure into a custom-blown glass burn chamber. Ignition of the RP/BR produces a dense white aerosol smoke. Negative pressure caused by a vacuum motor downstream from the inhalation chamber causes the following sequence of air flow -- intake air moves from the burn chamber through plastic intake hosing to the inhalation chamber, through the inhalation chamber to the exhaust line and RP/BR exhaust filters, through the filters to the vacuum and out a ceiling-exhaust vent.

a. RP/BR extruder/generator subsystem

Figure 3 is a schematic drawing of the RP/BR extruder/generator subsystem. Operation requires loading the extrusion cylinder with a formulated RP/BR and hexane mixture. This material is then extruded automatically under approximately 300-1000 psi pressure using a hydraulic cylinder (Enerpac, Butler, WI) connected to a metering pump (Eldex, Menlo Park, CA). The RP/BR bead (approx. 2 mm dia.) is extruded into a custom-blown glass burn chamber where it is ignited to produce the RP/BR aerosol. A small envelope of nitrogen (N_2) gas is bled continuously into the RP/BR extrusion tip to prevent a "backburn" of RP/BR (see N_2 tank and N_2 tubing in Fig. 2).

b. Inhalation chamber subsystem

The RP/BR aerosol moves from the glass burn chamber to the apex of the inhalation chamber via flexible, wire-ribbed PE tubing (approx. 2-in. dia. and 100-mil thick).

The inhalation chamber is a standard stainless steel unit (36 x 36 x 36 in.) with autoclave door (Bertke and Young, Cincinnati, OH). The internal chamber has three shelves containing four (12 x 12 x 12-in) stainless steel wire mesh animal cages each. A polyvinyl-chloride (PVC) drain and valve is plumbed to the bottom of the chamber and connected to a floor drain for flushing the interior of the chamber.

c. Air-flow/exhaust-filter subsystem

At the base of the chamber, aerosol is exhausted via a standard PVC pipe (2 in. o.d.) to a seven-bank, DX-grade filter unit (Balston Filter Products, Lexington, MA). The filters remove over 99 percent of the aerosol and associated contaminants leaving the chamber (Holmberg et al. 1985). Next, air flows via flexible, wire-ribbed PE tubing (2-in. dia.) to the vacuum source (Dayton Electrical Mfg. Co., Chicago, IL) and exits the System via similar tubing through a ceiling vent. A 30-gallon PVC shroud covers the vacuum source to prevent any residual smoke products from entering the room; this shroud is also vented to the ceiling vent via PE tubing.

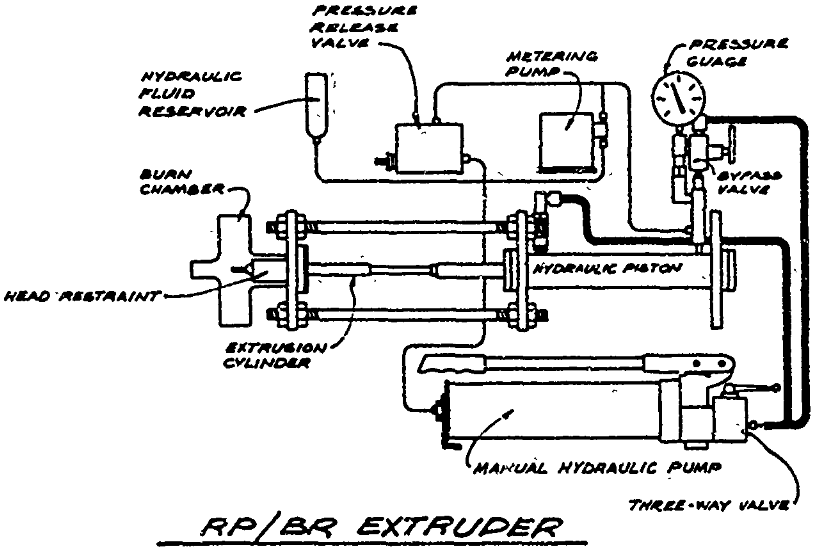


Figure 3. Schematic diagram of the RP/BR extruder/generator subsystem (from Holmberg et al. 1985).

Regarding aerosol movement, air flow rates were calibrated using a Fleisch Pneumotachograph Air Pressure Gauge (OEM Medical, Inc., Richmond, VA). Pressure-calibrated values for this pneumotachograph were: 1mm water equals 92.22 l/min and 5mm water equals 461.1 l/min. With the pneumotachograph mounted (air tight) in the exhaust line between the chamber and the filter unit, numerous determinations were made of the air pressure drop. These recordings allowed precise settings to be made of air flow using the Dayton Vacuum (air mover) with a magnahelic guage (Dwyer Instruments, Inc., Michigan City, ID). The effect of these air flow changes on a second magnahelic mounted to record the air pressure drop across the 1-in. orifice plate between the air intake line and the apex of the chamber was plotted.

Figure 4 is a graph of the air flow by pressure drops recorded during calibration for the orifice-plate magnahelic guage. As shown, air flow rates of 500 l/min and 250 l/min agreed with pneumotachograph settings for these air flow rates (i.e., 1.166 in. water and 0.29 in. water, respectively).

2. Equipment/Environmental Deficiencies

Approximately three months were devoted to the conduct of pilot burns intended to familiarize personnel with the operation of the Original System. Although most subsystems functioned perfectly, several undesirable equipment/environmental conditions were detected. These equipment/environmental deficiencies involved: (a) lack of a humidity-control subsystem for the intake air supply, (b) lack of a HEPA-filter subsystem for the intake air, (c) lack of a temperature-control subsystem for the intake aerosol, and (d) lack of acid-resistant tubing between the RP/BR burn chamber and the inhalation chamber. Each deficiency led to specific modifications of the Original System which eventually improved operation during RP/BR burns.

B. Modified System And Operating Conditions

Figure 5 is a technical illustration of the Modified RP/BR Extruder and Inhalation Chamber System. All modifications were made to the Original System's air supply ahead of the inhalation chamber; the air intake became a "closed, filtered, cooled and humidified source." Except for these modifications, all functions of the Original RP/BR Extruder and Inhalation Chamber System were the same as previously described.

1. Humidification of Intake Air

Air to the laboratory room housing the System was not humidified. Daytime relative humidity (RH) in Denver is variable, i.e., 40 percent average annual RH during daylight h, with monthly extremes of 34 percent for July and September and 49 percent for January and November (NOAA 1985). Additionally, in-chamber RH has been reported to be a determining factor of H_3PO_4 acid concentration and aerosol particle formation with RP/BR (Brazell, Moneyhun, and Holmberg 1984)--maintenance of in-chamber RH between 40 and 60 percent is recommended.

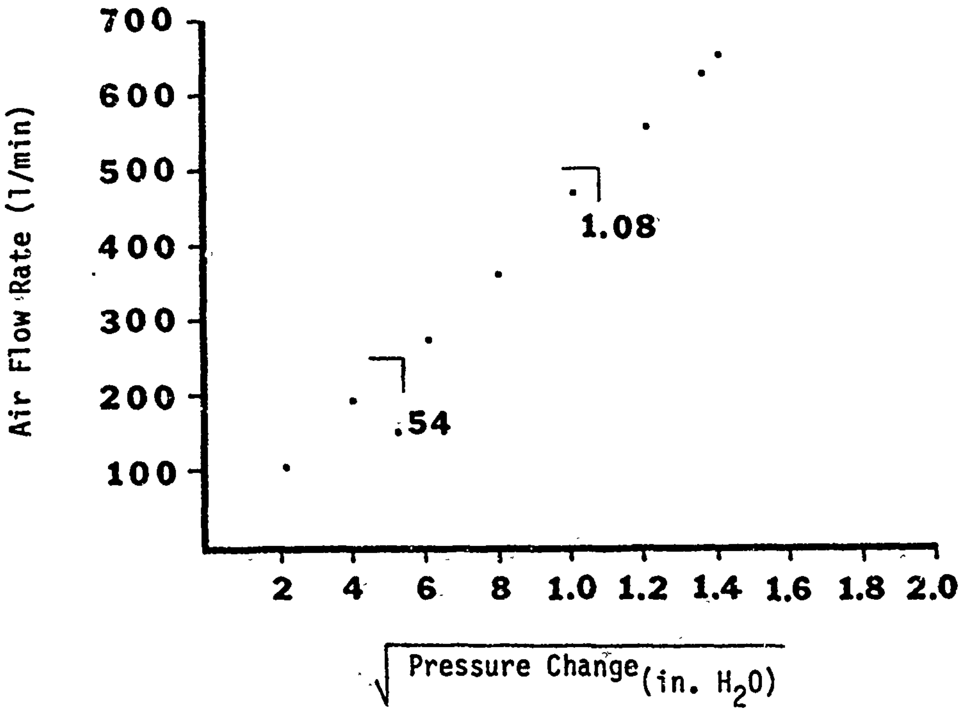


Figure 4. Plot of the air flow rate by pressure relationship for the RP/BR inhalation chamber. (Note.-- Calibration was performed using a pneumotacograph; squared values for 0.54 and 1.08 (i.e., 0.39 and 1.16) are magnahelic settings used to produce a 250 l/min and 500 l/min air-exchange rate, respectively.).

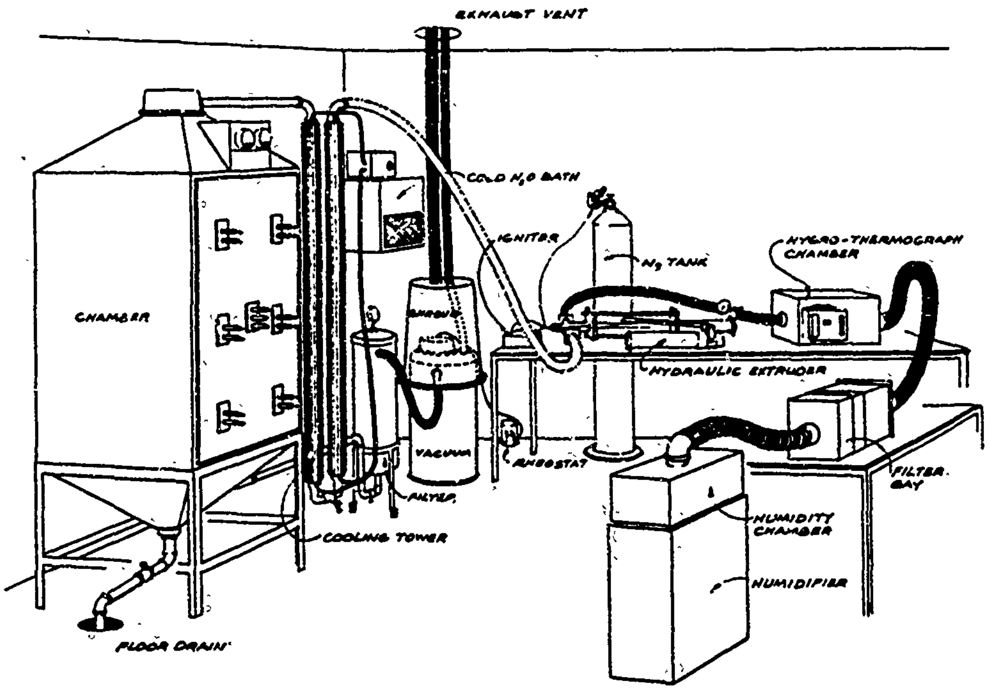


Figure 5. Technical illustration of the RP/BR Extruder and Inhalation Chamber System as modified by DWRC Staff. (Note.-- Components of the System are scaled relative to the perspective, i.e., 2.54 cm equals 0.6 m, but the locations of some components have been drawn to improve the visual display.).

Humidification of the intake air was accomplished using a commercial console humidifier (Emerson Electric Co., St. Louis, MO) with a custom Plexiglas humidity-collection chamber (61 x 30.5 x 30.5-cm) located over the humidifier's exhaust (Fig. 5). Humidity output was regulated manually with a variable auto transformer (Staco Energy Products, Dayton, OH); RH of intake air was monitored by the operator using a Hygro-thermograph (The Bendix Corp., Baltimore, MD) located in the custom-made Plexiglas Hygrothermograph Chamber (62.3 x 31.8 x 32.4 cm). The Hygro-thermograph was calibrated prior to each day's RP/BR burns using a sling psychrometer (Belfort Instrument Co., Baltimore, MD). Humidity was added or not added to the intake air so as to maintain greater than 40 percent RH in the intake air; the addition of humidity rarely produced a chamber RH reading exceeding 60 percent.

A special RH-recording port in the main air exhaust line exiting the inhalation chamber permitted assessment of in-chamber RH. Briefly, an approximately 20-cm length of 10.16-cm (o.d.) clear Plexiglas tubing was plumbed vertically into the exhaust pipe. This tube was sealed at the top with a blank PVC nipple. A standard wet/dry bulb thermometer was inserted into the tubing for RH measurements, and RH was determined using standard charts corrected for altitude (U.S. Department of Commerce, 1965).

Table 1 summarizes in-chamber RH and temperature measurements for a sample of separate wet-/dry-bulb estimates collected during 37 burns in Task 1. As shown, RH averaged over 39 percent for each data set at the respective target concentrations studied. Although certain RH values ranged as low as 30 and 32 percent, these were atypical. Additionally, temperature data reflect the excellent control which the "cooling tower" provided for chamber air--mean temperatures consistently between 20°-30°C.

It should be noted that systematic comparison of chamber-RH values will be made using both the wet-/dry-bulb procedure as well as a dessicant-trap procedure in subsequent tests. The trap procedure consists of gravimetric estimates of water vapor collected using 19.5-cm long by 2.4-cm diameter plastic tubes packed with anhydrous calcium sulfate (Drierite). The ends of the trap are sealed with Teflon flow valves. The trap will be weighed immediately before and after collection of a 10-min aerosol sample (i.e., 1 l/min flow rate filtered both upstream and downstream). Pre- versus post-weight changes in the trap will be used to determine RH based upon known weights of water vapor in a l of saturated air at respective temperatures. Preliminary comparisons of "wet-/dry-bulb" and "dessicant-trap" estimates of in-chamber RH indicate that these procedures yield close agreement.

2. Filtration of Intake Air

Air to the laboratory room housing the System was filtered for large particulates only and provided at the rate of 15 exchanges per h. From the humidity-collection chamber, air was routed through a 1.09-m

Table 1. Ranges and means (± S.D.) for inhalation chamber relative humidity and temperature at each of the Task 1 RP/BR burn concentrations[1].

Air Flow (l/min)	Aerosol Target Concentration (mg/l)	Number of Measurements	Relative Humidity (%)	Temperature (C)
500	0.4	7	Range = 44-56 X(± S.D.)=49.2(±4.5)	Range = 19.5-21.5 X(± S.D.)=20.1(±0.7)
	1.5	7	Range = 40-50 X(± S.D.)=45.0(±3.0)	Range = 20.5- 21.5 X(± S.D.)=21.0(±0.5)
	3.0	5	Range = 30-44 X(± S.D.)=39.1(±4.3)	Range = 21.5 - 24.0 X(± S.D.)=22.2(±0.9)
250	3.0	7	Range = 38-56 X(± S.D.)=46.7(±6.5)	Range = 20.0 - 21.5 X(± S.D.)=20.8(±0.5)
	4.5	7	Range = 37-48 X(± S.D.)=41.3(±3.3)	Range = 20.5 - 23.0 X(± S.D.)=21.5(±0.89)
	6.0	4	Range = 32-48 X(± S.D.)=40.0(±6.7)	Range = 22.5-24.0 X(± S.D.)=23.1(±0.5)

---

[1] Relative humidity data are based upon wet-bulb/dry-bulb thermometer readings at the exhaust port approximately 60-65 min into each RP/BR burn; temperature data are dry-bulb thermometer readings taken in the upper-right portion of the chamber at this time.

length of 10.16-cm (i.d.) flexible PVC tube to a custom-made Plexiglas filter bay (33 x 33 x 15.3-cm). This collected the humidified air prior to passage through an Absolute Filter Unit (Young and Bertke Co., Cincinnatti, OH) which contained a pleated coarse filter (American Air Filter, Louisville, KY), a charcoal bed and a HEPA filter (Mine Safety Appliances CO., Pittsburgh, PA). Next, the humidified, filtered air flowed through a 1.22-m section of 10.16-cm (i.d.) flexible PVC pipe to the Hygro-thermograph Chamber previously described, and on to the RP/BR burn chamber via a 1.57-m section of 7.5-cm diameter PVC tube.

3. Cooling of Intake RP/BR Aerosol

Temperature of the room air supplied to the System was preconditioned to $22^{\circ} \pm 2^{\circ}C$. Control of ambient room temperatures during extreme hot and cold weather was augmented with two supplemental 15,000 BTU window-mounted air conditioners (White-Westinghouse Appliance Co., Pittsburgh, PA) and two Chromalox electric space heaters (Emerson Electric Co., St. Louis, MO), respectively.

In addition to the replacement of plastic intake tubing with a 2.47-m length of 5.6-cm diameter flexible stainless steel pipe to prevent acid-caused deterioration, a U-shaped, 5-m length of 6.35-cm diameter stainless steel pipe was run from the end of the acid-resistant flexible pipe to the apex of the inhalation chamber. The base of the U-shaped column consisted of a custom-molded stainless connector with a 5.6-cm (o.d.) valve and faucet (14.5-cm-long, 1.9-cm i.d.) to permit drainage of condensate. The legs of the U are made of 5.6-cm stainless steel pipe. Each leg of this U-shaped pipe was surrounded by 10.16-cm diameter PVC pipe, with the ends sealed using a special rubber adapter that was clamped to both the stain-less steel and PVC (top and bottom of columns) by standard radiator clamps. This formed a water-tight sealed compartment. Each column was plumbed at the top and bottom with 0.8-cm PE laboratory hose that ran from the reservoir of a cold-water bath (Messgerate-Werk Lauda, West Germany) to the top of each column via a PE hose with a plastic T-joint at the point of insertion into each leg of the cooling column. The water flowed down each column, exiting at the base and joining via another T-joint for return of the water to the bath's reservoir via a 0.8-cm diameter section of PE hose. All water-supply hoses and the PVC water columns were covered with a 2.54-cm-thick foam pipe insulation to enhance cooling.

Use of the temperature-control subsystem was a decision of the operator. Generally, circulation of water was not required when ambient room temperatures were less than $21^{\circ}C$ or low-concentration RP/BR burns were planned (i.e., < 3.0 mg/l). Circulation of $1^{\circ}C$ water through the subsystem allowed inhalation chamber temperatures to be regulated at between 19.5° and $24^{\circ}C$ during all RP/BR burns (see Table 1).

4. Installation of Acid-resistant Intake Air Tubing

As described, the original 2.3-m, 5.08-cm diameter flexible plastic intake tubing was replaced with a 2.47-m length of 5.6-cm flexible stainless steel pipe and the added U-shaped stainless steel piping needed for the cooling column. A 16-cm long, 90-degree stainless steel coupling (5.6-cm i.d.) was welded to this flexible pipe for attachment to the RP/BR burn chamber; and, a 47-cm-long (5.6cm i.d.), 90-degree coupling was welded to the "downstream end" for attachment to the chamber intake. This eliminated acid-caused deterioration of intake lines.

III. CHARACTERIZATION OF THE IN-CHAMBER AEROSOL DISTRIBUTION AND ATMOSPHERE QUALITY

Task 1 also involved a study to (1) assess ambient carbon monoxide (CO) levels within and nearby the Laboratory in which the inhalation chamber was housed and (2) characterize the distribution of RP/BR aerosol within the inhalation chamber and the quality of chamber air.

Because of the close proximity of the DWRC Laboratory to a heavily traveled highway (U.S. Rt. 6), ambient CO levels both within and nearby the Laboratory were monitored at the start of the Project. Measurements of CO were made at selected locations between 11 August and 4 September 1986.

The study to characterize RP/BR distribution within the inhalation chamber was conducted similar to Aranyi (1983a). Aerosol samples were collected from 12 cage sites and the chamber center. Aerosol was collected onto fiberglass filters using air flow pumps (1 l/min) for later gravimetric and titration analysis. Selected respiratory and contaminant gases were measured using either air-tight-syringe volume samples (i.e., gas chromatography) or industrial-hygiene-type analyzer tubes. Following aerosol- and gas-sample collections, aerosol particle size measurements for RP/BR target concentrations of 4.5 mg/l or less were made at respective cage locations and the center of the chamber using a cascade impactor microbalance.

Conduct of the Ambient CO Evaluation and In-chamber RP/BR Aerosol and Air Quality Study involved repeated measurements of a diverse set of aerosol and gaseous variables. Table 2 lists these variables and the analytical technique used to measure each variable.

A. Methods and Procedures

1. Ambient Carbon Monoxide Evaluation

Single or multiple CO measurements were made between 0800 and 1700 h on 16 days between 11 August and 4 September 1986. Measurements were performed in Room 158 (i.e., RP/BR Inhalation-chamber Room), Room 12 (i.e., office of the researcher conducting the measurements), on the South Loading Dock of DWRC, and beneath the U.S. Route 6 Viaduct located near DWRC (i.e., approximately 0.5 km northeast).

Table 2. List of ambient carbon monoxide and in-chamber RP/BR aerosol and atmospheric gas variables plus the respective techniques used to measure each variable.

Variable	Technique
Ambient Carbon Monoxide	
Carbon Monoxide (CO)	Ecolyzer® Carboxyhemoglobin Analyzer System
In-chamber RP/BR Aerosol and Air Quality	
Aerosol Mass	Gravimetric Analysis
Phosphoric Acid Deposition	Titration Analysis
Aerosol Opacity	ORNL Infrared Detector
Aerosol Particle Size	QCM Cascade Impactor
Respiratory Gases	
Oxygen (O_2)	Gastec Analyzer Tube
Carbon Dioxide (CO_2)	Gastec Analyzer Tube
Contaminant Gases	
Carbon Monoxide (CO)	Gastec Analyzer Tube
Phosphine (PH_3)	Gas Chromatography
Hexane (C_6H_{14})	Gas Chromatography

Data collections involved the use of an Ecolyzer® Carboxyhemoglobin Analyzer System (Energetics Science, Hawthorne, NY). This instrument was calibrated once per week using a 20 ppm CO gas mixture (Gastech Inc., Newark, CA). Operation and maintenance of the instrument adhered to procedures provided in the manufacturer's Instruction Manual (Energetics Science, Hawthorne, NY). Each sampling for CO required approximately three min. The Ecolyzer was always positioned at the same measurement site.

2. In-chamber RP/BR Aerosol and Air Quality Study

Delineation of the in-chamber RP/BR aerosol distribution and air quality involved six sets of variables: (a) aerosol mass, (b) phosphoric acid (H_3PO_4) titration, (c) aerosol opacity, (d) aerosol particle size, (e) respiratory gases (O_2 and CO_2), and (f) contaminant gases (CO, PH_3, C_6H_{14}). Throughout the research, each cage contained a quart-sized, cylindrical plastic bottle (13-cm x 9 cm o.d.) to simulate the disruption in air flow caused by the presence of animals.

Figure 6 is a schematic illustration showing the comprehensive data collection schedule used within each RP/BR burn to obtain measurements of environmental conditions, aerosol variables, and atmosphere quality variables. As shown, each burn was comprised of a fixed-length, one-h segment for the assessment of variations in aerosol concentrations, plus an additional 30 to 45 min for particle size determinations.

Data collections also can be viewed as having involved pre-burn, burn, and post-burn events, with certain measurements collected precisely on schedule and other measurements collected within approximate time intervals. Specifically, measurements of equipment/room conditions plus aerosol mass were obtained within plus or minus four min of the times illustrated in Figure 6. Gas chromatography samples (i.e., PH_3 and C_6H_{14}) were collected five min prior to burn and within 30 to 35 min after ignition of RP/BR. Gastec Analyzer Tubes (i.e., CO, O_2, CO_2) were typically drawn anytime during the final 35 min of burn. Particle size determinations were a special case because of the requirement to obtain equipment stability with the Cascade Impactor prior to data collection; these measurements were generally accomplished within 65 to 100 min after ignition of RP/BR (i.e., following the main 60-min data collection).

Both spatial and temporal variations in RP/BR concentration were evaluated using the aerosol mass, phosphoric acid deposition, and particle size variables. Spatial homogeneity/heterogeneity refers to the variations in RP/BR aerosol concentration (mg/l) observed at the 12 cage sites and center of chamber during a continuous one-h burn. Temporal homogeneity/heterogeneity refers to the within-burn variations in RP/BR aerosol concentration (mg/l) observed near the center of chamber during three, 10-min sampling periods (i.e., 20-30, 35-45, and 50-60 min after ignition). For research purposes, an *a priori* criterion of 20 percent maximum deviation of aerosol measurements among cage sites and the center of chamber was used to judge acceptable uniformity of aerosol distribution.

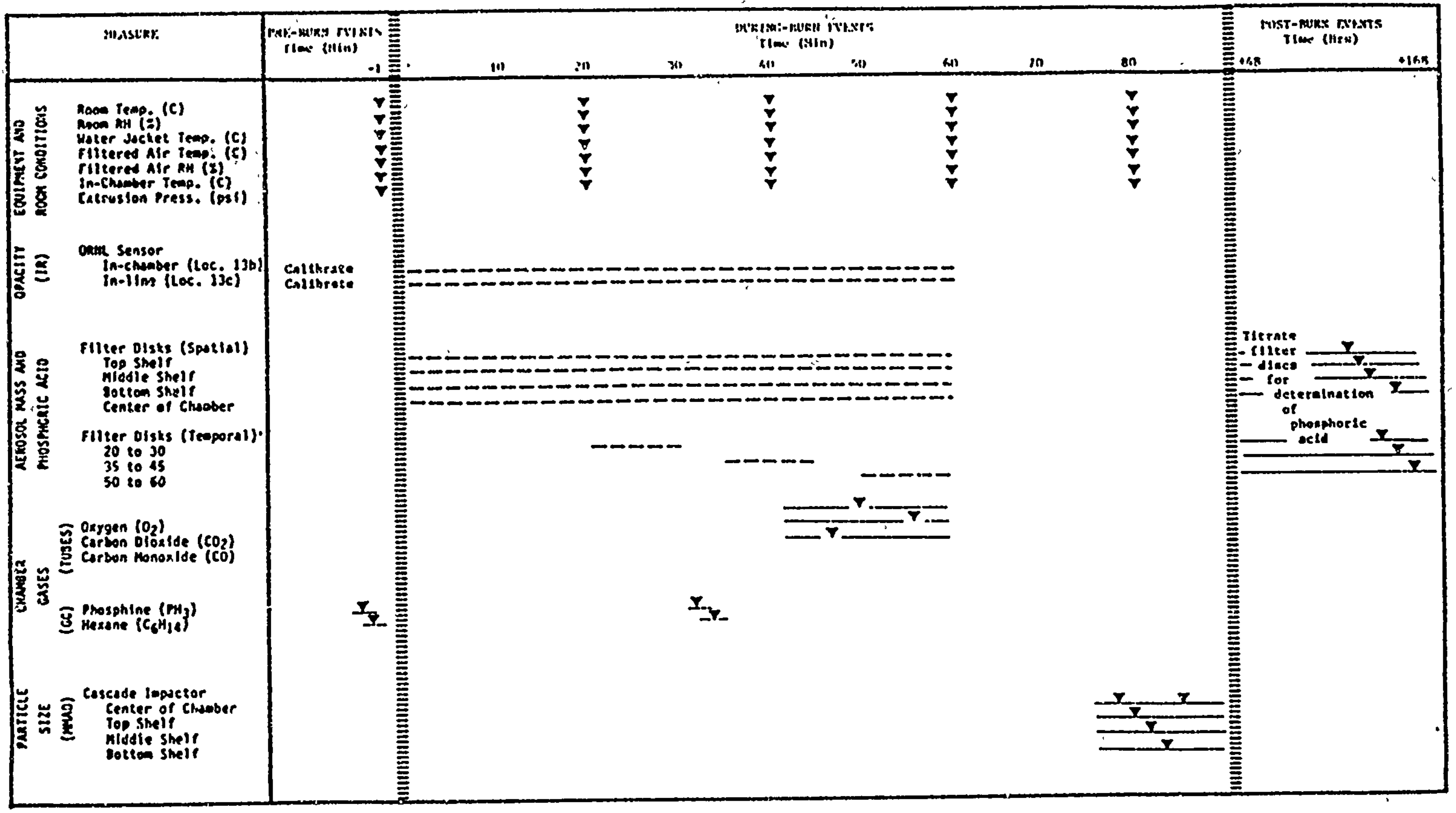


Figure 6. Schematic illustration showing the extensive sampling schedule required for each RP/BR burn. (Note.-- "Arrows" designate discrete type measurements collected at fixed times; "arrows above solid lines" designate discrete-type measurements collected at fixed, but variable, times within the period represented by the line; and "dashed lines" represent long-term, continuous-type sampling of aerosol.).

Regarding spatial homogeneity/heterogeneity, each burn involved the collection of aerosol onto filter discs from four chamber locations per RP/BR burn (see Fig. 7). During each burn, one sampling tube was inserted into a designated cage site on the Top, Middle, and Bottom Shelf; whereas the fourth tube was always projected to the center of chamber (location 13a). Actual cage and center of chamber sampling locations are illustrated in Figure 8. The use of identical length stainless steel sampling tubes and flexible plastic tubing ensured that aerosol traversed the same distance from each sampling site to a respective collection filter. The sampling order of cage sites was randomized, but then fixed for all cells of the design (see Table 3). A series of four burns were required to sample the aerosol concentrations and particle size distributions characterizing the 12 cage sites. Opacity measurements were always monitored at the center of chamber (i.e., sampling tubes 13b and 13c).

Only two aerosol measurements were used to quantify temporal homogeneity/heterogeneity -- aerosol mass and H_3PO_4 deposition. Because stable RP/BR concentrations would not occur until the aerosol equilibrated within the chamber, filter collections were scheduled during the latter part of each one-h burn period. Filter disc collections were drawn from the center of chamber (sampling tube 13c) between 20 to 30, 35 to 45, and 50 to 60 min of each burn.

a. Aerosol mass

This refers to the mass of RP/BR aerosol, particulates, water vapor, etc. per unit volume (wt/v) of chamber atmosphere. It is an index of the amount of material present in the inspired chamber air.

Collection of RP/BR aerosol samples required construction and assembly of special aerosol-sampling equipment (Fig. 9). In order to sample aerosol from respective chamber locations, the autoclave-type door to the inhalation chamber was removed. A Plexiglas panel (127- x 87- x 0.9-cm) was cut to fit the chamber doorway. A border of nonconductive (dead-air space) rubber insulation (2.0-cm wide x 2.5-mm thick) was cemented to the internal side of the panel and the external surface of the doorway to create an air-tight seal when joined. The door was mounted to the chamber doorway using 16 commercial spring clamps (i.e., spaced at about one ft. intervals along the door's perimeter).

Detailed measurements of the cage sites and the geometric center of the chamber were used to project locations for 15, 2.0-cm-diameter drill holes onto the Plexiglas panel. Six pairs of drill holes corresponding to the two cages located in the left and right halves of each shelf were made in the panel (see Fig. 9). Each pair of holes was aligned vertically (2.7-cm center-to-center distance) on the horizontal midpoints of the front six cages. The lower drill holes of each pair corresponded to an insertion point approximately 15-cm above each shelf for the front six cages; whereas, the upper drill holes corresponded to an insertion point approximately 17.7-cm above each shelf for the rear six cages. Three additional vertically-placed drill holes were cut into the Plexiglas (2.7-cm center-to-center distance) such that the middle port corresponded

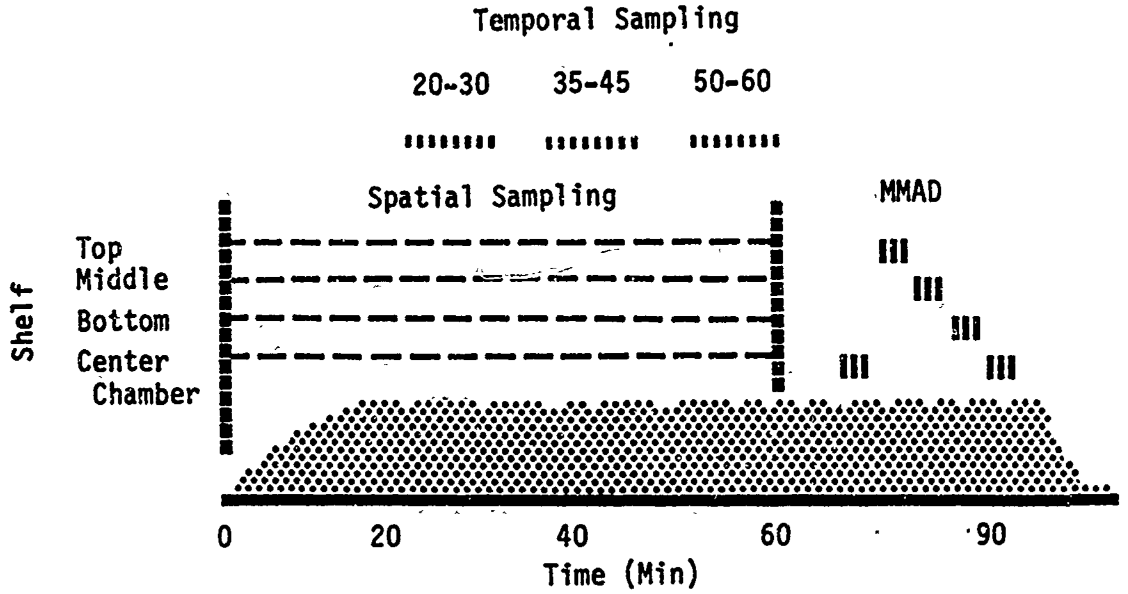


Figure 7. Schematic illustration depicting aspects of the spatial- and temporal-sampling scheme used for the collection of aerosol mass, phosphoric acid deposition, and particle size data (MMAD) during each RP/BR burn. (Note.-- Spatial sampling was accomplished by collecting four, 60-minute filter pads from a cage location on the Top, Middle, and Bottom Shelves of the inhalation chamber, plus the center of the chamber. Temporal sampling was accomplished by collecting three, 10-minute filter pads from the center of the chamber between 20-30, 35-45, 50-60 min. Particle size measurements were made during approximately a 30-min burn-continuation period following the 60-min spatial-sampling period.).

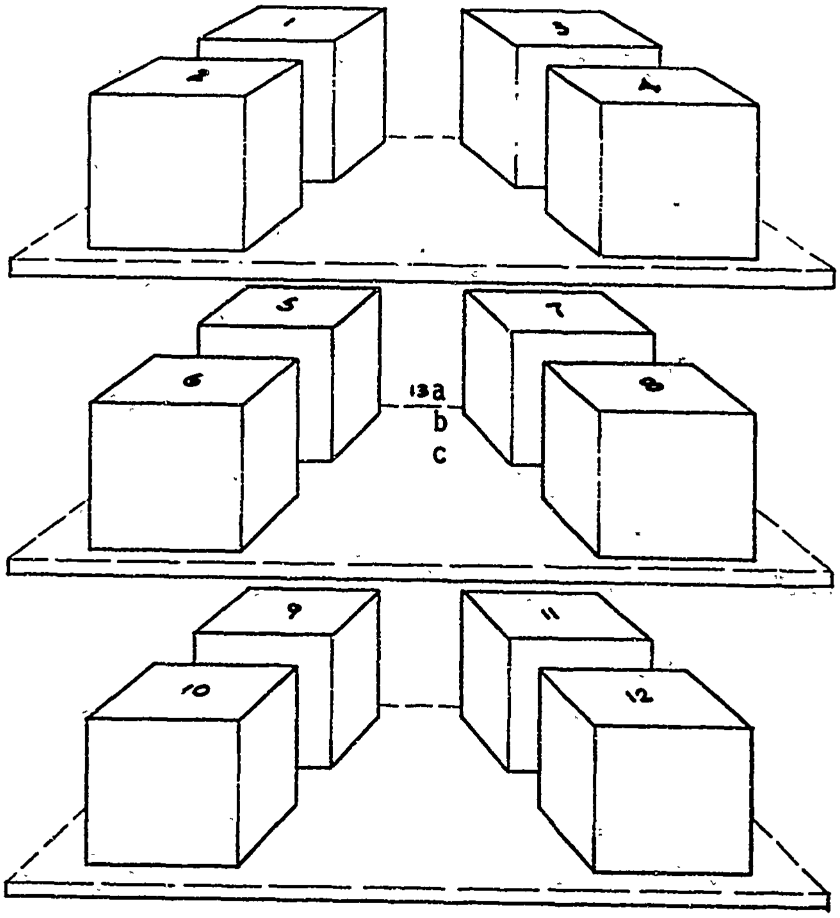


Figure 8. Schematic illustration showing the interior of the inhalation chamber with 12 cage and three center of chamber aerosol sampling locations.

Table 3. Sampling order for cage sites and center of chamber used to assess spatial homogeneity/heterogeneity of RP/BR aerosol concentrations and particle size distributions.

	RP/BR SAMPLING LOCATION			
	BURN			
SHELF	1	2	3	4
TOP	3	1	4	2
MIDDLE	5	6	8	7
BOTTOM	12	10	9	11
CENTER CHAMBER	13a	13a	13a	13a

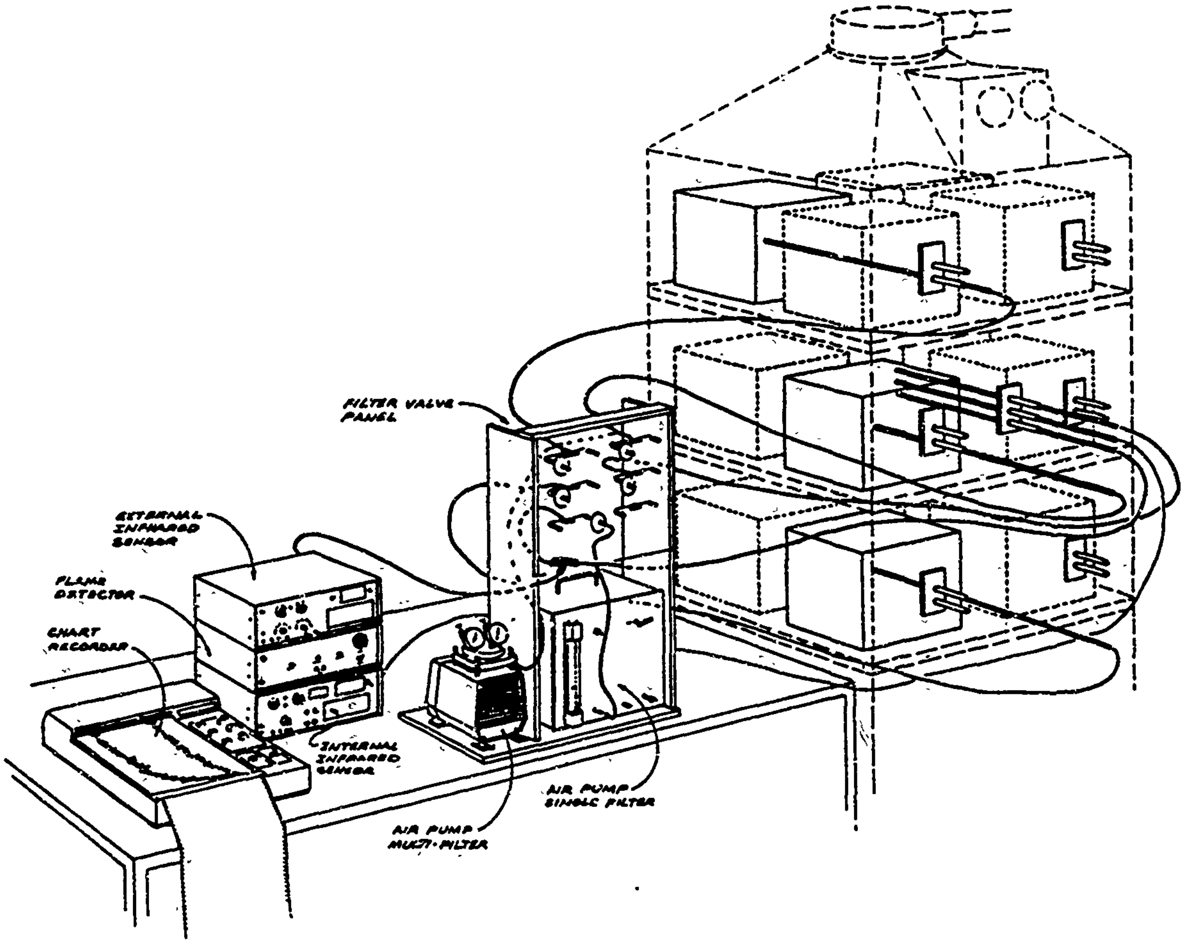


Figure 9. Technical illustration of the Aerosol Sampling System. (Note.-- The within-chamber and in-line infrared sensors with chart recorder are not part of the System.).

to the straightline projection from the geometric center of the chamber. Each hole was plumbed with a 2.1-cm long PVC connector and threaded-chrome nipple/reducer (0.9-mm i.d.) on the inner side. The external PVC connector was then plumbed with a 12.7-cm long by 2.54-cm-diameter PVC pipe that was again threaded with a chrome nipple/reducer (0.9-mm i.d.). A PE bevel was placed inside the reducer to hold and seal the stainless steel sampling tubes. This door design proved air tight when tested with helium using a sensitive gas detection meter.

Identical lengths (1-m-long x 7.5-mm i.d.) of stainless steel tubing served as rigid sampling tubes for drawing aerosol from designated chamber locations. For each RP/BR burn, three of these tubes were inserted through the PVC pipe in the Plexiglas panel to three designated cage sites (i.e., one per shelf per burn); and, three tubes were inserted through the panel to sampling zones near the center of the chamber. Identical lengths of non-toxic plastic tubing (154.5-cm-long x 0.8-cm i.d.) were connected to the external ends of five stainless steel tubes and run to the common line of separate stainless steel tri-valves (Whitey Co., Highland Heights, OH) on a custom-built sampling-valve panel (Fig. 9).

The sampling-valve panel was a custom-built wooden stand containing a Plexiglas mounting panel (40.5 x 42.5 x 0.6-cm) that had six stainless steel tri-valves attached to the Plexiglas at roughly 22-cm center-to-center horizontal distances. Three of these were used to regulate RP/BR aerosol flow from designated cage sites (i.e., one per shelf) via the sampling tubes; two were used to regulate aerosol flow from the center of the chamber during spatial and temporal sampling; and one was a main control valve regulating the other five valves. These tri-valves were used to direct vacuum pressure across a fiberglass filter disc during aerosol data collections.

Aerosol collections were made using 45-mm-diameter acrylic filter holders (Phipps and Bird Co., Richmond, VA) and 45-mm-diameter Borosilicate-glass-filter discs (Phipps and Bird Co., Richmond, VA). A 45-mm-diameter Buna-n Rubber O-Ring was used to seal the filter discs within the filter holders. Certain of the filter holders were machined and threaded to a 0.625 in. diameter at the center to hold a Millipore Limiting Flow Orifice (Millipore Corp., Bedford, MA); these Orifices were guaranteed to provide a uniform flow rate (i.e., 1 l/min ± 5%) for sampled aerosol. Filter holders were subsequently mounted onto the downstream legs of respective tri-valves on the sampling-valve panel. In-line sampling connections were made air tight with Teflon tape.

Vacuum pressure for the aerosol sampling system was created by two different air-flow pumps. Four filter holders containing the Millipore Limiting Flow Orifices were connected to a Millipore Vacuum Pressure Pump (Millipore Corp., Bedford, MA) through four of the stainless steel tri-valves using lengths of 0.3-mm i.d. plastic tubing. These filters corresponded to the three cage-site-sampling tubes and the center-of-chamber-sampling tube. One unmodified filter holder was connected to a custom-built vacuum pressure pump (ORNL, Oak Ridge, TN) using similar tubing. This filter holder corresponded to the bottom sampling tube projecting from the center of chamber; and, it was also calibrated to provide a uniform flow rate (i.e., 1 l/min ± 5%) for the collection of RP/BR aerosol during three successive 10-min periods of each burn.

Aerosol mass of RP/BR was measured using a gravimetric analytic technique. As indicated, RP/BR aerosol was collected on 45-mm diameter fiberglass discs using the aerosol sampling system. Filter discs were stored in a ventilated Nalgene bottle (1l) and conditioned to the chamber-room atmosphere for at least 24 h prior to use. Filter holders were thoroughly dried and handled with low-static cotton or rubber gloves at all times. Filter discs were loaded into holders using clean metal tweezers. All aerosol samples were collected at constant flow rates (1 l/min).

Conduct of the gravimetric technique involved weighing the assembled filter holder, filter disc, and O-ring on a Sartorius Analytical Balance (Brinkman Instruments Co., Westbury, NY) immediately prior to and following aerosol collection. The difference between the pre- and post-weight of respective filters was the mass of the aerosol accumulated during the sampling period.

b. Phosphoric acid (H_3PO_4) titration

Titration analysis was used to determine the amount of H_3PO_4 contained on filter-disc collections of RP/BR aerosol. This measure is considered representative of the total phosphorous content of the aerosol (Burton et al. 1982).

Following gravimetric analysis, filter discs collected from respective sampling locations were deposited into covered plastic Petri dishes (Miles Laboratories Inc., Naperville, IL). Single discs were collected at RP/BR burn concentrations below 4.5 mg/l; whereas, two successive discs (30-min each) were collected at concentrations of 4.5 and greater (i.e., to avoid saturation of discs). A number of unused, "blank" discs were also stored in this manner for purposes of quality assurance analyses. Petri dishes with filter discs were stored in a ventilated cabinet for between 48 and 168 h to allow for complete hydrolysis of the acids on the discs. This aging process was used because at the time of aerosol collection there are several acids present (i.e., H_3PO_4, polyphosphorics and cyclic polyphosphorics). With storage, the larger phosphoric acid molecules hydrolyze to H_3PO_4 (Burton et al. 1982).

Titration analysis involved the use of a Radiometer DTS-800 Multititration System (Radiometer America Inc., Cleveland, OH). Upon removal of the filter from storage, each disc was extracted using 60-ml of boiled deionized water in a 400-ml glass beaker agitated with a magnetic stir bar for 10 min. When two pads were involved, the solutions were combined after extraction. Subsequently, a 20-ml sample of the extracted solution was pipeted into a 22 to 45-ml disposable sample cup (Radiometer America Inc., Cleveland, OH) and titrated using either a 0.1N or 0.01N sodium hydroxide (NaOH) titrant (Fischer Scientific, Fair Lawn, NJ). The titrator was programmed to calculate mg of H_3PO_4 in the total extracted sample by inflection-point titration. The formula used to make the calculation for single filter pads is as follows:

$$\text{Total mg } H_3PO_4 = \frac{(\text{ml titrant to 1st inflection})(\text{meq/ml titrant})}{\text{ml of sample}} \times \text{Factor}$$

where, Factor refers to a unique titration constant based on (3 x ml of sample x formula wt. in mg/meq of H_3PO_4) or (3 x 20 x 98). Assuming that hydrolization to H_3PO_4 is complete, the first inflection point is a direct measure of the total number of phosphorous atoms (i.e., mg of H_3PO_4) present in the extracted sample. If only H_3PO_4 (not other polyphosphoric acids) is present, equal amounts of NaOH are required to titrate to the first as from the first to the second inflection point. Comparisons of H_3PO_4 standard solutions indicated that a storage period of at least 48 h for filter discs allowed sufficient hydrolization--all analyzed samples yielded equivalence of first and second inflection points.

c. Aerosol opacity

The density of RP/BR aerosol within the inhalation chamber was monitored continuously during the burns using one or two versions of the ORNL Aerosol Sensor (Higgins, Gayle, and Stokely, 1978; Holmberg et al. 1985). These sensors consisted of a light-emitting diode mounted beside, but optically separated from, a phototransistor. Aerosol particles scatter the infrared light, raising the mv output of the transistor and providing an analog record onto a chart recorder (Cole Palmer Instrument Co., Chicago, IL). The first version (used during all Task 1 RP/BR burns) was a standard version; it consisted of a 16-cm-long metal probe which was inserted into the stainless steel sampling tube (location 13b) until it protruded near the center-of-chamber (see Fig. 9). This sensor was connected to the sensor control unit and two-pen chart recorder. The second version (used concurrently with the first during approximately the final two-thirds of the Task 1 burns) consisted of a photosensor mounted in the center of a "T-shaped" segment of 0.95-cm diameter rubber tubing (i.e., 17-cm-long). This segment of rubber tubing was inserted into the sampling line running from the bottom sampling tube (location 13c) from the center of the chamber to the stainless steel tri-valve used with the ORNL flow pump (see Fig. 9.). It measured the opacity of aerosol flowing through this sampling tube.

Because the phototransistor section of the sensor is responsive to room light, especially wavelengths in the near infrared part of the spectrum, the Plexiglas sampling panel used to cover the door of the inhalation chamber had to be darkened. This was accomplished by painting sections of the panel black (i.e., surrounding the pipes plumbed into the panel for insertion of aerosol sampling tubes as well as the perimeter of the sampling panel). Velcroix strips were glued to these blackened areas of the panel approximately 4-cm from the painted edges. A template of approximately 7-mm-thick cardboard was then cut to match the panel with cutouts for the sampling tubes; and, Velcroix strips were attached to matching surfaces. This produced a totally darkened interior of the chamber when the cardboard was positioned over the Plexiglas and appropriate Velcroix strips tamped together.

Each sensor probe has its own characteristic sensitivity due to manufacturing variations and must be individually calibrated against an actual gravimetric aerosol estimate. This was done by comparing gravimetric filter samples of the aerosol taken over a known time period to the integrated sensor response provided by the digital counter on the readout module. However, for current purposes, opacity data were not quantitatively analyzed; rather, chart paper plots of the sensor measurements were handled descriptively--the infrared recordings were used to document the RP/BR burns.

d. Aerosol particle size

Estimates of the mass median aerodynamic diameter (MMAD) of RP/BR aerosol particles were made during a 30- to 45-min, post-aerosol collection period. These measurements were made using a Piezo-electric Quartz-Crystal-Micro-Balance (QCM) Cascade Impactor (California Measurements Inc., Sierra Madre, CA). All MMAD measurements were conducted according to the manufacturer's instructions.

For current purposes, the MMAD sampling procedure involved five successive measurements of RP/BR aerosol from each of three cage sites (i.e., 1/shelf), with two center of chamber readings (i.e., location 13a) obtained for purposes of reliability comparison at the start and end of the sampling order. The QCM Cascade Impactor was allowed to warm up and stabilize according to the manufacturer's instructions. Quartz crystals were also tested for the proper stability range (± 1 Hz) prior to each sampling period and cleaned, greased, and re-calibrated as required. Aerosol sampling involved connecting the respective stainless steel sampling tube to the ORNL flow pump (i.e., 16 l/min flow) via a high concentration slide valve on the QCM Cascade Impactor. The stainless steel tube was connected to the slide valve with a 105-cm-length (0.75-cm i.d.) of non-toxic, plastic tubing; whereas, the connection from the Impactor to the flow pump involved a 130-cm-length (0.30-cm i.d.) of plastic tubing with an in-line 45-mm-diameter aerosol filter to protect the pump's diaphragm from phosphoric acid. The aerosol sampling rate was 4.3 l/min. Aerosol for each respective sample was circulated a minimum of 90 sec prior to intake of a 10-sec sample to be injected into the stack of quartz crystals using the slide valve. Each MMAD reading then required an additional 90 sec to distribute the 300 μl aerosol sample for crystal sensing as governed by the Impactor. Upon completion of this temporal sequence, paper tape output containing (1) a histogram of the relative mass of aerosol detected for each stage, (2) the number of 300 μl samples taken (i.e., typically N=1), (3) the total aerosol mass (mg/m^3) accumulated on all stages, and (4) the change in frequency (Hz) and mass accumulated for each stage (mg/m^3) was printed. Actual determinations of MMAD and geometric standard deviation for each sampling and RP/BR burn were completed using a graphical analysis procedure (Log particle size diameter versus probability of sampling detections) outlined by Chuan (1986).

e. Respiratory gases

Oxygen and CO_2 levels within the inhalation chamber were measured regularly throughout Task 1. This involved use of the Gastec Gas Detection System (Gastec Inc., Newark, CA)--a standard industrial-hygiene-type analyzer tube and pump system.

The Gastec System consists of a 19-cm long (4.5-cm o.d.) cylindrical vacuum pump capable of drawing either 50- or 100-ml (± 5%) volumes of air per stroke. The end of the pump contains a 2-cm long (0.5-cm i.d.) rubber inlet for insertion of a 14-cm-long (0.5-cm o.d.) gas analyzer tube. Specific gas analyzer tubes are produced for the detection and measurement of over 160 toxic and non-toxic gases.

For current purposes, the Gastec pump was cleaned and maintained in accordance with the manufacturer's instructions (see Appendix A). The vacuum draw was calibrated approximately once every 60 tube collections using a standard bubble-flow meter (Supelco Inc., Bellefonte, PA 16823); calibration also adhered to the manufacturer's recommended procedure and was judged acceptable to ± 5% of 100 ml volume per min. Oxygen and CO_2 were measured using Gastec Analyzer Tubes ÷31 (% O_2) and 2LL(ppm CO_2), respectively.

Sampling was conducted between 30 to 60 min following ignition of the RP/BR using a sampling port located on the right side of the inhalation chamber. This threaded port was fitted with a PVC plug having a 0.75-cm-diameter center hole also fitted with a cone-shaped rubber stopper as an air seal. Upon selection of the specific analyzer tube, the ends were broken off and the tubes inserted into the rubber inlet of the Gastec pump (i.e., Tube 2LL inserted directly, but Tube ÷31 mounted ahead of an appropriate "scrubber tube" that was inserted into the pump). The analyzer tube was then inserted through the sampling port, and a seal was created between the PVC sampling port and rubber inlet of the pump by manual pressure. Next, the required volume and number of pump strokes were performed as precribed on the specifications sheet (see Appendix A).

Upon completion of the sampling sequence, the tube was withdrawn, the sampling port sealed with the rubber cone-shaped plug, and the farthest migration of dye determined using the graduated markings on the side of the analyzer tube. Dual, independent estimates of dye migration were made for a number of the analyzer-tube collections by two staff conducting each RP/BR burn. Actual percent O_2 and ppm CO_2 were corrected for atmospheric pressure at 1646 m (5400 ft.) elevation based upon the following formula:

$$\text{Corrected Analyzer Tube Value} = \text{Actual Tube Value} \times \frac{760 \text{ mm Hg}}{628 \text{ mm Hg}}.$$

f. Contaminant gases

Periodic checks for three contaminant gases were performed: CO, phosphine (PH_3), and hexane (C_6H_{14}).

Carbon monoxide within the inhalation chamber was measured regularly throughout Task 1 using the Gastec System (see Appendix A). Sampling procedures were identical to those described for O_2 and CO_2. Gastec Analyzer Tube ILL (ppm CO) was used, with each ppm reading of CO again corrected using the formula cited for the respiratory gases.

Periodic checks of pre- and post-RP/BR ignition air samples for the presence of PH_3 and C_6H_{14} were analyzed using gas chromatography (GC). Phosphine analyses were conducted using an MT-220 GC equipped with a flame photometric detector (phosphorous filter, 526 nm) and an 18 x 1/4-in aluminum column packed with Chromasorb 102. Hexane samples were analyzed using a GC equipped with a mass selective detector and a 12 m x 0.2 mm i.d. capillary column (methyl silicone, 0.32 µm film thickness).

Collection of aerosol samples were made using 5 ml and 50 µml Pressure Lock Gas Syringes (Alltech Associates Inc., Deerfield, IL). A 51-cm length (0.6-cm i.d.) of rigid plastic hosing was permanently inserted into the inhalation chamber through the second sampling port (i.e., 80-cm from base) on the right-front side of the chamber. A rubber septum was glued into the external portion of this hose. The internal portion of the hose was curved towards the center of the chamber and rested on top of the second tier of animal cages. A cylindrical PE plug made the hose an airtight attachment when not in use.

To collect a sample, a 130-cm length (0.30-cm i.d.) of flexible plastic tubing with Teflon tape was inserted onto the external end of the plastic sampling hose. This was attached to the ORNL vacuum pump, with a 45-mm filter assembly included in-line to protect the pump from H_3PO_4. Chamber air or aerosol was evacuated through the hose at a rate of one l/min. After a minimum of two min had elapsed, the Pressure Lock Syringe was inserted into the rubber septum and a volume of air or aerosol extracted.

Immediately following extraction of a 5 ml and 50 µl syringe from the sampling septum, the samples were analyzed by GC. Estimates of ppb PH_3 and ppm C_6H_{14} were determined using a "sample versus PH_3- and C_6H_{14}-standard-comparison procedure." The GC outputs of air or aerosol samples were compared to a 12-14 ppm PH_3 in nitrogen (Scientific Gas Products, Fremont, CA) and a 100 ppm C_6H_{14} in nitrogen gas concentration (Scott Specialty Gases, San Bernadino, CA). Respective excursions in GC output were then used to compute detection quantities of the PH_3 or C_6H_{14} in the air or aerosol samples.

B. Experimental Designs and Data Analyses

1. Ambient Carbon Monoxide Evaluation

No formal experimental design was used in the collection of ambient CO data. As stated, single or multiple measurements of ppm CO were made between 0800 and 1700 h on 16 days between 11 August and 4 September 1986 at three locations within the DWRC Laboratory and at a major automobile-traffic artery nearby. The ppm CO readings were summarized descriptively (i.e., means, standard deviations, ranges) to characterize relative amounts of CO associated with each sampling location.

2. In-chamber RP/BR Aerosol and Air Quality Study

A total of 64 RP/BR burns were conducted as part of the In-chamber RP/BR Aerosol and Air Quality Study. Of these, 48 burns served as the final balanced data set for statistical analyses involving aerosol mass, H_3PO_4 titration, and aerosol opacity. Sixteen burns were repeated due to a variety of problems associated with the initial data collections (i.e., incorrect sampling procedures, equipment failures). Statistical analyses for the particle size data were also based on the final 48-burn data set, but additional missing data for these burns led to the use of procedures involving unbalanced data. For purposes of air quality assessments, respiratory and contaminate gas measurements from all 64 burns were included in summary statistics.

Spatial variation in aerosol mass, phosphoric acid deposition, and aerosol particle size within the inhalation chamber was assessed using a three-way, fixed-effects model (Winer 1971). The specific design was a 6 (RP/BR Extrusion) x 4 (Burn) x 3 (Shelf) analysis of variance (ANOVA), with Burn and Shelf treated as repeated measures factors. This design confounded air-flow rate (i.e., 250 and 500 l/min) within the Extrusion Factor, but provided for an exact solution of the variance attributable to the 12 cage sites by inclusion of a Burn x Shelf interaction term. Independent RP/BR billets used to produce the smoke for each burn were viewed as the basic elements of the design.

Extrusion rates of RP/BR were selected arbitrarily to approximate high, moderate, and low chamber concentrations at the 500 and 250 l/min air flow rates. Calibration of the extrusion pump (i.e., metering pump; see RP/BR Extruder/Generator Subsystem) was accomplished based upon a series of initial 10-min filter pad collections of aerosol mass for gravimetric analysis. Specifically, two RP/BR billets were burned (August 25 and 26, 1986) at a variety of extrusion rates with 500 l/min air-flow, and two billets were burned (September 3, 1986) at various extrusions with 250 l/min air flow. Following stabilization of the ORNL sensor recordings, a 10-min filter pad was collected and weighed. Subsequently, the extrusion setting was altered and the process repeated. These gravimetric determinations were plotted (see Fig. 10) and used to select micrometer settings for the extrusion pump that were equal to "target concentrations" of 0.4, 1.5, and 3.0 mg/l for 500 l/min and 3.0, 4.5, and 6.0 mg/l for 250 l/min air flow rates, respectively. These "target con-

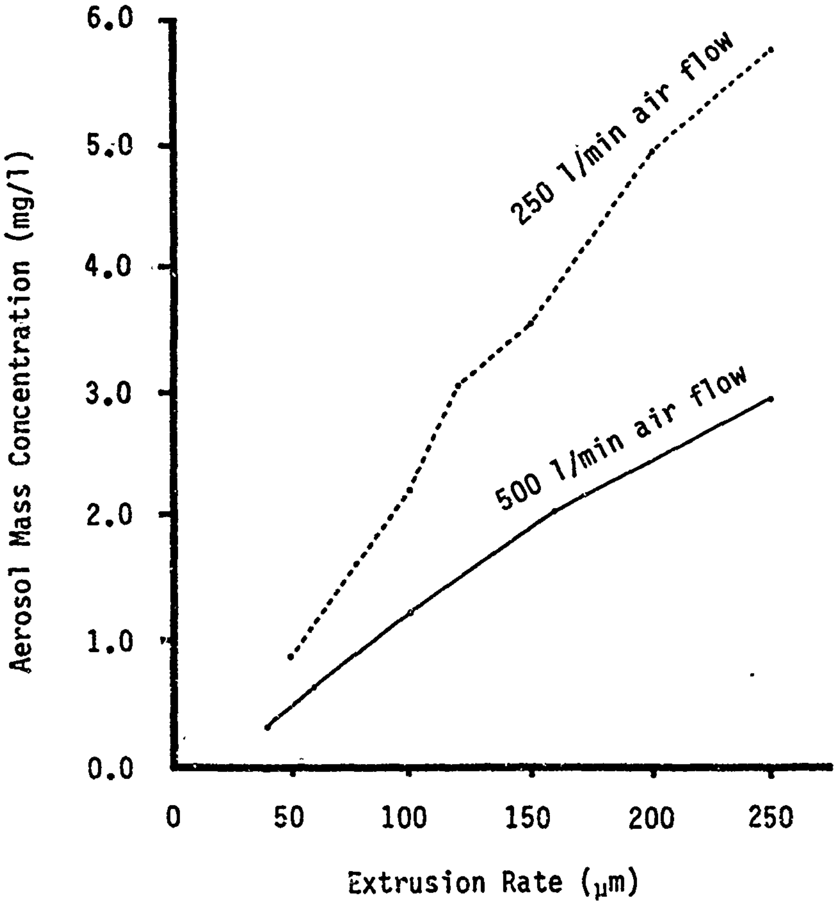


Figure 10. Graph of the 10-min filter pad estimates (gravimetric analysis) of RP/BR aerosol concentration used to calibrate the hydraulic extruder pump; appropriate settings were selected to produce "target concentrations" of 0.4, 1.5, and 3.0 mg/l and 3.0, 4.5, and 6.0 mg/l RP/BR levels within the chamber at the 500 and 250 l/min air flow rates, respectively.

centrations" corresponded to micrometer settings of 43, 123, and 243 for the 500 1/min air flow rate and 125, 180, and 270 for the 250 1/min rate.

The spatial distribution of aerosol within the chamber was examined using six dependent variables: (1) aerosol mass (mg of aerosol), (2) H_3PO_4 deposition (mg of titrated H_3PO_4), (3) aerosol particle size (µm mass median aerodynamic diameter), (4) difference in aerosol mass between respective cage sites and chamber center within burn ($\pm$ percent difference in mg of aerosol), (5) difference in H_3PO_4 deposition between respective cage sites and chamber center within burn ($\pm$ percent difference in mg H_3PO_4), and (6) difference in particle size between respective cage sites and the final center chamber reading within burn ($\pm$ percent difference in µm mass median aerodynamic diameters). Two complete replications in a balanced design were used for the aerosol mass and H_3PO_4 deposition variables; whereas, two incomplete replications in an unbalanced design were analyzed for the particle size variables. The aerosol mass and the H_3PO_4 data were analyzed using the PROC ANOVA Program of the Statistical Analysis System (Helwig and Council 1979); whereas, the particle size data were analyzed using the PROC GLM Program of this System (Helwig and Council 1979). Significant effects were further analyzed using the post-hoc Duncan Multiple Range Test (Waller and Duncan 1969).

Temporal variations in aerosol mass (mg) and H_3PO_4 deposition (mg) were assessed using a three-way, fixed-effects model (Winer 1971). This design was a 6 (RP/BR Extrusion) x 2 (Replication) x 3 (Sampling Period) analysis of variance, with Sampling Period treated as a repeated measures factor. Replication refers to the first and second set of 24 burns that made up the final data set; this factor was included in analyses of effects due to the relatively greater degrees of freedom with these data (i.e., the spatial data exhausted additional degrees of freedom due to the Burn and Shelf Factors). Significant effects were further assessed using the post-hoc Duncan Multiple Range Test (Waller and Duncan, 1969).

Additionally, a number of linear regression equations were fitted to certain air quality data, and correlation coefficients were used to assess agreement among investigators conducting these measurements.

C. Results and Discussion

1. Ambient Carbon Monoxide Evaluation

Results for this evaluation were straightforward. Essentially, no detectable ambient CO was observed at the DWRC Laboratory; whereas, detectable levels of CO were found consistently at the Route 6 Viaduct.

Table 4 presents a set of descriptive statistics associated with the CO readings at the four measurement sites. Detections within Room 158 (Inhalation Chamber Room) were limited to three, 3 ppm detections; whereas, CO detections in Room 12 and at the Southeast Loading Dock yielded two, 1 ppm and one, 6 ppm values, respectively. The only consistent positive detections occurred beneath the Route 6 Viaduct located approximately 0.5 km northeast of DWRC; these averaged 9.6 ppm for the 20 measurement sessions.

Table 4. Descriptive statistics characterizing the results of ambient CO data (Ecolyzer) measured at four sites within and nearby the DWRC Laboratory.

Variable	Site			
	Room 158 (Inhalation Chamber Room)	Room 12 (Office)	Southeast Loading Dock	Route 6 Viaduct
CO (ppm)[1]				
Mean	0	-0.8	-0.6	9.6
S.D.	1.5	1.2	1.8	6.5
Range	-3 to +3	-3 to +1	-3 to +6	-2 to +23
Number of Samplings	36	21	19	20

[1] Slight "off-zero" (negative or positive) measurements of < 3 ppm are possible with the Model CO/Hb 200; these deviations from zero are to be corrected from carboxyhemoglobin values during actual respiratory measurements. For current purposes, the off-zero values reflect deviations attributed to "drift" of electronic components.

Results of the Ambient CO Evaluation indicate that the DWRC facilities used for RP/BR research are free from CO contamination. The Center's location at approximately 5,400 ft. elevation, coupled with its proximity to one of the major "poor-air cities" in the U.S. (i.e., Denver -- approximately 16 km to East), warranted this work. Although low levels of CO were regularly measured at a traffic thoroughfare about 0.5 km northeast, these detections proved inconsequential. Near zero measurements were recorded for a total of 76 checks at three separate locations within or adjacent to the research area. The absence of measurable CO in the laboratory confirms that animals held for behavioral/physiological studies during Tasks 2 and 3 will be unexposed to any debilitating effects of this contaminate prior to and following RP/BR treatments.

2. In-chamber RP/BR Aerosol and Air Quality Study

a. Aerosol mass

(1) Spatial homogeneity/heterogeneity.-- Although a number of statistically significant effects were found for the 1-h accumulated aerosol mass data, detailed inspection showed these effects to be limited to specific sampling locations and not biologically significant. Any disparities from the homogeneous distribution of aerosol mass were much less than the 20 percent maximum heterogeneity criterion imposed to determine sufficiency of the aerosol distribution within chamber.

Results of the ANOVA for total mg of aerosol collected during each 60-min burn revealed that the Shelf x Burn interaction ($F = 4.01$, $df = 6/48$, $p < .003$), as well as the Shelf ($F = 15.06$, $df = 2/48$, $p < .0001$) and Extrusion ($F = 1111.76$, $df = 5/24$, $p < .0001$) main effects, were significant.

The Shelf x Burn interaction is graphically displayed in Figure 11. Post-hoc Duncan Range Tests confirmed that the high and low mean mg collections for the Top Shelf during Burns 2 and 3 and the Bottom Shelf during Burn 1 accounted for this interaction. Recall that filter-collection sites (cages) were confounded with Burn. Burn 1 coincided with aerosol collections from Cages 3, 5, and 12; whereas, Burns 2 and 3 coincided with Cages 1, 6, and 10 and Cages 4, 8, and 9, respectively (see Table 3 and Fig. 8). The high mg mass values for the Top Shelf were collected from Cage Sites 1 (178.6) and 4 (176.2); and, the low mg value for the Bottom Shelf was traced to Cage 12 (161.5). Actually, mean mass values for Cages 1 and 4 were not different from other Top and Middle Shelf cage sites, but were different from Bottom Shelf sites. Cage Site 12 was significantly lower than all other Cage means. Cage 12 was located at the bottom right front of the chamber -- adjacent to the sampling port used to collect Gastec Analyzer Tube readings. The frequent insertion/withdrawl of Gastec Tubes during respiratory and contaminant gas measurements is believed to have permitted a small vortex of room air to enter the chamber, thereby diluting the aerosol collections in the vicinity of Cage 12.

The significant main effect for Shelf confirmed that consistently lower aerosol mass values were obtained for cage sites near the bottom

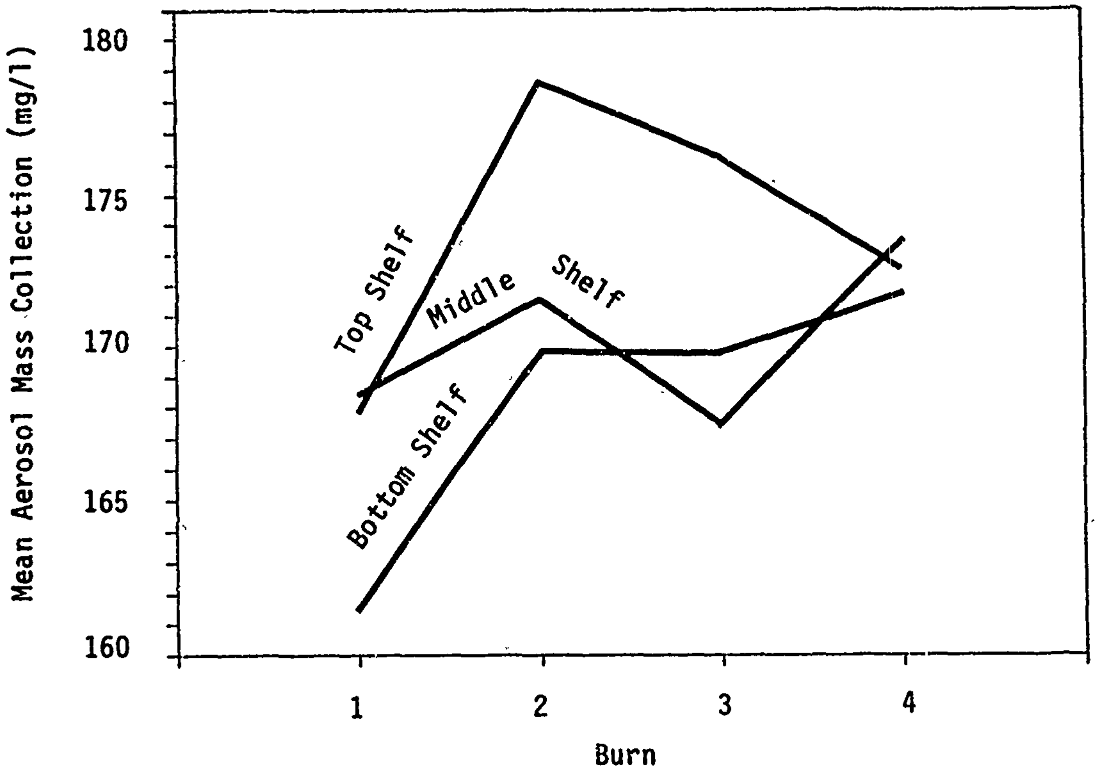


Figure 11. Mean RP/BR aerosol mass collections (i.e., total 1-h samples) from respective shelves of the inhalation chamber during the four burns required to sample the 12 cage locations; this represents the Shelf X Burn interaction found for the gravimetric analysis. (Note.--Cage site is confounded with Burn; specific cage locations sampled for each Burn are: Burn 1 = 3, 5, 12; Burn 2 = 1, 6, 10; Burn 3 = 4, 8, 9; and Burn 4 = 2, 7, 11.).

of the chamber. Mean ($\pm$ S.D.) mg mass values for the Top, Middle, and Bottom Shelves were 174 ($\pm$ 102.5), 170 ($\pm$ 99.7), and 168 ($\pm$ 98.9) mg, respectively. Duncan Range Tests showed that the Bottom Shelf yielded lower values, with no differences between the Top and Middle Shelves. This translates as: the Bottom Shelf yielded a -1 and -3 percent lower average 1-h mass value than the Middle and Top Shelves, respectively. This difference is considered to be statistically interesting, but of limited biological concern. The -3 percent disparity in aerosol mass between the Top and Bottom Shelves converts to 2.9 versus 2.8 average mg/l concentrations for 1-h exposures. This is unlikely to produce significantly different behavioral or physiological effects, except under extremely lengthy or multiple exposure periods. The result further explains the interaction effect, substantiating our interpretation that intermittent influxes of air associated with the insertion and removal of Gastec Analyzer Tubes probably diluted the aerosol at the bottom of the chamber (i.e., in particular Cage Site 12).

As expected, the Extrusion effect indicated that the six designated RP/BR extrusion settings produced smoke having significantly different amounts of aerosol mass. This demonstrates the repeatability and control of RP/BR mass afforded by the extrusion pump. Figure 12 is a graph of the aerosol mass means obtained at each extrusion setting. Mean ($\pm$ S.D.) mg aerosol collections for the three extruder settings at the 500 l/min air flow rate were 21.6 ($\pm$ 2.3), 85.8 ($\pm$ 5.6), and 186.0 ($\pm$ 15.9) mg; whereas, similar values for the three extrusion settings at the 250 l/min air flow were 165.7 ($\pm$ 7.1), 240.0 ($\pm$ 9.8), and 325.6 ($\pm$ 18.7) mg, respectively. Duncan Range Tests confirmed that the six extruder settings produced significantly different masses of aerosol, but that the mass produced by the 243 µm extrusion setting at 500 l/min air flow was greater than that produced by the 270 µm extrusion used at 250 l/min air flow. This is inconsequential to the Study. Our aim was to determine the relationship between selected RP/BR extrusion settings and amount of aerosol produced. Whether a given setting at 500 l/min air flow causes greater or lesser mass than a setting at 250 l/min air flow is of no concern, particularly since no interaction occurred between the Extrusion Factor and other variables in the design. Dividing each of the mean values by 60 (i.e., 1 l/min sampling for 60 min) indicated that the mean aerosol concentrations associated with each of the extrusion settings were: 0.36, 1.43, and 3.10 mg/l for 500 l/min air flow and 2.77, 4.00, and 5.43 mg/l for 250 l/min air (see Fig. 12 and Table 5).

Regarding the ANOVA for percentage difference in aerosol mass collections between cage sites and the center of chamber within each burn, no significant effects were found. Mean percentage differences for respective cage sites and center chamber (i.e., Burn x Shelf interaction cells) ranged between 0.0 and -4.1 percent. Interestingly, most mean percentage differences were negative, indicating a bias for center chamber aerosol mass collections to be greater than cage-site collections.

The current data and aerosol sampling scheme are somewhat unique. Although prior researchers have studied the characteristics of RP/BR aerosol generated using a similar generator system (Aranyi 1983a; Brazell

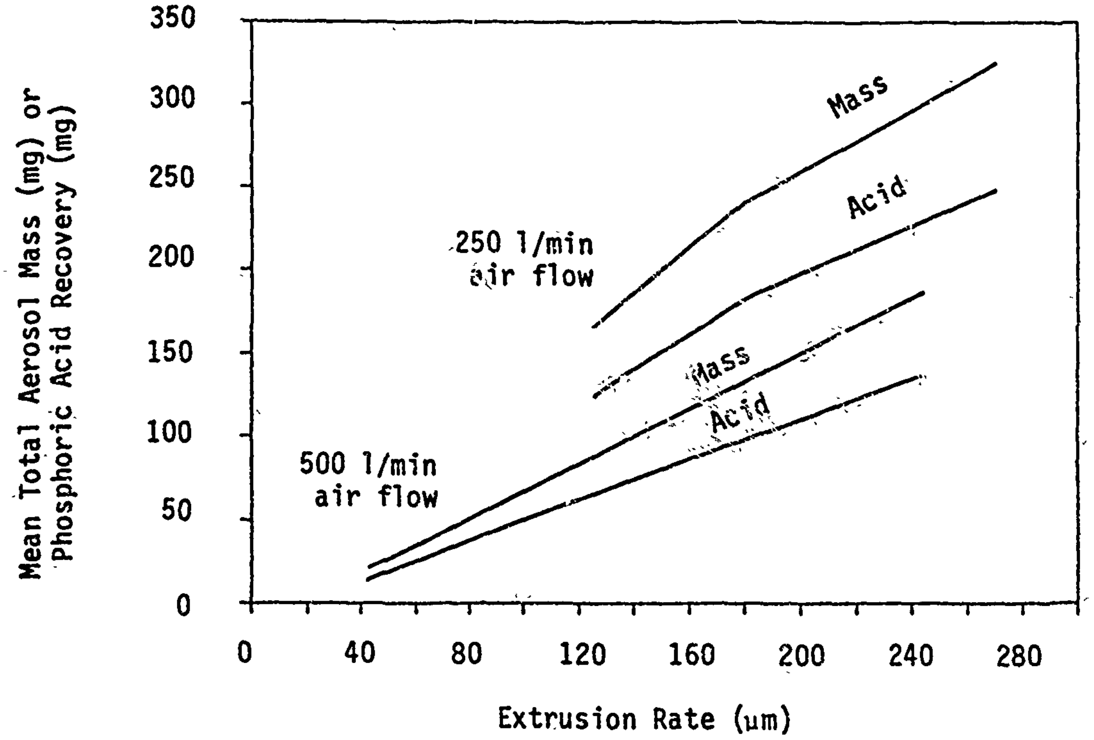


Figure 12. Mean aerosol mass weights (gravimetric analysis) and mean phosphoric acid depositions (titration analysis) associated with the RP/BR burns conducted at each of the three extrusion settings at 500 l/min (i.e., 43, 123, and 243 μm) and 250 l/min (i.e., 125, 180, and 270 μm) air flow. (Note.-- The overlap of the mass and acid values at the 243 and 125 μm extrusion settings for 500 and 250 l/min air flow rate, respectively, reflects the non-transitive function reported for total mg aerosol mass and phosphoric acid results -- Extrusion main effect.).

et al. 1985), frequent sampling of contaminant gases through a side port in the chamber wall was not conducted in these studies. Aranyi (1983a) used a similar sampling scheme, but reported no significant spatial effects. We contend that the current design and analysis is more powerful than previous approaches, revealing a subtle, highly-localized dilution effect caused by fresh air entering the chamber during frequent sampling for contaminant gases with Gastec Analyzer Tubes.

Despite the minor departures from strict homogeneity of aerosol mass cited, we consider the current results to be statistically -- not biologically -- significant. As mentioned, all effects observed were less than the *a priori* 20 percent maximum heterogeneity criterion invoked for our evaluation. The computed difference for the Bottom Shelf and Cage Site 12 mean mg aerosol mass from other Shelves and Cage Sites equaled -3 percent and -10 percent, respectively. To eliminate potential questions of heterogeneous RP/BR concentrations, we opt to not use Cage Site 12 for the exposure of animals in Task 2 and Task 3 studies. This will ensure consistent RP/BR exposure conditions for animals in later experiments.

(2) Temporal homogeneity/heterogeneity.-- The mg of aerosol mass collected during three 10-min periods (i.e., 20-30, 35-45, and 50-60 min within-burn) were extremely uniform. The ANOVA for mg of aerosol mass on these 10-min filter collections yielded only an Extrusion main effect ($F = 471.19$, $df = 5/36$, $p < .0001$); no other main or interaction terms were significant. Again, the Extrusion effect simply confirmed that aerosol masses combined over the three filters were significantly different at the respective extrusion settings -- results identical to that for spatial homogeneity/heterogeneity tests.

Although no significant Period effect was found for these aerosol mass collections, Table 5 presents the mean (± S.D.) mg aerosol mass collected during the 20-30, 35-45, and 50-60 min periods within burns; note the consistency among these means within extrusion settings.

b. Phosphoric acid (H_3PO_4) titration

(1) Spatial homogeneity/heterogeneity.-- The ANOVA for total mg of H_3PO_4 recovered from the 60-min aerosol mass filter collections yielded two significant main effects: Shelf ($F = 7.73$, $df = 2/30$, $p < .001$) and Extrusion ($F = 2481.28$, $df = 5/24$, $p < .0001$). These effects were similar to those obtained for aerosol mass (gravimetric analysis), except that no Shelf x Burn interaction was found for the H_3PO_4 titrations. Typically, H_3PO_4 mass recoveries were approximately three-fourths those of total aerosol mass.

Examination of the Shelf main effect revealed that consistently lower H_3PO_4 recoveries were found for filters collected from locations on the Bottom Shelf. Mean (± S.D.) mg H_3PO_4 for the Top, Middle, and Bottom Shelves were 130 (± 77.6), 130 (± 76.9), and 126 (± 75.4), respectively. This pattern of H_3PO_4 titrations corroborates the interpretation given for aerosol mass. Namely, a three percent dilution of H_3PO_4 in the

Table 5. Mean (± S.D.) aerosol mass weights (gravimetric analysis) collected for the three 10-min sampling periods.

Air Flow Rate	Extrusion Setting	Aerosol Mass Concentration	Mean (+ S.D.) mg Aerosol Mass for Sampling Periods		
(l/min)	(µm)	(mg/l)	20-30 min	35-45 min	50-60
500	43	0.36	3.9 (± 0.9)	2.9 (± 1.1)	3.5 (± 0.6)
	123	1.43	14.5 (± 3.2)	14.9 (± 1.1)	16.0 (± 0.6)
	243	3.10	32.1 (± 3.0)	32.0 (± 2.8)	32.0 (± 3.2)
250	125	2.77	29.7 (± 3.8)	29.4 (± 2.1)	30.0 (± 4.5)
	180	4.00	43.0 (± 2.3)	45.0 (± 3.6)	44.0 (± 3.2)
	270	5.43	57.0 (± 2.2)	58.4 (± 3.3)	59.4 (± 3.2)
----------Combined-------------------			30.6 (± 17.7)	30.4 (± 18.6)	30.8 (± 18.5)

atmosphere existed near the base of the chamber -- a result probably caused by the repeated sampling of gases from the lower sampling port on the chamber sidewall. Unlike the results found for aerosol mass, however, no Shelf x Burn interaction occurred with these data. This suggests that no specific Cage Sites were associated with the decreased H_3PO_4.

The main effect of Extrusion for H_3PO_4 titration produced an identical pattern of means as that for aerosol mass (Fig. 12). Range Tests showed that all mean mg depositions were significantly different from each other. Although this result is of minor importance to the homogeneity/heterogeneity issue (i.e., in the absence of Extrusion interactions with other factors), it indicates that different amounts of H_3PO_4 were produced at the designated extrusion settings and that the highest and lowest settings at the 500 and 250 l/min air flow rate produced different amounts of H_3PO_4 which were reversed in order. Similar to aerosol mass, this result simply demonstrates the exclient capability of the ORNL System to produce reliable concentrations of RP/BR, but the imperfect calibration obtained for the 243 µm (500 l/min air flow) and 125 µm (250 l/min air flow) extrusion settings.

Table 6 presents a comparison of mean aerosol mass collections and mean H_3PO_4 depositions for the designated extrusion pump and air flow rates used in Task 1. As indicated, mean recoveries of H_3PO_4 were between 70 and 76 percent of mean mass weights for respective extrusion settings. These data conform well to data published by Brazell et al. (1985) which showed that RP/BR aerosol mass concentrations of 1.3 and 3.5 mg/l yielded H_3PO_4 concentration recoveries of 1.0 and 2.8 mg/l, respectively -- between 77 and 80 percent H_3PO_4. Thus, results strongly imply that aerosol generation with the Modified RP/BR Extruder and Inhalation Chamber System at DWRC produced RP/BR aerosols typical of ORNL-generated aerosols.

The ANOVA for percentage differences between H_3PO_4 depositions on filters collected at designated cage sites and center chamber within each burn yielded only one significant effect -- Shelf (F = 10.32, df = 2/30, p <.0002). Mean percentage differences for the overall Top, Middle, and Bottom Shelf titration values were -0.3, -0.9, and -3.6 percent, respectively. Again, these differences confirm that decreased H_3PO_4 was associated with the Bottom Shelf and that the effect was of small magnitude.

(2) Temporal homogeneity/heterogeneity.-- The mg titrations of H_3PO_4 from filter disks collected during three 10-min sampling periods within burns yielded three significant ANOVA terms: Replication x Extrusion (F = 5.40, df = 5/36, p <.0008), Period (F = 5.43, df = 2/10, p <.0064), and Extrusion (F = 1889.94, df = 5/36, p <.0001). Because the Extrusion term represents the combined H_3PO_4 quantities titrated from the three 10-min filters -- a 30-min collection analogous to one-half of the "spatial collection" -- only the Period and Replication x Extrusion effects will be described here.

Duncan Multiple Range Tests showed that the mean mg H_3PO_4 value for the 20-30 min sampling period was slightly greater than mean mg

Table 6. Comparison of aerosol mass versus H_3PO_4 deposition on filter discs at the six RP/BR extrusion settings used in Task 1.

Air Flow (l/min)	(RP/BR) Extrusion Setting (µm)	Aerosol Mass (mg)	Aerosol Concentration (mg/l)[1]	H_3PO_4 Titration (mg)	Concentration of H_3PO_4 (mg/l)[1]	Percent H_3PO_4 of Aerosol Mass
500	43	21.6	0.36	15.2	0.25	70
	123	85.8	1.43	64.1	1.07	75
	243	186.0	3.10	137.3	2.29	74
250	125	165.7	2.77	124.3	2.07	75
	180	240.0	4.00	183.2	3.05	76
	250	325.6	5.43	247.9	4.13	76

[1] Concentrations derived based upon 60-min collection at 1 l/min.

recoveries for the 35-45 and 50-60 min periods. Specific mean (± S.D.) H_3PO_4 quantities for these periods were 22.7 (± 13.9), 22.2 (± 14.1), and 22.2 (± 14.1) mg, respectively. Thus, greater acid was found on the first 10-min filter collection than on the second and third within-burn filters.

Explanation of the temporal differences in H_3PO_4 is best tied to the dynamics of how the chamber filled with aerosol and the selection of 10-min sampling times. As will be described for opacity measurements, the density of aerosol within the chamber increased rapidly to a set level during the initial 10 min period following ignition of the RP/BR. The opacity charts then displayed a "rounded top," with smoke densities increasing to a broad peak during 11 to 30 min after ignition. Subsequently, opacity either stabilized or gradually declined with intermittent perturbations until cessation of the burn. The fact that a filter disk was collected during the time of the "rounded top" (i.e., 20-30 min) is believed to account for the elevated H_3PO_4 deposition in the first temporal-sampling period. Delaying the collection of the first filter, or eliminating it altogether, would have undoubtedly yielded temporally homogeneous readings. The current result characterizes the "increasing-concentration phase" of the RP/BR burns.

Figure 13 is a schematic illustration of the appropriate cell means comprising the Replication x Extrusion interaction. Duncan Multiple Range Tests showed that within Replications, mean H_3PO_4 titrations were different among burns conducted at certain extrusion settings. The interaction appeared to result from the reversal of mean H_3PO_4 values for filters collected during burns at the 125 and 180 μm (i.e., 250 l/min air flow) and 243 μm (i.e., 500 l/min air flow) extrusion settings. Replication 1 mean mg H_3PO_4 values were greater than those for Replication 2 at the 125 and 180 μm settings, but less H_3PO_4 was titrated from the filter collected during the Replication 1 than Replication 2 burns at the 243 μm extrusion.

Despite statistical significance of the Replication x Extrusion interaction, this finding is not unduly troublesome to the temporal homogeneity/heterogeneity issue for several reasons. First, remember that no such interaction occurred for aerosol mass (gravimetric analysis). Second, the cell means involved in the Replication x Extrusion term represent H_3PO_4 quantities that were combined over the three 10-min samplings (i.e., 20-30 plus 35-45 plus 50-60 min filters). Finally, the actual size of H_3PO_4 differences contributing to the interaction were relatively low; percentage differences of the Replication 1 versus Replication 2 means at the 125, 180 and 243 μm extrusion settings were +11, +6, and -8 percent, respectively.

c. Aerosol opacity

Opacity data were treated descriptively. That is, individual tracings of the chart recordings obtained using the ORNL infrared sensors were prepared to illustrate the patterns and durations of RP/BR smoke densities associated with each of the burns. It should be recalled that two separate

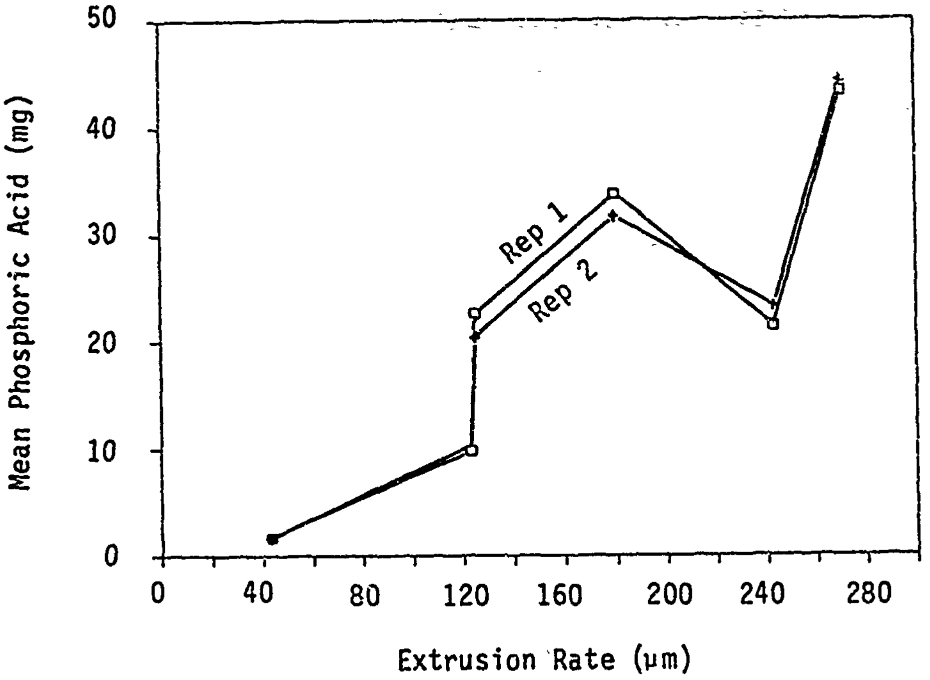


Figure 13. Graph of the Replication X Extrusion interaction for phosphoric acid titration recoveries from the three 10-min filter disks used to assess temporal homogeneity/heterogeneity at the six RP/BR extrusion settings (i.e., 43, 123, 125, 180, 243, and 270 µm).

sensors were used: (1) a within-chamber sensor near the center of the chamber (location 13b) and (2) a special, in-line sensor located in the temporal aerosol-collection tube (location 13c; see Fig. 9).

Figure 14 presents an actual tracing of the dual chart recordings obtained using the within-chamber and in-line sensors on November 17, 1986. Particulars of this burn were: 500 l/min air flow, 243 µm RP/BR extrusion, Burn 3 sampling configuration (i.e., aerosol collections from Locations 4, 8, 9, and 13a), and 15 cm/h chart speed. This RP/BR (Billet 33 from Batch 3, Can E-7) was ignited at 2:22 pm and extinguished at 3:46 pm (MST). The computed aerosol mass concentration based upon the 60-min filter collected from Location 13a (center chamber) was 2.83 mg/l. This burn yielded opacity tracings highly representative of most burns. Collection of measurements and operation of equipment was error free and in accordance with procedures. Although the two tracings reflect the same burn, some important similarities and differences associated with the two sensors should be noted.

Regarding Figure 14, note the lateral 0.5 cm offset of the pens; this is a feature of the chart recorder to allow clear presentation of two functions. Following RP/BR ignition, both tracings rise abruptly for 12 to 15 min, the characteristic increase in smoke density associated with "chamber fill." The momentary dip in the tracings immediately after ignition indicates a "flame out" and re-ignition of the RP/BR. At the time of "chamber fill", both tracings display a "rounded top" -- the characteristic elevated opacity associated with the stabilization of smoke density at fixed extrusions with the RP/BR metering pump. Except for several momentary drops and gradual perturbations, both tracings then display fairly stable opacities for the remainder of the burn. Six momentary declines in the tracing of the in-line sensor were caused by operator switching of filter-sampling valves at the start and end of the 20-30, 35-45, and 50-60 min temporal filters. The within-chamber tracing runs the full length of the 84-min burn; whereas, the in-line sensor tracing stops after 65 min due to cessation of aerosol collections for temporal homogeneity/heterogeneity assessments. Upon cessation of the sampling pump (i.e., in-line sensor) and RP/BR flame (i.e., within-chamber sensor), smoke-density readings abruptly decline to near zero. Smoke residues probably account for the lack of a zero opacity trace near the end of the in-line sensor measurement. The within-chamber sensor tracing shows that some aerosol remained after 8 min of post-flame venting; typically, 10-15 min was allowed to exhaust aerosol from the chamber at the end of Task 1 burns.

Tracings of the within-chamber sensor measurements collected during the 48 RP/BR burns composing the balanced data set for Task 1 are presented in Figures 15a and 15b. Figure 15a illustrates the 24 tracings obtained for each of the 8 burns conducted at the 43, 123, and 243 µm extrusion settings with 500 l/min air flow; whereas, Figure 15b presents the 24 tracings made during each of the 8 burns conducted at the 125, 180, and 270 µm extrusion setting with 250 l/min air flow. The x-axis represents real time (min), while the y-axis is scaled to reflect relative opacity.

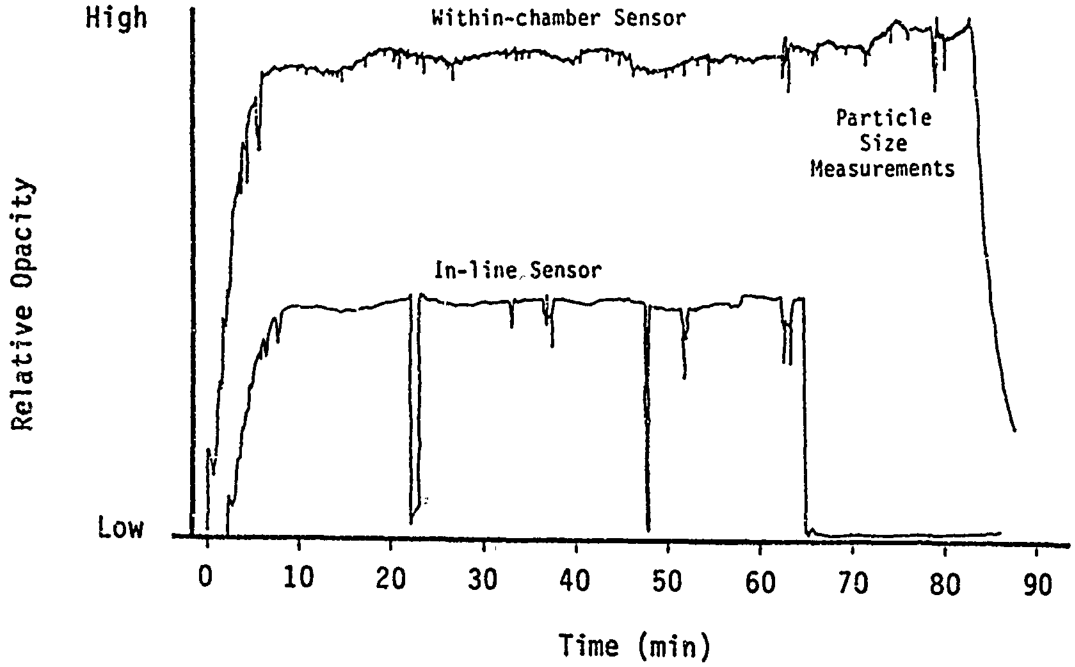


Figure 14. Tracings of the within-chamber and in-line infrared sensor recordings obtained for a burn at 243 µm with 500 l/min air flow on November 17, 1986. (Note.-- This burn yielded a concentration of 2.83 mg/l averaged for the main 60 min aerosol mass collection period.).

That the within-chamber sensor was responsive to changes in aerosol concentration is obvious from an initial glance at the sets of tracings displayed in Figure 15a and 15b. Increased RP/BR extrusion settings generally produced elevated infrared opacity tracings. Of course, considerable variability in smoke density is evident both within and between burns at each extrusion setting.

Detailed inspection of Figure 15a reveals several characteristic features of the RP/BR burns conducted at 500 l/min air flow. Burns at the 43 and 123 µm extrusions involved single billets; whereas, half of the burns conducted at the 243 µm extrusion required the ignition of two billets to accomplish the particle size measurements. The RP/BR was simply spent faster at the 243 µm extrusion, requiring a second billet to be loaded in order to allow collection of particle size data.

The smoke concentration (mg/l) represented by the tracings at each extrusion setting were not monotonically related to the position of a given tracing on the y-axis. Tracings that produced relatively higher or lower opacity charts varied in actual aerosol mass (i.e., gravimetric estimates associated with 60 min filter collections were not perfectly correlated with the opacity index). This finding reflects measurement error and the lack of precise calibration of the chart recorder.

Inspection of Figure 15b also shows the variability in opacity tracings apparent within extrusion settings. Wide ranges of opacity are evident at the 125, 180, and 270 µm extrusions. These data reflect the inability of the sensor to maintain calibration between burns, particularly at the high concentration burns. The shortened charts at the 270 µm extrusion reflect the absence of particle size measurements during these burns. No particle measurements were made at the 5.43 mg/l smoke concentration because the cascade impactor could have been damaged by such massive particle loadings on the sensor crystals.

d. Aerosol particle size

Computations of specific mass median aerodynamic diameters (MMADs) of particles assumed that cascade impactor measurements adhered to a logarithmic (Log) normal distribution. The size of the particles corresponding to the median was used in later statistical analyses.

Figure 16 presents representative Log plots of MMAD values based on cascade impactor measurements collected at four chamber locations during three selected RP/BR burns in Task 1. These burns involved the "Burn 2 sampling configuration." Locations 1, 6, and 10 plus 13a were sampled for aerosol generated during 43 and 123 µm extrusions at 500 l/min air flow and 180 µm extrusion at 250 l/min air flow. Particle sizes ranged between MMAD values of 0.01 and 3.2 µm for these extrusions. The MMADs for the four sampling locations were clustered within extrusion settings (burn), but were distinct for the different extrusion settings. Ranges of MMAD values for the four sampling sites at each extrusion rate were: 43 µm extrusion = 0.23 to 0.27 µm, 123 µm extrusion = 0.45 to 0.59 µm, and 180 µm extrusion = 0.70 to 0.97 µm.

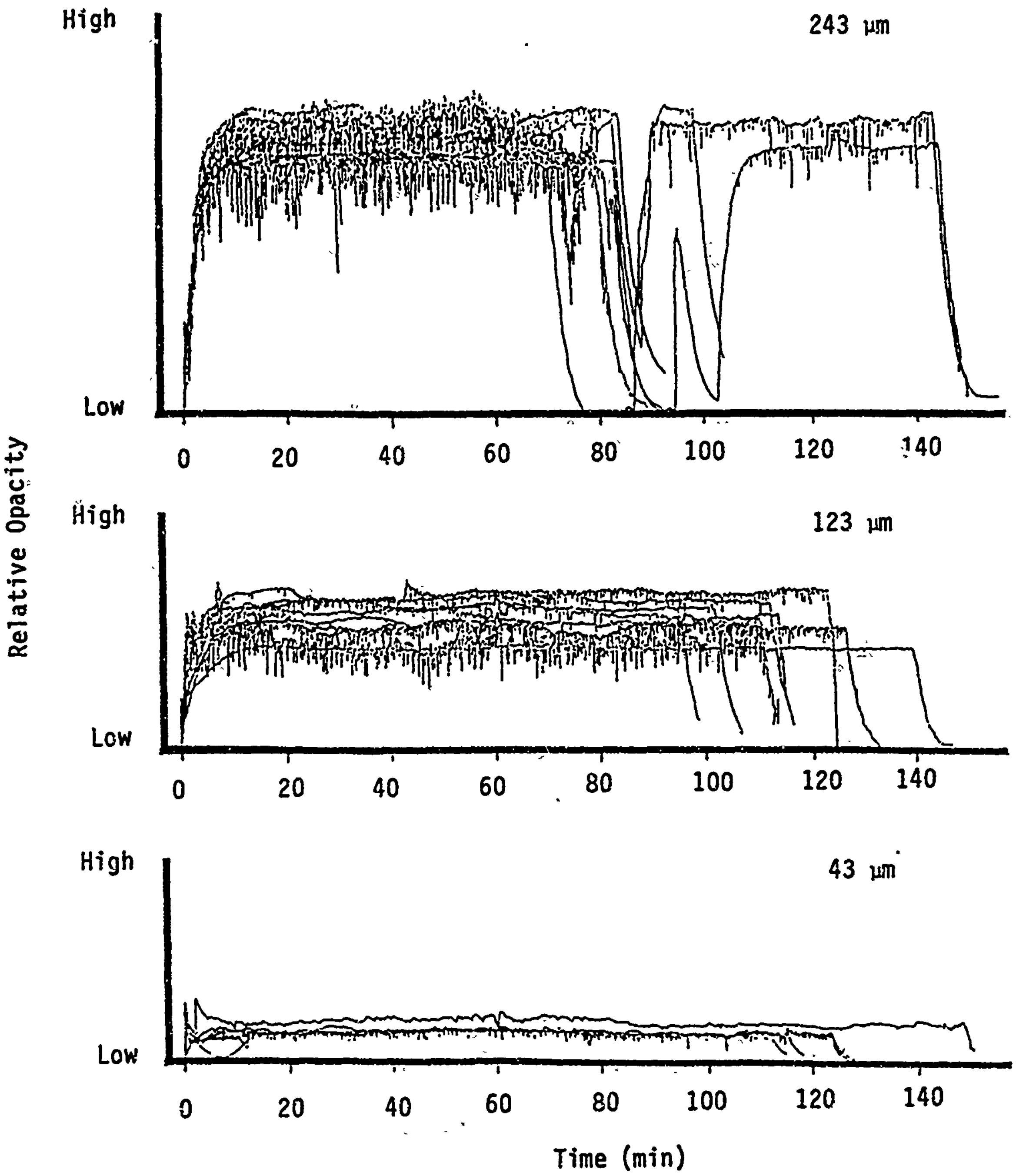


Figure 15a. Tracings of the within-chamber sensor recordings obtained for respective RP/BR burns at the 43, 123, and 243 µm extrusion settings with 500 l/min air flow. (Note.-- Careful inspection of the tracings reveals that only seven charts occur for the 43 and 243 um burns; these and other incongruities are due to incorrect chart speeds -- no tracings.).

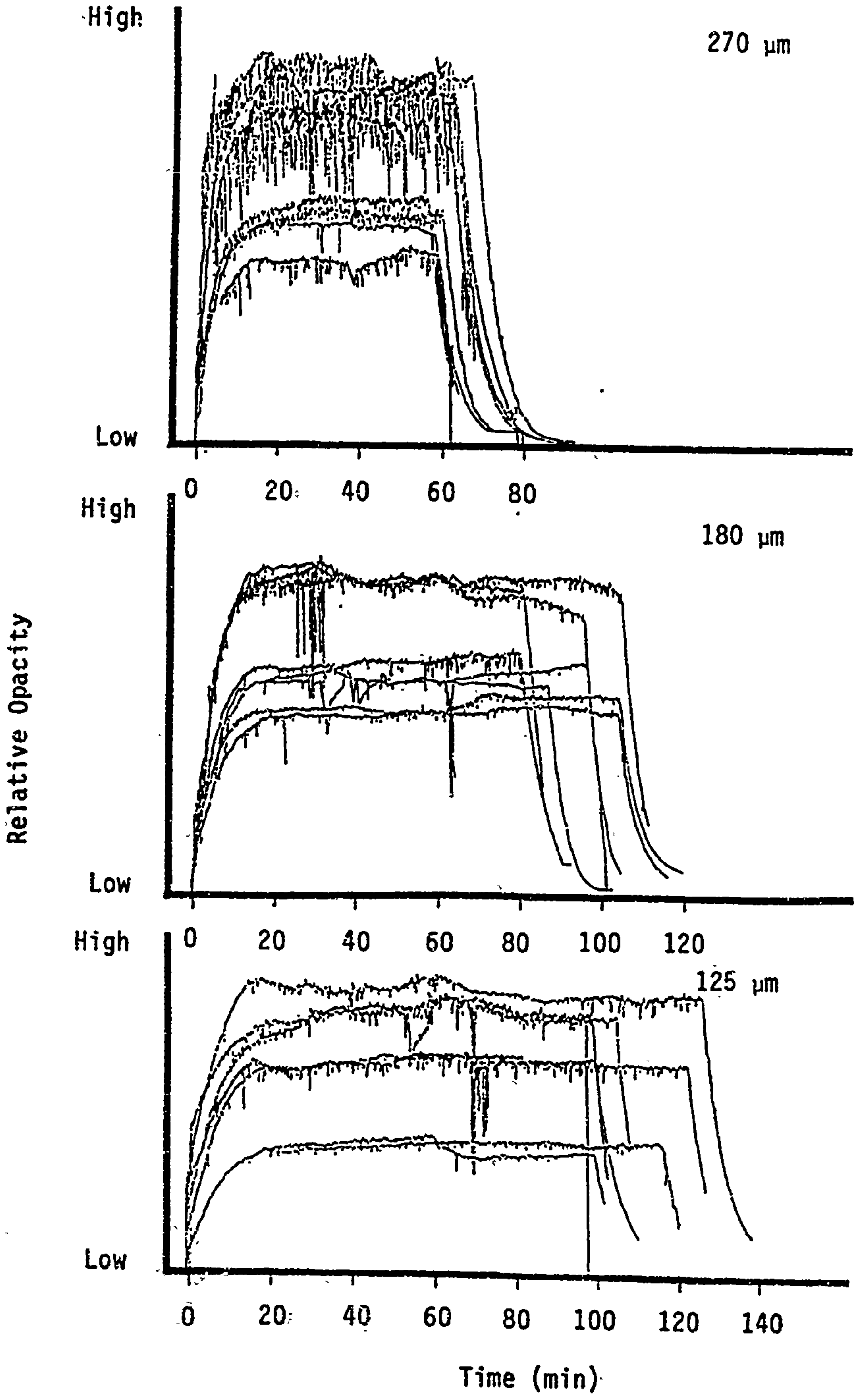


Figure 15b. Tracings of the within-chamber sensor recordings obtained for respective RP/BR burns at the 125, 180, and 270 µm extrusion settings with 250 l/min air flow.

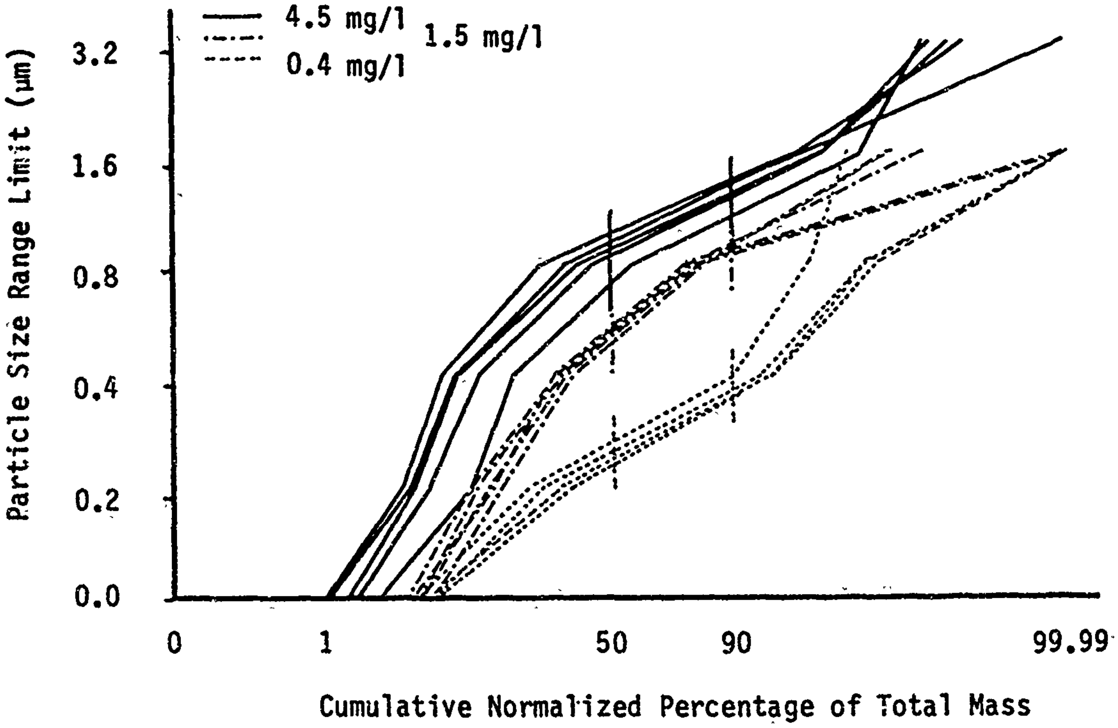


Figure 16. Representative plots of mass aerodynamic diameter distributions for three selected burns of RP/BR intended to produce target concentrations of 0.4, 1.5, and 4.5 mg/l within the inhalation chamber (Note.-- The Burn 2 sampling configuration involved particle size estimates drawn from Cage Sites 1, 6, and 10 plus Chamber Center.).

The ANOVA to assess spatial differences in aerosol particle size within the chamber yielded significant main effects for Extrusion (F = 37.7, df = 4/12, p <.0001) and Shelf (F = 11.4, df = 2/24, p <.0003). These results were readily interpreted.

Extrusion-caused differences in mean MMAD values were associated with the 500 l/min air flow condition. Duncan Multiple Range Tests showed that mean (± S.D.) MMADs of 0.22 (± 0.02), 0.41 (±0.08) and 0.72 (± 0.14) µm for the 43, 123, and 240 µm extrusion settings at 500 l/min air flow were significantly different from each other. Average MMADs of 0.71 (± 0.10) and 0.74 (± 0.10) µm for the 125 and 180 µm extrusions at 250 l/min air flow were neither different from each other nor the 240 µm extrusion mean at 500 l/min air flow, but these values also were greater than the means recorded for 43- and 123-µm-extrusions at 500 l/min. Thus, these results confirm that current aerosol generations were characterized by average MMADs of between 0.22 and 0.74 µm. Within limits, faster extrusion rates of RP/BR (burn rates) produced aerosols having larger MMADs.

Comparison of these particle size values with previously published data conducted with similar RP/BR extrusion-type smoke-generator systems yielded only rough agreement. Aranyi (1983a) reported average particle sizes of 0.31 to 0.33 µm, 0.42 to 0.42 µm and 0.53 to 0.61 µm for a series of RP/BR burns at 500 l/min air flow yielding 0.27 to 0.32 mg/l, 0.50 to 0.61 mg/l and 1.04 to 1.08 mg/l aerosol mass concentrations, respectively. Our findings for similar burn conditions indicate that means of 0.22 and 0.41 µm were associated with aerosol mass concentrations of 0.36 and 1.43 mg/l RP/BR. The lowest concentration burns produced MMADs approximately 10 µm less than that of Aranyi; whereas, our moderate concentration burns at 500 l/min air flow led to MMADs equivalent to those of Aranyi at about 0.5 mg/l less aerosol mass. Additionally, Brazell et al. (1985) reported an MMAD value of 0.47 µm for a series of RP/BR burns conducted at a concentration of 3.99 mg/l and 250 l/min air flow -- a particle size nearly 0.30 µm less than our observed 0.74 µm at 4.00 mg/l aerosol mass concentration.

Similar to the results for aerosol mass and H_3PO_4 titration, the Shelf Factor also accounted for a significant portion of variance in particle size. Particle sizes were greater for samples collected on the Bottom Shelf of the chamber. Mean (± S.D.) MMAD values for the three shelves were: Top = 0.52 (± 0.23) µm, Middle = 0.52 (± .23)µm, and Bottom = 0.55 (± 0.24)µm. Duncan Range Tests confirmed that the Bottom Shelf mean was greater than the others, with the Top and Middle Shelves yielding undifferentiated mean MMADs. This result is viewed to reflect the growth of aerosol particles with time and passage from the apex to base of the chamber. Aranyi (1983a) reported a similar "size gradient" from apex to base during her homogeneity/heterogeneity assessments.

Regarding the ANOVA for percentage difference in MMAD values between cage sites and the center of chamber, only the Shelf main effect proved significant (F = 5.08, df = 2/22, p <.0131). Mean percentage differences of the Top, Middle, and Bottom Shelf values from Chamber Center were +1.6, +1.0, and +8.6 percent, respectively. Duncan Range Tests between these means yielded the pattern of results described for raw MMAD (µm). Percentage deviations of particles from Chamber Center were significantly greater and positive for Cage Sites 9, 10, 11, and 12 (i.e., Bottom Shelf), a result that is consistent with the Shelf effect previously described.

In summary, although disparities of 0.10 to 0.30 µm exist between current results and the data of other authors at similar RP/BR concentrations, we conclude that these particle size values are typical for RP/BR smoke at this wide range of concentrations. The range of MMAD values obtained (i.e., 0.22 to 0.74 µm) is well within the respirable range of 0.1 to 10 µm (Phalen 1984). Similar to Aranyi (1983a), none of the significant statistical effects for Extrusion or Shelf will be of consequence to the inhalation and deposition of RP/BR particles into the tracheobronchial region of animals.

e. Respiratory gases

Limited statistical analyses of the Gastec Analyzer Tube data for respiratory gases were conducted. Results of the O_2 and CO_2 measurements were interpreted based upon summary statistics (i.e., means, standard deviations, ranges) within each RP/BR "target concentration" by air flow condition. Unlike the data for the ANOVAS, all available measurements were used in these descriptions. An estimate of CO_2 production attributable to RP/BR combustion was calculated for each "target concentration" by subtracting a mean ambient CO_2 reading for Room 158 (i.e., Chamber Room). Additionally, estimates of inter-rater reliability coefficients for O_2- and CO_2-Tube readings were calculated.

Table 7 presents mean (± S.D.) and range statistics for Gastec Tube detections of O_2 (%), CO_2 (ppm), and CO_2-From-Burn (ppm) measurements. Estimates of O_2 and CO_2 were collected during 37 and 40 of the 64 total RP/BR burns conducted in Task 1, respectively -- a 58 and 63 percent incidence of sampling.

Results indicate that sufficient O_2 to sustain 12 adult animals of either species was present within the chamber during all RP/BR burns at the six "target concentrations." Mean O_2 readings fluctuated between 20.6 and 21.8 percent for RP/BR burns conducted at the respective extrusion settings.

Carbon dioxide levels also were within acceptable limits for sustaining 12 adult test animals during RP/BR exposure. Average CO_2 values ranged between 496 and 840 ppm for burns at the six RP/BR extrusion settings. Several extreme readings characterized the data obtained at the 243 µm extrusion setting with 500 l/min air flow (i.e., 1210 ppm) and at the 180 and 270 µm setting with 250 l/min air flow (i.e., 908 and 968 ppm). Despite these extremes, however, CO_2 typically made up between 0.5 to 0.8 percent of chamber air during burns -- levels sufficiently below the 1.0 percent threshold concentration often associated with respiratory dysfunction.

Table 7. Mean (± S.D.) and range statistics for O_2 (%), CO_2 (ppm), and CO_2-From-Burn (ppm) obtained for RP/BR burns using Gastec Analyzer Tubes (i.e., Tube Nos. 31 and 2LL)[1].

Air Flow Rate (l/min)	RP/BR Extrusion Setting (μm)	Mean Aerosol Mass Concentration (mg/l)	O_2 (%)	Number of Samples	CO_2 (ppm)	CO_2-From-Burn[2] (ppm)	Number of Samples
500	43	0.36	x=21.4(±0.54) R=20.6-21.8	5	x=496(±27) R=484-545	42	5
	123	1.42	x=21.4(±0.85) R=20.6-23.0	7	x=635(±94) R=453-726	181	7
	243	3.10	x=21.6(±0.46) R=21.2-22.4	7	x=840(±208) R=605-1210[3]	386	8
250	125	2.77	x=21.5(±0.6) R=21.2-22.4	4	x=611(±12) R=605-629	157	4
	180	4.00	x=21.8(±1.0) R=20.6-24.2	9	x=746(±100) R=605-908	292	9
	270	5.43	x=20.6(±2.7) R=15.7-21.8	5	x=787(±126) R=605-968	334	7

[1] All values corrected for elevation of DWRC above sea level:

$$\text{Actual Tube Value} \times \frac{(760 \text{ mm Hg})}{(628 \text{ mm Hg})}.$$

[2] A corrected mean of 454 ppm CO_2 was obtained for three Room 158 CO_2 readings; this was subtracted from the respective within-chamber means to estimate CO_2 production associated with each extrusion setting.

[3] An outlier of 1210 ppm exaggerated this mean.

Inspection of the CO_2-From-Burn data (Table 7) for the six extrusion settings indicates acceptable levels of CO_2 production associated with RP/BR combustion. Previous data from ORNL for burns yielding 2.2, 4.0, and 5.3 mg/l average aerosol mass produced 150, 220, and 370 ppm CO_2 at 250 l/min air flow, respectively (Brazell et al. 1985). Current data for this air flow condition at 2.8, 4.0, and 5.4 mg/l average concentrations are shown to produce 157, 292, and 334 ppm CO_2, respectively. Thus, our results are in excellent agreement, implying that the Modified RP/BR Extruder and Inhalation System functioned similar to other ORNL Systems.

f. Contaminant gases

Because contaminant gases were either rarely detected or only detected in minor quantities, statistical analyses of these data were limited. Results of the GC analyses for detection of PH_3 and C_6H_{14} were treated descriptively -- actual frequencies of detections/non-detections and obtained quantities were tabled. Gastec Analyzer Tube draws for CO were assessed based upon mean, standard deviation and range statistics within each RP/BR concentration by air-flow condition. Additionally, an estimate of inter-rater agreement for CO-tube readings was computed using correlational analysis, and the relationship between RP/BR concentration and CO was determined using a linear regression analysis.

Table 8 presents actual frequencies of sampling and amounts of PH_3 and C_6H_{14} detected with 44 separate GC analyses of aerosol taken both prior to and during 22 of all 64 RP/BR burns. This represented a 34 percent incidence of sampling (i.e., 22 pre-burn and 22 during-burn checks). Detections of PH_3 and C_6H_{14} were found in only 18 percent (4 of 22) and 5 percent (1 of 22) of the samples collected during RP/BR burns, with all detections yielding negligible quantities of these gases. Interestingly, all detections occurred in samples collected at the 250 l/min air flow.

The four PH_3 detections occurred in samples taken during actual RP/BR burns. Detections ranged between 30 and 54 ppb -- quantities only 10 to 18 percent of the 1985-86 Short-term Threshold Limit Value of 300 ppb for a one-h exposure (NRC 1977). The rare, negligible detections of PH_3 indicate that this compound is of no consequence to planned studies with animals.

Four detections of C_6H_{14} were found; however, only one of these occurred in a sample that was collected during an RP/BR burn and yielded 7 ppm -- a negligible amount of the compound. Three detections occurred in samples taken prior to ignition of RP/BR with only filtered, humidified air circulating through the chamber. These pre-burn detections are attributed to volatilization of C_6H_{14} from RP/BR extruded from the burn tip prior to ignition. Approximately eight percent C_6H_{14} by weight is mixed with RP/BR during formulation to soften the product. Detections for these "pre-burn" samples ranged between two and nine ppm -- negligible quantities compared to the 50 ppm for a one h exposure specified as the 1985-86 Short-term Threshold Limit Value for C_6H_{14} (NRC 1977). Together, these data indicate that infrequent, negligible amounts of C_6H_{14} should characterize RP/BR burns during subsequent studies involving animals; but, these quantities

Table 8. Phosphine (PH_3) and hexane (C_6H_{14}) gas chromatography detection frequencies (plus respective ppb or ppm values) obtained for 22 RP/BR burns.

Air Flow Rate	Contaminate Gas			
	PH_3		C_6H_{14}	
	Pre-Burn	During-Burn	Pre-Burn	During-Burn
500 l/min	ND (n=9)	ND (n=9)	ND (n=9)	ND (n=9)
250 l/min	ND (n=13)	ND (n=9)	ND (n=10)	ND (n=12)
		D (n=4) (30,51,51, and 54ppb)	D (n=3) (2,2, and 9ppm)	D (n=1) (7ppm)

ND = Non-Detection
D = Detection
n = Number of samples collected
Limit of detection for PH_3: 30 ppb/5 ml; 1985-86 Short-term Threshold Limit Value: 300 ppb.
Limit of detection for C_6H_{14}: 2.0 ppm/50 µl; 1985-86 Short-term Threshold Limit Value: 50 ppm

should have no deleterious behavioral or physiological consequences to test animals.

Table 9 presents mean (± S.D.) and range statistics for Gastec Tube detections of CO (ppm) by RP/BR burn concentrations and air-flow rates. A total of 42 (i.e., 66% sampling incidence) Gastec Tubes were drawn during the 64 RP/BR burns in the total Task 1 data set. Mean ppm quantities were 8.8, 15.7, 22.4, 32.7, 28.3, and 35.5 for the 0.36, 1.43, 3.10, 2.77, 4.00, and 5.43 mg/l burn concentrations, respectively. A correlation coefficient of 0.90 was computed for a subsample of 12 CO tubes that were read independently by two staff members (i.e., R. T. Sterner and K. A. Crane); this was judged to be a satisfactory index of inter-rater agreement for CO measurements.

Figure 17 is a scatterplot showing the best-fit linear regression line between RP/BR concentration (mg/l) as determined by gravimetric analysis and corresponding CO (ppm) detections. The linear regression function obtained was: $Y = -0.04809 + 0.12907x$; this yielded a coefficient of determination (R^2) of 0.642 with residual error of 1.1189. We also know that CO values correlated .80 with RP/BR concentrations. Thus, the linear function accounted for about 64 percent of the variance in the scatterplot.

Measurable CO was found in 100 percent of the tubes drawn; however, 85 percent of detections gave ppm readings below the 35 ppm standard for a maximum one-h exposure as set by EPA (NRC 1977). Seven (i.e., 15%) actual CO detections exceeded this standard (see Footnote 2, Table 9), with five occurring at the fastest RP/BR extrusion and slowest air flow condition (i.e., 270 µm extrusion and 250 l/min air flow). Solving the regression equation for the associated RP/BR concentration based on gravimetric analysis that would yield 35 ppm CO, a within-chamber concentration of 4.47 mg/l would be predicted to produce this Short-Term Limit Threshold. Although high CO exposure represents a potential confounding variable for later behavioral/physiological studies, use of reduced RP/BR exposure concentrations would circumvent this potential problem.

D. Conclusions

We conclude that the objectives for Task 1 have been satisfied. Results show that the RP/BR-generating equipment functions well. Chamber-exposure conditions meet *a priori* criteria established for the homogeneous distribution of aerosol, with sufficient respiratory gases and low levels of contaminates characterizing most burns. Aerosol particle sizes are within the respirable range. The animal-housing area is free from CO. Essentially, Tasks 2 and 3 should proceed. Specific conclusions pertinent to each of the key objectives for Task 1 follow:

1. Development of RP/BR Extruder and Inhalation Chamber System

The Modified RP/BR Extruder and Inhalation Chamber System has been shown to provide a satisfactory environment for the exposure of prairie dogs and rock doves to controlled doses of RP/BR smoke. The addition of a humidified and filtered air supply in advance of the RP/BR burn chamber produces

Table 9. Mean (± S.D.) and range statistics for CO (ppm) obtained for 42 RP/BR burns using Gastec Analyzer Tubes (i.e., Tube No. 1LL)[1].

Air Flow Rate (l/min)	RP/BR Extrusion Setting (µm)	Mean Aerosol Mass Concentration (mg/l)	Number of Samples	CO (ppm)[2]
500	43	0.36	6	x = 8.8 (± 3.5) R = 6 - 16
	123	1.43	7	x = 15.7 (± 4.6) R = 10 - 22
	243	3.10	8	x = 22.4 (± 6.6) R = 18 - 38
250	125	2.77	4	x = 32.7 (± 3.1) R = 29 - 36
	180	4.00	9	x = 28.3 (± 7.2) R = 18 - 36
	270	5.43	8	x = 35.5 (± 6.4) R = 27 - 48

---

[1] All ppm values corrected for 5400 ft. elevation (i.e., 628 mm Hg) in accordance with manufacturer recommendations for Tube 1LL. The formula for this correction is: Actual Tube Value X $\frac{760 \text{ mm Hg}}{628 \text{ mm Hg}}$.

[2] The EPA standard for CO is 35 ppm maximum for a one h average exposure (NRC, 1977). Seven CO-tube readings exceeded this standard; specific ppm readings and air flow/aerosol target concentration conditions were: 500 l/min and 3.0 mg/l = 38 ppm; 250 l/min and 3.0 mg/l = 36 ppm; and, 250 l/min and 6.0 mg/l = 36, 36, 36, 38, and 48 ppm.

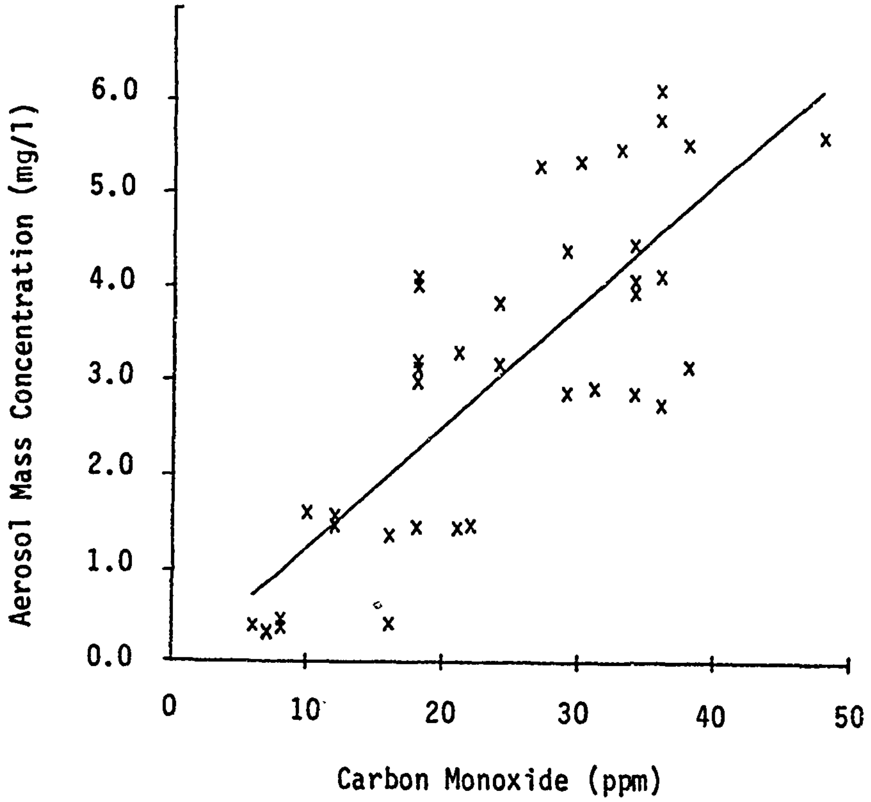


Figure 17. Scattergram and fitted linear function for the relationship between RP/BR concentration and carbon monoxide values as determined with Gastec Analyzer Tube 1LL.

particulate- and contaminate-free air having between 39 and 49 percent average RH. Installation of a 5-m length of water-cooled stainless steel tubing between the RP/BR burn chamber and the inhalation chamber allows average in-chamber temperatures to be maintained at 20 to 23°C. The RP/BR extruder produces reliable aerosol concentrations for each of three selected extrusion settings at both 500 l/min and 250 l/min air flow.

2. Check for Ambient CO in the Laboratory

Results of the Ambient CO Evaluation confirm that the DWRC facilities are free from CO. Near zero ppm readings of CO characterized the 76 checks made at three locations within or adjacent to the research and animal-housing areas.

3. Spatial and Temporal Uniformity of Aerosol in the Chamber

Results for aerosol mass, H_3PO_4 titration and particle size indicate that the within-chamber RP/BR atmosphere are within the 20 percent maximum heterogeneity criterion. Although a number of statistically significant effects were obtained, further inspection showed these to be limited to specific sampling locations and less than the 20 percent criterion established to define acceptable homogeneity. The ANOVAS for aerosol mass, H_3PO_4 titration and particle size each yielded significant Shelf effects, with cage locations on the Bottom Shelf characterized by less aerosol mass, less H_3PO_4, and larger particle size. Specific findings of note include: (a) Cage Site 12 adjacent to the sampling port used for Gastec Analyzer Tube measurements yielded 10 percent lower aerosol mass values than other sites, (b) an overall "dilution effect" occurred for mean aerosol mass and H_3PO_4 titration values collected from the Bottom Shelf of the chamber (i.e., about -3 percent), and (c) a slight gradient of larger aerosol particle sizes occurred from the Top to Bottom Shelf of the chamber -- 0.52 to 0.55 µm, respectively. We interpret the lower mass and acid values to reflect aerosol dilution from periodic bursts of air entering the chamber in the vicinity of the Bottom Shelf during insertion and withdrawl of Gastec Analyzer Tubes -- an artifact of the air quality assessments. Although these data do not violate the imposed heterogeneity criterion, we plan to utilize only Cage Sites 1 to 11 (i.e., exclude Cage Site 12) during animal exposures in Tasks 2 and 3. The particle size gradient is of no concern because all particles are within the typical respirable range.

4. Air Quality Within the Chamber

Oxygen and CO_2 levels within the chamber are always at sufficient levels for normal respiratory functions. The average O_2 and CO_2 levels range between 20.6 and 21.8 percent and between 496 and 840 ppm (i.e., 0.49 and 0.84 percent) respectively.

The presence of PH_3 and C_6H_{14} contaminants is extremely rare, and these occur in only negligible quantities. Conversely, CO occurs in measurable amounts during practically all RP/BR burns, with average readings increasing from 8.8 to 35.5 ppm between the slower (43 µm) and faster

(270 µm) RP/BR extrusion rates. Average levels of CO are consistently higher under conditions of 250 l/min air flow; mean CO ranged between 32.7 and 35.5 ppm at 250 l/min air flow versus 8.8 and 22.4 ppm at 500 l/min air flow in Task 1.

To avoid the buildup of high levels (i.e., > 35 ppm) of CO during RP/BR burns in Task 3, RP/BR aerosol concentrations for an average 60-min exposure will be limited to a maximum 4.00 mg/l (i.e., 180 µm extrusion) at 250 l/min air flow. This would be predicted to yield an average CO value of 31 ppm for this exposure condition.

IV. REFERENCES

Aranyi, C. 1983a. Research and Development on Inhalation Toxicologic Evaluation of Red Phosphorous/Butyl Rubber Combustion Products. Phase I Report, Contract DAMD17-82-C-2121, IIT Research Institute, Chicago, IL. 97pp.

__________. 1983b. Research and Development on Inhalation Toxicologic Evaluation of Red Phosphorous/Butyl Rubber Combustion Products. Phase II Report, Contract DAMD17-82-C-2121, IIT Research Institute, Chicago, IL. 113pp.

Brazell, R. S., J. H. Moneyhun, and R. W. Holmberg. 1984. Chemical and Physical Characterization of Phosphorous Smokes for Inhalation Exposures and Toxicology Studies. ORNL/TM-9571 (AD-A153824) U. S. Army Project Order Nos. 9600, 0027, and 1897, Oak Ridge National Laboratory, Oak Ridge, TN. 61pp.

Burton, F. G., M. L. Clark, R. A. Miller and R. E. Schirmer. 1982. Generation and characterization of red phosphorous smoke aerosols for inhalation-exposure of laboratory animals. American Industrial Hygiene Association Journal, 43(10), 767-772.

Chuan, R. L. 1986. Personal Communication: Example Calculation of Mass Median Diameter Using Log-probability Paper. California Measurements Inc.: Sierra Madre, CA. 4pp.

Helwig J. T. and K. A. Council (Eds.). 1979. Anova and Duncan. The SAS User's Guide. SAS Institute Inc.: Cary, NC. pg. 121-130, 190-194.

Higgins, C. E., T. M. Gayle, and J. R. Stokely. 1978. Sensor for detection of tobacco smoke particulates in inhalation exposure systems. Beitrage zur Tabakforschung International, 9(4),185.

Holmberg, R. W., J. H. Moneyhun, and T. M. Gayle. 1985. A System for the Continuous Generation of Phosphorous Aerosols from Red Phosphorous-Butyl Rubber. Final Report, ORNL/TM-9649 (AD-A157342), Oak Ridge National Laboratory, Oak Ridge, TN. 37pp.

Jenkins, R. A., T. M. Gayle, J. S. Wike, and D. L. Manning. 1981. The Sampling and Chemical Characterization of Concentrated Smokes. In Sampling and Analysis of Toxic Materials in the Atmosphere, ASTM Boulder Symposium Proceedings, Boulder, CO., Aug. 2-6, 1981, American Society for Testing and Materials (Tech. Publ. 786), Philadelphia, PA. Pg. 153-166.

Jenkins, R. A., W. Holmberg, J. S. Wike, J. H. Moneyhun and R. S. Brazell. 1983. Chemical Characterization and Toxicologic Evaluation of Airborne Mixtures: Chemical and Physical Characterization of Diesel Fuel Smoke. Final Report, ORNL/TM-9196 (AD-A-142718), Oak Ridge National Laboratory, Oak Ridge, TN.

National Research Council (NRC). 1977. Carbon Monoxide. National Academy of Sciences: Washington, D.C. 139pp.

Phalen, R. F. 1984. Inhalation Studies: Foundations and Techniques. CRC Press, Inc.: Boca Raton, FL. 277pp.

U.S. Department of Commerce. 1985. Local Climatological Data: Annual Summary with Comparative Data -- Denver, Colorado. ISSN 0198-7682, National Oceanic and Atmospheric Administration: Asheville, NC. 7pp.

________________. 1965. Relative Humidity and Dew Point Table. TA No. 454-0-3B (9-65), Environmental Science Services Administration: Washington, D.C. 3pp.

Waller, R. A. and D. B. Duncan. 1969. A Bayes Rule for the Symmetric Multiple Comparison Problem. Journal of the American Statistical Association, 64, 1489-1499.

Winer, B. J. 1971. Statistical Principles In Experimental Design. Mc Graw-Hill: New York. pg. 559-571.

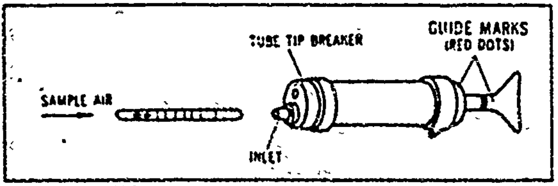
V. APPENDIX

A. Operating Guide For The Gastec Analyzer Pump And Specifications For CO, CO_2, And O_2 Analyzer Tubes.

(We thank Sensidyne, Inc., the U.S. distributor for Gastec Corp., for permission to print these instructions/specifications.)

OPERATING INSTRUCTIONS
GASTEC PRECISION GAS DETECTOR SYSTEM

SAMPLING & MEASUREMENT PROCEDURE:



1. Break tips off a fresh detector tube by bending each tube end in the tube tip breaker of the pump.
2. Insert tube securely into pump inlet with arrow on tube pointing toward pump.
3. For twin tubes, connect Ⓒ marked ends with rubber tubing after breaking each end. Insert analyzer tube into pump with arrows on tubes pointing toward pump. See figure below.

4. Make certain pump handle is all the way in. Align red dots on pump body and handle.
5. Pull handle out to desired stroke. Handle can be locked on either 1/2 pump stroke (50 cc) or 1 pump stroke (100 cc).
6. Read concentration at the interface of stained-to-unstained reagent when staining stops. Unlock handle by making 1/4 turn and return it to starting position.
7. In case more pump strokes are indicated in instruction sheet in each box of tubes, take additional sample by repeating pump strokes without removing tube.

CORRECTION FOR TEMPERATURE, HUMIDITY OR PRESSURE:

Calibration of the Gastec detector tubes is normally based on a tube temperature of 20°C (68°F), approximately 50% relative humidity, and normal atmospheric pressure.

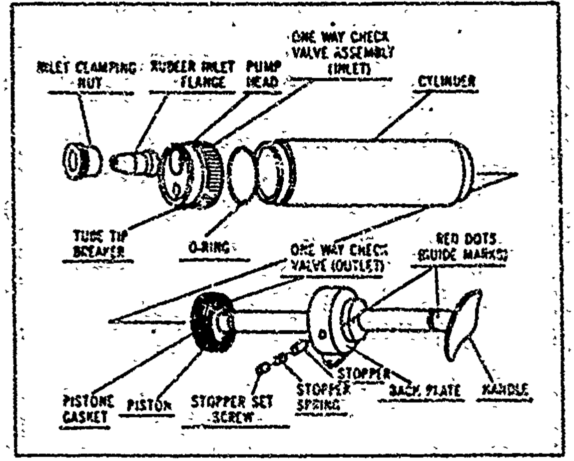
1. No correction is normally required for tube temperatures of 0°—40°C (32°—104°F) and for relative humidity range of 20—90%.
2. Where detecting reagent is abnormally sensitive to temperature or humidity, correction table or chart is provided in each box of tubes. In this case, tube reading must be corrected using correction table or chart.
3. Tube reading is proportional to absolute pressure. To correct for pressure, multiply by

$$\frac{760}{\text{Atmospheric Pressure (mmHg)}}$$

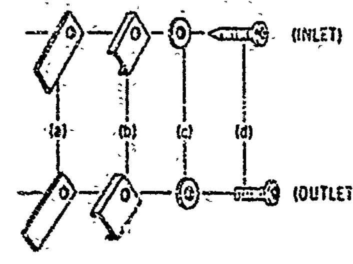
GASTEC PUMP PERFORMANCE

DESCRIPTION OF PUMP

Construction of pump is illustrated below. Pump pulls the highest vacuum (8.1" of Hg). It eliminates flow-rate orifice which may cause malfunction of pump by clogging or leaking orifice. Friction-proof piston gasket (lubricant seal packing) provides completely leakproof sampling at all times.



ONE-WAY CHECK VALVE ASSEMBLY



(a) Rubber valve flap
(b) Valve retainer
(c) Plain washer
(d) Valve screw

CHECKING PUMP PERFORMANCE

A. Visually check rubber inlet flange for cracks or tears. Replace if damaged. Tighten inlet clamping nut.

B. <u>Valve Leak Check</u>

1. Insert a fresh sealed detector tube into pump. Misalign red dots on pump and handle. Pull several fairly rapid continuous full pump strokes.
2. Pull handle out 6 mm (1/4 inch) and hold in this position for 1 or 2 seconds.
3. Release handle.
4. If handle returns to within 1.5 mm (1/16 inch) of fully closed position, continue to step C.

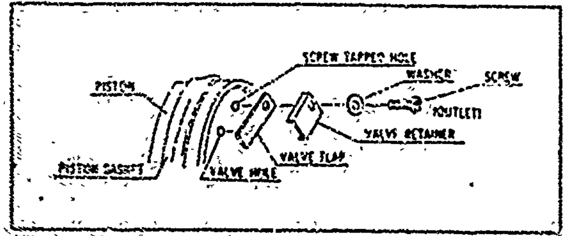
5. If handle does not return to within 1.5 mm (1/16 inch) of fully-closed position (or less), perform the following Valve Lubrication instruction outlined below.

C. Field Volume Check

1. Insert a fresh sealed detector tube into pump.
2. Align red dots on pump body and handle.
3. Pull handle firmly and at a moderate speed until handle locks into position. Wait 1 minute.
4. Unlock handle by turning it and guide it back.
   TO PROTECT PUMP STOPPER from breakage, do not release the handle and allow it to spring back when conducting a leak test. Make sure to hold your hand onto the handle and guide it back.
5. Pump handle should return to within 6 mm (1/4 inch) of the fully closed position.
6. If pump handle does not close to within 6 mm (1/4 inch) or less, follow lubrication instructions and retest.

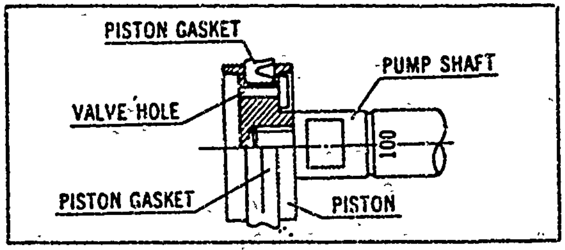
D. Lubrication Instructions (Perform Laboratory Volume Check "E" after each lubrication)

1. Valve Lubrication
   a. Unscrew back plate and withdraw piston from pump cylinder.
   b. Remove check valve from piston.
   c. Clean valve and piston with lint-free cloth. Proper valve cleansing is as follows: Place cloth flat on desk. Wipe rubber valve flap in a flat position across cloth. Do not bend the rubber flap valve.
   d. Apply a small amount of grease evenly around the valve opening to form a thin film. A thin film is nearly invisable.
   e. Replace valve assembly loosely in the same manner as removed.



   f. Before tightening the screw, align valve so that valve hole is in center of valve flap.
   g. Then push the rectangular valve retainer all the way toward loose end of valve flap.
   h. Now tighten screw. If a torque driver is available, tighten to 0.8 Kg-cm. Otherwise, be careful not to overtighten screw. When tightened, screw must not deform rectangular valve retainer.

2. Piston Gasket Lubrication
   a. Wipe off piston and cylinder with a clean lint-free cloth.
   b. Remove piston gasket with a small bladed screwdriver. Take care not to cut gasket.
   c. Clean slot in piston with lint-free cloth. Wipe off rubber gasket.
   d. Wipe an ample supply of grease into gasket slot on piston and inside gasket.
   e. Replace gasket making sure that open side of gasket is toward pump handle.



   f. With the excess grease from piston slot, wipe around outside of gasket and piston.
   g. Wipe an ample amount of grease into cylinder at the area of piston entrance.
   h. Insert piston slowly into the cylinder. Work the piston back and forth slowly in the cylinder several times.
   i. Now screw back plate firmly onto cylinder.
   j. Repeat leak tests.
   k. If any leak remains, replace piston gasket.
   l. Only if a leak persists, go to procedure below.

3. Pump Head Lubrication
   a. This is only necessary where all previous procedures have failed to correct a leak.
   b. Visually check pump head "O" ring for cracks.
   c. Replace "O" ring if cracked.
   d. Place a light coat of grease on pump cylinder head screw threads and the "O" ring.
   e. Insert new "O" ring.
   f. Screw pump head firmly on to "O" ring and make sure "O" ring is seated uniformly. Overtightening pump head may push "O" ring out of place. Do not overtighten.
   g. Wipe off excess grease.

E. Laboratory Volume Check (To be performed at least after each lubrication)

The Gastec pump can be checked periodically to assure that 100 ± 5 ml are being sampled.

1. Arrange a graduated 100 ml soap film flow meter in a volume test mode.
2. Insert a fresh Gastec tube into the Gastec pump. The tube must be broken at both ends (ready for use).
3. Attach the Gastec tube to top of soap film flow meter with rubber hose. Make sure there are no leaks.
4. Pull pump handle out full to lock at one stroke in normal sampling manner.
5. Wait until the bubble stops moving and read the volume evacuated.
6. If the volume evacuated is other than 100 ± 5 ml, proceed to lubrication instruction and retest.

GASTEC

OXYGEN DETECTOR TUBE NO. 31

The Gastec Detector Tube No.31 provides a rapid fully quantitative analysis of the concentration of OXYGEN in air with an accuracy telerance of ±25 utilizing the Gastec Multi-Stroke Gas Sampling Pump.

PERFORMANCE:

Calibration Scale	6—24% (based on 1/2 pump stroke)
Measuring Range	6—24%
Number of Pump Stroke	1/2 pump stroke only (50 ml)
Detecting Limit	6% at half pump stroke (50 ml)
Sampling Time	1 minute per pump stroke
Color Change	Black—White
Shelf Life	2 years

MEASUREMENT PROCEDURE:

1. Break tips off a fresh analyzer tube and a HCl scrubber tube by bending each tube end in the tube tip breaker of the pump.
2. Connect the ends of the analyzer tube and HCl scrubber tube, marked with ©, using a rubber tubing supplied. Insert the HCl scrubber tube securely into the rubber inlet of the pump with the arrows on the twin tubes pointing toward the pump.
3. Make certain the pump handle is all the way in. Align the red guide marks on the shaft and pump body.
4. Pull the handle until it locks at half pump stroke (50 ml). Wait 1 minute.
5. Read concentration at the interface of the stained-to-unstained reagent when staining stops.

CORRECTION FOR TEMPERATURE, HUMIDITY AND PRESSURE:

Calibration of the Gastec Detector Tube No.31 is based on a tube temperature of 20°C (68°F) and not the temperature of gas being sampled, approximately 50% relative humidity and normal atmospheric pressure. No correction is required for tube temperature of 0—40°C (32°—104°F) and for relative humidity range of 0—100%. To correct for pressure, multiply tube reading by

$$\frac{760}{\text{Atmospheric Pressure (mm)}}.$$

CALIBRATION AND ACCURACY:

The Gastec Detector Tube No.31 is carefully calibrated as an integreal part of the manufacturing process. Calibration and accuracy test are performed using cylinder bottle standard gas.

DETECTION PRINCIPLE:

Oxygen reacts with titanium trichloride and produces titanium dioxide and hydrogen chloride and produces white color stain.

INTERFERENCES:

Ammonia, hydrogen chloride, hydrogen sulfide, sulfur dioxide, nitrogen dioxide, halogens, Carbon dioxide, and Carbon monoxide do not give any effect on tube reading.

SEE OPERATING INSTRUCTIONS INCLUDED WITH THE GASTEC MULTI-STROKE GAS SAMPLING PUMP.

Manufacturer : Gastec Corporation, Yokohama Japan
86K-31-5

Printed in Japan

GASTEC

CARBON DIOXIDE
EXTRA LOW RANGE TUBE NO. 2LL

The Gastec Detector Tube No. 2LL provides a rapid, fully quantitative analysis of the concentration of CARBON DIOXIDE in air with an accuracy tolerance utilizing the Gastec Multi-Stroke Gas Sampling Pump.

PERFORMANCE:

Calibration Scale	300−5,000 ppm (based on 1 pump stroke)		
Measuring Range	100−300 ppm	300−5000 ppm	4600−11,500 ppm
Number of Pump Strokes	3	1	1/2
Detecting Limit*	30 ppm	—	—
Shelf Life	3 years		
Sampling Time	3 minutes/pump stroke		
Color Change	White−Purple		

*The minimum detectable concentration.

MEASUREMENT PROCEDURE:

1. Break tips off a fresh detector tube by bending each tube end in the tube tip breaker of the pump.
2. Insert the tube securely into the rubber inlet of the pump with the arrow on the tube pointing toward the pump.
3. Make certain the pump handle is all the way in. Align the red guide marks on the shaft and housing of the pump.
4. Pull the handle all the way out until it locks on 1 pump stroke (100 ml). Waiting until staining stops.
5. Read concentration at the interface of the stained-to-unstained reagent.
6. If the stain length extends over the highest calibration mark, use 1/2 stroke reading (50 ml) and obtain the true concentration by multiplying the tube reading by 2.3.
7. If the stain does not attain to the first calibration mark, repeat above sampling procedure 2 more times. Obtain true concentration by dividing by 3.
8. To unlock the pump, turn the handle 1/4 turn in either direction.

Carbon Dioxide CO_2

Tube Reading (ppm)	300	500	1,000	2,000	3,000	4,000	5,000
1/2 Pump Stroke (50 ml)	—	—	—	4,600	6,900	9,200	11,500

CORRECTION FOR TEMPERATURE, HUMIDITY OR PRESSURE:

Calibration of the Gastec detector tube No. 2LL is based on a tube temperature of 20°C (68°F) and not the temperature of the gas being sampled, approximately 50% relative humidity, and normal atmospheric pressure. No correction is required for tube temperatures of 0°−40°C (32°−104°F) and for relative humidity range of 10−90%. To correct for pressure, multiply by

$$\frac{760}{\text{Atmospheric Pressure (mmHg)}}.$$

CALIBRATION AND ACCURACY:

The Gastec detector tube No. 2LL is carefully calibrated as an integral part of the manufacturing process. Calibration and accuracy test are performed using combinations of standard reference of known concentrations and dynamic gas flow system, and wet chemical colorimetric technique (Barium hydroxide-Phenolphtalein method) or gas chromatographic technique.

DETECTTON PRINCIPLE:

Carbon dioxide reacts with hydrazine to form carbonic acid monohydrazide, which discolors redox indicator (crystal violet).

$$CO_2 + N_2H_4 \longrightarrow NH_2 \cdot NH \cdot COOH$$

INTERFERENCES:

Interferent	Concentration	Result	Comment
Ammonia	Up to 1,000 ppm	No effect	At more than 1,000 ppm, gives minus error
Carbon monoxide	Up to 500 ppm	"	
Sulfur dioxide	Up to 30 ppm	"	
Nitrogen dioxide	Up to 30 ppm	"	
Chlorine	Up to 20 ppm	"	

DANGEROUS AND HAZARDOUS PROPERTIES:

Threshold Limit Value-Time Weighted Average by ACGIH (1985): 5,000 ppm (7−8 hours)

SEE OPERATING INSTRUCTIONS INCLUDED WITH THE GASTEC MULTI-STROKE GAS SAMPLING PUMP.

Manufacturer: Gastec Corporation, Yokohama, Japan
85E-2LL-3

Printed in Japan

GASTEC

CARBON MONOXIDE
EXTRA LOW RANGE DETECTOR TUBE NO. 1LL

The Gastec Detector tube No. 1LL provides a rapid, quantitative analysis of the concentration of CARBON MONOXIDE in air with a minimum accuracy of ±25% using the Gastec Multi-Stroke Gas Sampling Pump

PERFORMANCE:

Calibration Scale	5—50 ppm (based on 2 pump strokes)
Measuring Range	5—50 ppm
Number of Pump Stroke	2
Correction Factor	Tube reading × 1
Detecting Limit*	1 ppm
Sampling Time	2 minutes per pump stroke
Color Chage	Yellow—Brown
Shelf Life	2 years

* Minimum detectable concentration.

MEASUREMENT PROCEDURE:

1. Break tips off a fresh detector tube by bending each tube end in the tube tip breaker of the pump
2. Insert the tube securely into the rubber inlet of the pump with the arrow on the tube pointing toward the pump.
3. Make certain the pump handle is all the way in. Align the red guide marks on the shaft and housing of the pump.
4. Pull the handle all the way out until it locks on 1 pump stroke (100 ml). Wait until staining stops. Repeat this sampling procedure one more time without removing the tube. For 2 pump stroke (200 ml) sampling, the handle must be turned 1/4 turn in either direction to unlock the pump so the handle can be returned to the starting position.
5. Read concentration at the interface of the stained-to-unstained reagent when staining stops after completion of 2 pump stroke (200 ml) sampling.

CORRECTION FOR TEMPERATURE, HUMIDITY ORPRESSURE:

Calibration of the Gastec detector tube No 1LL is based on a tube temperature of 20°C (68°F) and not the temperature of the gas being sampled, approximately 50% relative humidity, and normal atmospheric pressure. No correction is required for tube temperatures of 0-40°C (32°F—104°F) and for relative humidity range of 20—90%. To correct for pressure, multiply by

$$\frac{760}{\text{Atmospheric Pressure (mmHg)}}.$$

CALIBRATION AND ACCURACY:

The Gastec detector tube No. 1LL is carefully calibrated as an integral part of the manufacturing process. Calibration and accuracy test are performed using combinations of standard reference gas of known concentrations and dynamic gas flow system, and nondispersive infared adsorption (NDIR) or gas chromatographic technique.

DETECTION PRINCIPLE:

Carbon monoxide reduces potassium palladosulfite to liberate metalic palladium, which produces a brown stain.

$$CO + K_2Pd(SO_3Pd(SO_3)_2 \longrightarrow K_2SO_3 + Pd + CO_2 + SO_2$$

INTERFERENCES:

Interferent	Concentration	Result	Comment
Carbon disulfide	1/50 of CO conc.	Plus error	Also produces similar stain by itself
Acetylene	1/50 of CO conc.	"	"
Hydrogen sulfide	1/50 of CO conc.	"	"
Mercaptans	1/50 of CO conc.	"	"
Phosphine	1/10 of CO conc.	"	"
Phosgene	1/10 of CO conc.	"	"
Sulfur dioxide	1/10 of CO conc.	"	No stain by itself
Ethylene	Up to 0.1%	No effect	
Hydrogen	Up to 0.2%	"	
Nitrogen dioxide		"	

DANGEROUS AND HAZARDOUS PROPERTIES:

Threshold Limit Value-Time Weighted Average by ACGIH (1986): 50 ppm (7—8 hours)

Threshold Limit Value-Short Term Exposure limit by ACGIH (1986): 400 ppm (15 minutes)

SEE OPERATING INSTRUCTIONS INCLUDED WITH THE GASTEC MULTI-STROKE GAS SAMPLING PUMP.

Manufacturer : Gastec Corporation, Yokohama, Japan
86X-1LL-5
Printed in Japan

VI. DISTRIBUTION LIST

10 copies

Commander
U.S. Army Biomedical Research and
Development Laboratory
ATTN: SGRD-UBZ-C/Mr. Merrell
Fort Detrick
Frederick, MD 21701-5010

2 copies

Defense Technical Information Center
ATTN: DTIC-DDAC
Cameron Station
Alexandria, VA 22304-6145

1 copy

Commander
U.S. Army Medical Research and
Development Command
ATTN: SGRD-RMI-S
Fort Detrick
Frederick, MD 21701-5012