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

TASK 3 REPORT

(RP/BR Aerosol Effects upon the Spontaneous Activity, Startle Response, Pulmonary Function and Blood Chemistry/Hematology of Black-tailed Prairie Dogs (Cynomys ludovicianus) and Rock Doves (Columba livia))

PRINCIPAL INVESTIGATORS (Scientific)

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

PRINCIPAL INVESTIGATOR (Administrative)

R.D. Thompson

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This Task 3 Report describes research conducted to evaluate selected behavioral-physiological effects of multiple exposures to a red phosphorus-butyl rubber (RP/BR) military smoke product in 2 wildlife species -- black-tailed prairie dog (Cynomys ludovicianus) and rock doves (Columba livia). Results expand the Army's comparative database of sub-lethal consequences of RP/BR-smoke exposure.

Eight separate inhalation-chamber studies were conducted. Each study evaluated the effects of 4 or 2 successive 80-min RP/BR-aerosol exposures in prairie dogs and rock doves, respectively; and, each study involved selected spontaneous activity, startle response, pulmonary function, or blood chemistry/hematology variables. All studies involved a 3-phase paradigm (Pre-exposure, Exposure, and Post-exposure) and 3 RP/BR-smoke exposure groups (0.0, 1.0, and 4.0 mg/ℓ concentrations). Twenty-four prairie dogs or

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rock doves were sampled in each study (8/group), with sex included as a factor in each design. These studies assessed both immediate (<3h out-of-chamber) and acute post-exposure (<12 days after exposure) effects. During exposures, chamber atmospheric conditions were monitored for aerosol mass, phosphoric acid, aerosol opacity, particle sizes, respiratory and contaminant gases, temperature, and relative humidity.

Results indicated that multiple exposures of these species to RP/BR smoke caused a number of immediate and acute behavioral-physiological effects. Most of the effects were subtle, lasting only 1 to 3 days post exposure. Key results gleaned from the studies were:

Chamber Atmospheric Conditions.-- RP/BR aerosol uniformity, respiratory contaminant gases, and in-chamber conditions present during the 192 smoke exposures were of acceptable uniformity. Several specific atmospheric indices were: (a) steady-state aerosol mass concentrations ranged from 0.7 to 1.6 and 4.1 to 5.5 mg/ℓ across all 1.0 and 4.0 mg/ℓ RP/BR burns, (b) phosphoric acids typically accounted for 66 to 75 percent of the mass, (c) particle sizes were invariably ≤1 μm, (d) O_2 ranged between 28 and 23 percent, CO_2 ranged between 0.6 and 1.0 percent, CO never exceeded 20 ppm, and PH_3 and C_6H_{14} were insignificant and detected for only 3 burns.

Mortality/Clinical Symptomatology.-- None of the 96 prairie dogs died during the studies; however, several prairie dogs developed a hoarse bark post exposure. Four of the 96 rock doves died post exposure. All deaths were male doves, occurred within the 4.0 mg/ℓ Groups, and occurred within 3 to 8 days after the second RP/BR exposure session.

Spontaneous Activity.-- Exposure of prairie dogs to 4, 80-min 4.0 mg/ℓ concentrations of RP/BR aerosol was associated with reduced home-cage ambulatory activity (walking, jumping) during the 2-h periods immediately following the first 2 exposure sessions.

Exposure of rock doves to 2, 80-min 4.0 mg/ℓ target concentrations of smoke was linked with reduced horizontal (preening, wing flapping) and ambulatory activity immediately after the first exposure session.

Startle Response.-- Exposures of prairie dogs to RP/BR smoke caused no reliable immediate or acute differences in startle response.

Exposure of female rock doves to 4.0 mg/ℓ aerosol caused decreased latencies (hyper-sensitivity) to electronic photoflash stimuli -- an effect not found for male doves.

Pulmonary Function.-- RP/BR-smoke exposure caused no significant effects on any pulmonary function variable of prairie dogs.

There was a significant Concentration X Day interaction effect for carbon dioxide production (V_{CO_2}), respiratory exchange ratio (RER), and metabolic rate (MR) in rock doves. These interactions were associated with elevated responses of doves exposed to 1.0 mg/ℓ RP/BR concentrations shortly after exposures.

Blood Chemistry/Hematology.-- There were significant Day main effects for oxygen partial pressure (P_{O_2}), hemoglobin (Hb), oxyhemoglobin (O_2Hb), methemoglobin (MetHb) and packed cell volume (PCV) in prairie dogs. These were attributed to handling-induced stress.

There was a significant Sex X Concentration effect for Hb and MetHb in rock doves. Female birds exposed to 4.0 mg/ℓ RP/BR smoke had elevated Hb and MetHb values relative to males. Concentration X Day interactions effects characterized the heterophile and lymphocyte data for doves, with these "stress indices" elevated and decreased, respectively, shortly post exposure.

EXECUTIVE SUMMARY

The objective of this project was to determine behavioral and physiological effects of red phosphorus and butyl rubber (RP/BR) smoke exposure upon 2 species of wildlife -- Black-tailed Prairie Dogs and Rock Doves. The research was comprised of 3 tasks: Task 1 -- Inhalation Equipment Development/Ambient Carbon Monoxide (CO) Evaluation/Aerosol Distribution and Air Quality Study, Task 2 -- Effective Smoke Concentration Range-finding Determinations, and Task 3 -- RP/BR Aerosol Effects upon Spontaneous Activity, Startle Response, Pulmonary Function, and Blood Chemistry/Hematology of Black-tailed Prairie Dogs (Cynomys ludovicianus) and Rock Doves (Columba livia). This report describes the Task 3 research.

Eight separate inhalation-chamber studies were conducted. Each study evaluated the effects of 4 or 2 successive 80-min RP/BR-aerosol exposures in prairie dogs and rock doves, respectively; and, each study involved selected spontaneous activity, startle response, pulmonary function, or blood chemistry/hematology variables. All studies involved a 3-phase paradigm (Pre-exposure, Exposure, and Post-exposure) and 3 RP/BR-smoke exposure groups (0.0, 1.0, and 4.0 mg/ℓ concentrations). Twenty-four prairie dogs or doves were sampled in each study (8/group), with sex included as a factor in each design. The studies assessed both immediate (≤3 h out-of-chamber) and acute post-exposure (≤12 days after exposure) effects. During exposures, chamber atmospheric conditions were monitored for aerosol mass, phosphoric acid, aerosol opacity, particle sizes, respiratory and contaminant gases, temperature, and relative humidity.

Results indicated that multiple exposures of these species to RP/BR smoke caused a number of immediate and acute behavioral-physiological effects. Most of the effects were subtle, lasting only 1 to 3 days post exposure. The most pronounced effects occurred for animals/birds in the 4.0 mg/ℓ Groups. Rock doves showed greater debilitation and mortality than prairie dogs throughout the series of studies. Several spontaneous activity, startle response, pulmonary function, and blood chemistry/hematology variables reflected "chamber-confinement or handling stress," rather than "RP/BR-induced stress," per se.

The following are specific results gleaned from the 8 studies:

RP/BR-aerosol Uniformity.-- Atmospheric conditions present during the 192 chamber exposures were of acceptable uniformity. Several specific atmospheric indices were: (a) steady-state aerosol mass concentrations ranged from 0.7 to 1.6 and 4.1 to 5.5 mg/ℓ across all 1.0 and 4.0 mg/ℓ RP/BR burns, (b) phosphoric acids typically accounted for 66 to 75 percent of the mass, (c) particle sizes were invariably ≤1 μm, (d) O_2 ranged between 28 and 23 percent, CO_2 ranged between 0.6 and 1.0 percent, CO never exceeded 20 ppm, and PH_3 and C_6H_{14} were insignificant and detected for only 3 burns.

Mortality/Clinical Symptomatology.-- None of the 96 prairie dogs died during the studies; however, several animals developed a hoarse bark post exposure. Four of the 96 rock doves died post exposure. All deaths were male doves, occurred within the 4.0 mg/ℓ Groups, and occurred within 3 to 8 days after the

occurred within the 4.0 mg/ℓ Groups, and occurred within 3 to 8 days after the second RP/BR exposure session.

Spontaneous Activity.-- Exposure of prairie dogs to 4, 80-min 4.0 mg/ℓ concentrations of RP/BR aerosol was associated with reduced home-cage ambulatory activity (walking, jumping) during the 2-h periods immediately following the first 2 exposure sessions.

Exposure of rock doves to 2, 80-min 4.0 mg/ℓ target concentrations of smoke was linked with reduced horizontal (preening, wing flapping) and ambulatory activity immediately after the first exposure session.

Startle Response.-- Despite the occurrence of a Concentration X Trial Type interaction for the latency-to-peak (msec) data for prairie dogs, it was concluded that inhalation of 1.0 or 4.0 mg/ℓ RP/BR smoke caused no reliable immediate or acute differences in 3 startle response measures using either electrical foot shock (1.5 mA or 200 VAC) or pure tone (7.8 or 15 KHz) stimuli.

A complex Concentration X Trial Type X Session interaction characterized the latency-to-peak measure of female rock dove startle responses. Exposure to 4.0 mg/ℓ aerosol caused decreased latencies (hyper-sensitivity) to electronic photoflash stimuli. This effect was not found for male rock doves.

Pulmonary Function.-- RP/BR-smoke exposure had no significant effect on any pulmonary function variable of prairie dogs. While there was a significant Day main effect for respiratory exchange ratio (RER), it is viewed as indicative of a ventilatory response to chamber confinement or handling variables.

There was a significant Concentration X Day interaction effect for carbon dioxide production (Vco_2), respiratory exchange ratio (RER), and metabolic rate (MR) of rock doves. The interactions were associated with responses of doves exposed to 1.0 mg/ℓ RP/BR concentrations. There was also a significant Day main effect for oxygen consumption (Vo_2) -- an effect viewed as due to chamber confinement or handling variables and not RP/BR smoke.

Blood Chemistry/Hematology.-- There were significant Day main effects for oxygen partial pressure (Po_2), hemoglobin (Hb), oxyhemoglobin (O_2Hb), methemoglobin (MetHb) and packed cell volume (PCV) in prairie dogs. These probably reflect handling-induced stress during anesthesia and repeated blood collections.

There was a significant Sex X Concentration effect for Hb and MetHb of rock doves. Female birds exposed to 4.0 mg/ℓ RP/BR smoke had elevated Hb and MetHb values relative to males. RP/BR-smoke exposures to 4.0 mg/ℓ concentrations also caused significant Concentration X Day interaction effects for heterophiles and lymphocytes in rock doves that were indicative of transient stress.

FOREWORD

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R. Thompson 8/6/90

PI Signature _____ Date

PREFACE

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 Chemical Development/Registration, Mammal Control Research and Bird Control Research at the Denver Wildlife Research Center (DWRC), Science and Technology (S&T), Animal and Plant Health Inspection Service (APHIS), U.S. Department of Agriculture (USDA) as part of the requirements of Project Order 85PP5847.

This Task 3 Report describes the main findings of 8 behavioral-physiological studies intended to assess sub-lethal consequences of red phosphorus smoke inhalation upon representative wildlife species. It is the third in a series of 4 reports required to fulfill the Project Order.

Research conducted under Task 3 involved 4 independent sets of studies -- Spontaneous Activity, Startle Response, Pulmonary Function and Blood Chemistry/Hematology. Similar experiments were conducted with prairie dogs and rock doves. All studies involved a "pre-exposure, exposure, and post-exposure paradigm," using the same RP/BR-aerosol target concentrations (i.e., 0.0, 1.0 and 4.0 mg/ℓ aerosol) and numbers of exposures for respective species (i.e., prairie dogs received 4 successive 80-min per day exposures and rock doves received 2 successive 80-min per day exposures).

To facilitate conduct of the research, a separate investigator assumed responsibility for each set of studies. Dr. Ray T. Sterner (Research Psychologist) conducted the work involving spontaneous activity measurements; Dr. Stephen A. Shumake (Research Psychologist) performed the evaluations involving startle response measurements; Dr. R. Daniel Thompson (Research Physiologist) conducted the studies involving pulmonary function measurements; and, Mr. Brad E. Johns (Research Physiologist) performed the evaluations involving blood chemistry/hematology measurements. This responsibility continued throughout all phases of the research -- design, data collection, analysis, and report writing.

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Address of Authors: USDA/APHIS/S&T, Denver Wildlife Research Center, Building 16, Denver Federal Center, Denver, Colorado 80225-0266.

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Mr. Henry S. Gardner, Jr. was the Contracting Officer's Representative for the U.S. Army; his advice and guidance on numerous technical/administrative matters proved invaluable during the course of the Project.

Personnel at Oak Ridge National Laboratory (ORNL) provided important technical advice and support. Dr. Roger Jenkins provided key advice on RP/BR-aerosol chemistry and co-ordinated numerous support activities involved in the interagency agreement between DOE and USDA. Mr. Jack Moneyhun supervised the production, analysis, and delivery of the raw RP/BR product. Mr. Tom Gayle provided literature and advice concerning devices for the measurement of startle response in animals. To each of these men, plus many of their unnamed associates, go special thanks.

Mr. Ken Crane, Mr. Stan Gaddis, and Mr. Jeff Homan provided the main technical support at DWRC. Mr. Crane performed the myriad of activities associated with the operation and use of the RP/BR-aerosol and Filtered-air Inhalation Exposure Systems; he also conducted many of the routine chamber-atmosphere measurements (e.g., Gastec Analyzer Tube collections, temperature/relative humidity measurements). Mr. Gaddis assisted with the conduct of most of the RP/BR-aerosol exposure sessions, plus performed most of the aerosol particle size and phosphoric acid titration analyses. Mr. Homan was responsible for operation of the Oxymax 85 System and he performed many of the pulmonary function tests and other diverse duties. All of these individuals performed many hours of data reduction and tabulation.

Our appreciation is also extended to Dr. Albert Dale and members of his staff (Ms. Phyllis Harris, Ms. Jennifer Keller, Mr. Ray Linton and Mr. Jim Waychoff) for efficient care and maintenance of the prairie dogs and rock doves during conduct of the studies.

Special appreciation is due Dr. Rick Engeman for extensive statistical consultations and analyses. Ms. Paige Groninger and Ms. Mary Cameron provided data entry and data file preparation.

Ms. Dolores Steffen provided a number of the graphs presented in certain sections of the Report; and, Jerry Rosencranz (Full Spectrum, Lakewood, CO) provided the technical illustrations of the inhalation, aerosol, and spontaneous activity equipment.

We wish to also thank Ms. Jean Alleman, Ms. Ann Grove, and Mr. Ed Thurston for their help with a variety of budget details and miscellaneous administrative duties related to the Project Order and the ORNL (DOE/USDA) Interagency Agreement.

Finally, we are most grateful to Ms. Linda Leidecker and Ms. Barbara Healzer for their careful typing of the manuscript.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION

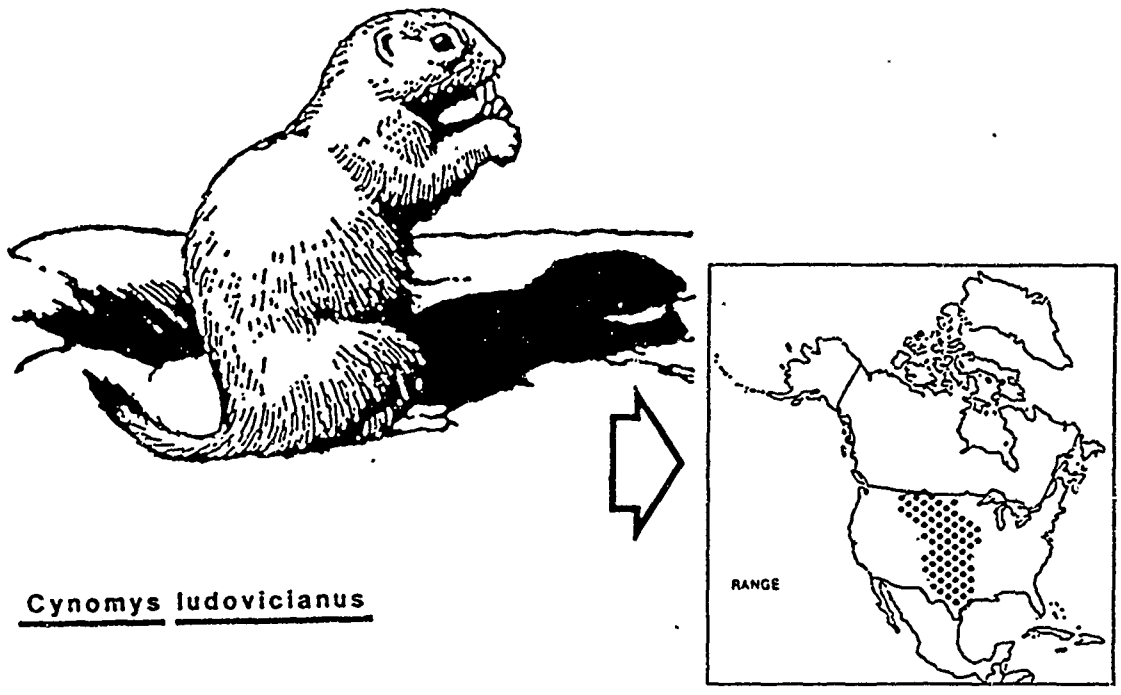
A grenade launching system utilizing red phosphorus butyl rubber (RP/BR) has been developed by the U.S. Army to mask the movements of troops and vehicles during combat (Burton, Clark, Miller, and Schirmer, 1982). This system fires a salvo of grenades that, upon detonation, produce a dense white smoke from the burning of the dispersed RP/BR product. The smoke consists almost entirely of phosphoric (H_3PO_4) and polyphosphoric acid (e.g., $H_4P_2O_7$)particles, with traces of hydrogen (H_2) and carbon monoxide (CO) (Brazell, Moneyhun, and Holmberg, 1984; Yon, Wentzel, and Bane, 1983).

The Health Effects Research Division, U.S. Army Biomedical Research and Development Laboratory (USABRDL) is responsible for determining health and environmental risks associated with munition and munitions by-products, including smokes and obscurants. Whereas the quantities of contaminants released into the environment are always of concern, health and environmental issues associated with contamination from these chemicals have become significant issues affecting the military in recent years. Both direct and residual effects of munitions and smokes have become potential sources of litigation involving military lands and watersheds.

Although environmental hazards posed by repeated use of RP/BR-smoke canisters and grenades on military reservations have been assumed to be negligible, pertinent data are limited. Scientific information on the nature and consequence of RP/BR aerosols includes prior reports on chemical characterization (Brazell et al., 1984; Holmberg, Moneyhun, and Gayle, 1985; Yon et al., 1983), animal toxicology (Aranyi, 1983a; Burton et al., 1982), and environmental fate (Van Voris, Cataldo, Ligote, Garland, McFadden, Frederickson, Li, Bean, Thomas, and Carlile, 1987). The current work is an effort to expand the data base of RP/BR aerosol effects.

Project Order 85PP5847 was aimed at measuring behavioral-physiological effects of RP/BR aerosol in 2 wildlife species --Black-tailed Prairie Dog (Cynomys ludovicianus) and Rock Dove (Columba livia). Research comprising the Project Order was divided into 3 tasks: Task 1--Inhalation Equipment Development/Ambient CO Evaluation/Aerosol Distribution and Air Quality Study, Task 2 -- Effective Smoke Concentration Range-finding Determinations, and Task 3 -- RP/BR Aerosol Effects upon the Spontaneous Activity, Startle Response, Pulmonary Function and Blood Chemistry/Hematology of Black-tailed Prairie Dogs (Cynomys ludovicianus) and Rock Doves (Columba livia). Figure 1 presents illustrations and distributional maps of the Black-tailed Prairie Dog and the Rock Dove.

Prairie dogs and rock doves were selected as representative wild mammalian and avian models to document potential toxicological, behavioral and physiological effects of RP/BR-smoke exposure. Results of inhalation chamber studies involving these species should indicate potential RP/BR-smoke effects upon a variety of North American wildlife species (common or endangered). These data should prove useful to the U.S. Army, and to the



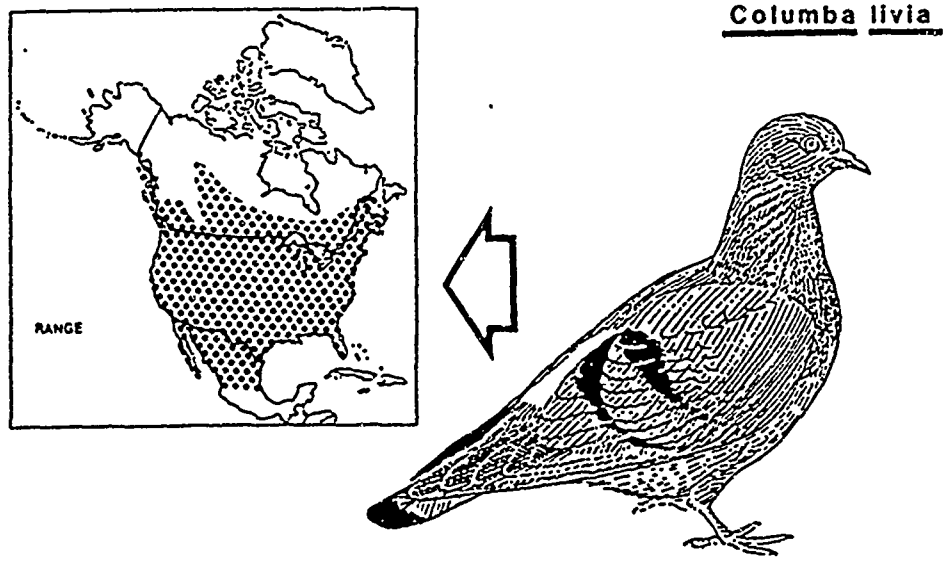


Figure 1. Illustrations of a black-tailed prairie dog (Cynomys ludovicianus) and a rock dove (Columba livia), with distributional range maps for each species shown as inserts.

public, in determining the environmental risks associated with frequent, repeated detonation of RP/BR grenades on military lands.

Task 3 inhalation-chamber studies were conducted between March and November 1988. The objective was to evaluate potential immediate and acute post-exposure consequences of multiple sub-lethal exposures to RP/BR aerosol upon selected behavioral-physiological variables in prairie dogs and rock doves.

II. EXPERIMENTAL APPROACH

Task 3 involved 8 inhalation chamber type studies. One study each was conducted to assess potential sub-lethal effects of RP/BR-aerosol inhalation upon selected spontaneous activity, startle response, pulmonary function and blood chemistry/hematology variables in prairie dogs and rock doves. All Task 3 Studies were designed to examine immediate (≤ 3 h out-of-chamber) and acute post-exposure effects (≤ 12 days) of multiple RP/BR-aerosol exposures. Similar research designs were used for studies conducted on both species.

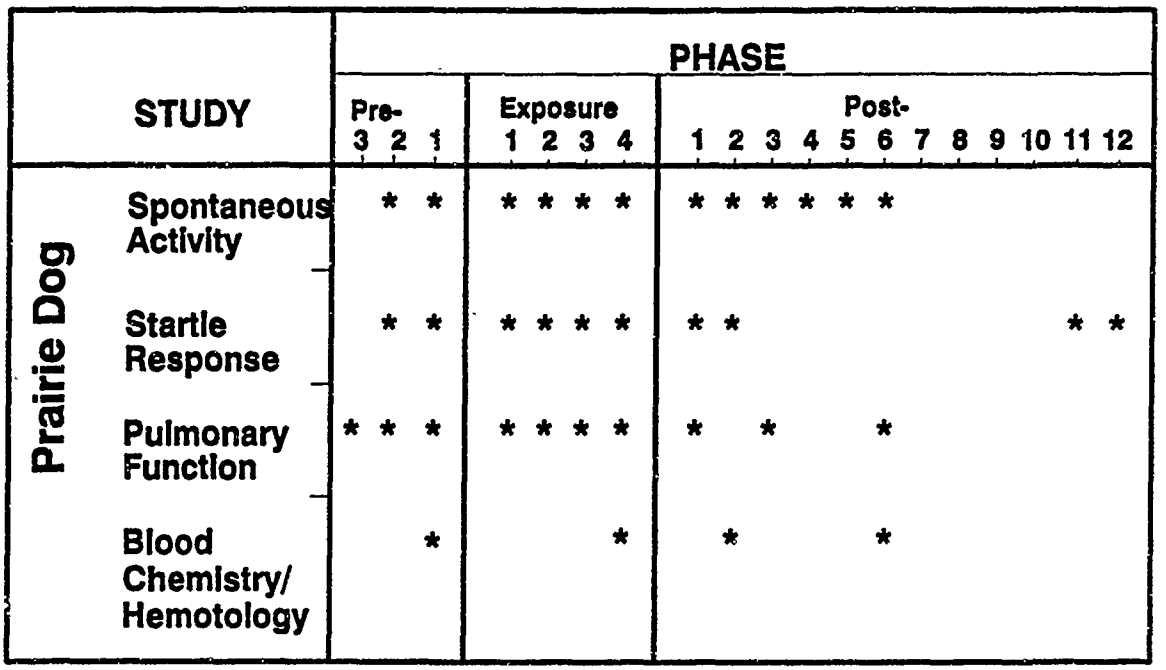
A. Pre-exposure/Exposure/Post-exposure Paradigm

All studies involved a sequential 3-phase paradigm -- Pre-exposure, Exposure, and Post-exposure Phases. These Phases corresponded to measurements which occurred preceding, during, and following the multiple-day exposures of animals to RP/BR aerosol or filtered air (control), respectively. Assessment of the potential effects of RP/BR-aerosol inhalation was based on shifts in measured responses that occurred temporally across these Phases. It should be noted that all Exposure Phase measurements were collected ≤ 3 h after the 80-min RP/BR exposures. No behavioral-physiological measurements were obtained during actual RP/BR-aerosol exposures.

Figure 2 is a schematic illustration of the paradigm relevant to the Spontaneous Activity, Startle Response, Pulmonary Function and Blood Chemistry/Hematology Studies. As shown, the specific behavioral-physiological variables were collected on similar days of each study; however, the frequency and occurrence of these data collections differed among the studies.

B. Experimental Designs

Each study involved a similar research design and used 24 animals. Three "common factors" inherent to the studies were: (a) RP/BR-aerosol/filtered-air concentration, (b) measurement sessions (days), and (c) sex of animals. Replications (i.e., the separate conduct of a design on several occasions with only a portion of the animals) were involved in all but the Startle Response Studies; however, this factor was omitted from most analyses due to limited degrees of freedom.



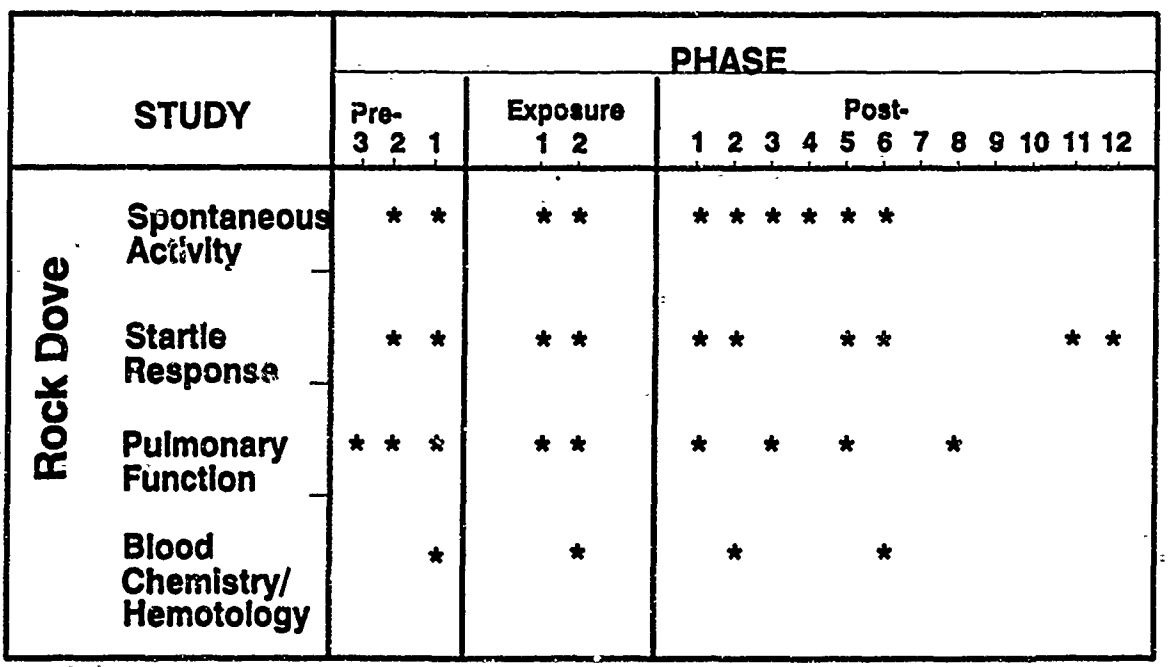


Figure 2. Schematic illustration of the research paradigm showing the days during each phase that respective measurements were collected for prairie dogs (top) and rock doves (bottom) in each behavioral-physiological study. (Note.-- The measurements made for the Exposure Phase occurred immediately after (within 2-3 h) animals or birds were removed from the inhalation chamber(s) -- no in-chamber behavioral-physiological data were collected).

Data were analyzed using analysis of variance (ANOVA). All studies with balanced, complete data were analyzed using the PROC ANOVA Program of the Statistical Analysis System (SAS) package of programs (SAS Institute, Inc., 1985) and the Type III sums of squares to determine significant effects. All unbalanced, missing-data designs (e.g., deaths, mis-sexed animals, omission of data) were analyzed using the PROC GLM Program of the SAS package (SAS Institute, Inc., 1985) and the Type III sums of squares to test for significant effects. Throughout all studies, statistical effects were tested at the 0.05 level of significance; where significant ANOVA terms occurred, post hoc Duncan Multiple Range Tests (0.05 level of significance) were used for pair-wise comparisons of respective means (Waller and Duncan, 1969).

1. Selected RP/BR-aerosol Concentrations (Plus Filtered Air) and Multiple-exposure Schedules

   All designs involved 3 separate groups of prairie dogs and rock doves which received 4- and 2-successive days of 80-min exposures, respectively, to target concentrations of either 0.0 (filtered air), 1.0, or 4.0 mg/ℓ RP/BR aerosol. Selection of these exposure/concentration schedules was based on results presented in Sterner et al. (1988) and Shumake et al. (1989).

   Exposure/concentration schedules were chosen so as to minimally and maximally challenge each species without causing mortality. For each species, we kept constant the numbers of exposures and concentrations for the 4 types of behavioral-physiological studies. This afforded some cross comparison of sub-lethal effects among the 4 types of variables. The following paragraphs detail the selection of the schedules.

   The maximal RP/BR-aerosol target concentration used in Task 3 Studies was 4.0 mg/ℓ with 250 ℓ/min air flow. This was determined from an examination of the observed in-chamber CO levels (Task 1) and mortality effects (Task 2). Computation of a linear regression equation between aerosol concentration (mg/ℓ) and CO (ppm) showed that a concentration of 4.47 mg/ℓ RP/BR aerosol was associated with the 1 h Short-term Limit Threshold of 35 ppm CO as set for human industrial exposure (National Research Council, 1977). Selection of a maximal 4.0 mg/ℓ (i.e., 180 μm extrusion setting) "target concentration" avoided confounding potential RP/BR-aerosol effects with those of CO. Additionally, results of Toxicity Range-finding Studies showed that 2 successive exposures to a 6.0 mg/ℓ and 3 successive exposures to a 3.0 mg/ℓ target concentration of RP/BR smoke produced some dove mortality (Shumake et al., 1989); thus, selection of a 2 exposure, 4.0 mg/ℓ aerosol schedule seemed appropriate for the current sub-lethal studies.

   The minimal target concentration was determined based on the capabilities of the RP/BR extruder. A 1.0 mg/ℓ concentration

(50 μm extrusion setting) at 250 ℓ/min air flow was near the minimum stable operating condition of the RP/BR extruder pump. This consistently produced a steady, slow burn of the RP/BR product, without "flame outs" and "re-lighting problems." Inclusion of a minimal aerosol exposure/concentration schedule was intended to provide a "point of reference" for comparison of sub-lethal effects produced by the 4.0 mg/ℓ concentration.

Finally, control groups of prairie dogs and rock doves were included in all studies. These groups were exposed to filtered air (0.0 mg/ℓ RP/BR-aerosol) for 4- and 2-successive daily exposures, respectively. Animals were placed in the Filtered-air Inhalation Chamber System for approximately 80-min.

2. Measurement Sessions (Days)

The term session is interchangeable with days of measurement, but the successive sessions did not necessarily occur on consecutive dates. During the Post-exposure Phase, most measurements were collected on intermittent dates (see Figure 2).

With the exception of blood chemistry/hematology variables, all studies involved, 2-3 Pre-exposure Sessions, 4 (prairie dogs) or 2 (rock doves) Exposure Sessions and 2-6 Post-exposure Sessions. Blood chemistry/hematology measurements were limited to 1 Pre-exposure, 1 Exposure and 2 Post-exposure Sessions; these few measurements were intended to reduce stress upon animals caused by the blood-sampling procedures. The longest chronological period encompassed by any study was 18 consecutive days (i.e., Prairie Dog Startle Response Study).

3. Sex of Animals/Birds

Sex of animals was included as a variable in all studies. All studies with prairie dogs involved balanced designs (n = 4 males and 4 females per RP/BR-aerosol or filtered-air group). This was aided by the easily recognized, external genitalia of the species.

All designs with rock doves were unbalanced, except for the Blood Chemistry/Hematology Study. Unbalanced designs resulted from some birds being mis-sexed using the cloacal examination method of Miller and Wagner (1955). A final determination of each bird's sex was made after the Post-exposure Phase based upon a laparotomy and internal inspection of each birds reproductive organs following euthanasia. These determinations typically revealed some errors of assignment (sexing) based on the cloacal procedure.

C. Actual Research Timelines

Figure 3 is a schematic illustration of the research timeline associated with the conduct of each behavioral-physiological study in Task 3. The actual conduct of the various behavioral-physiological

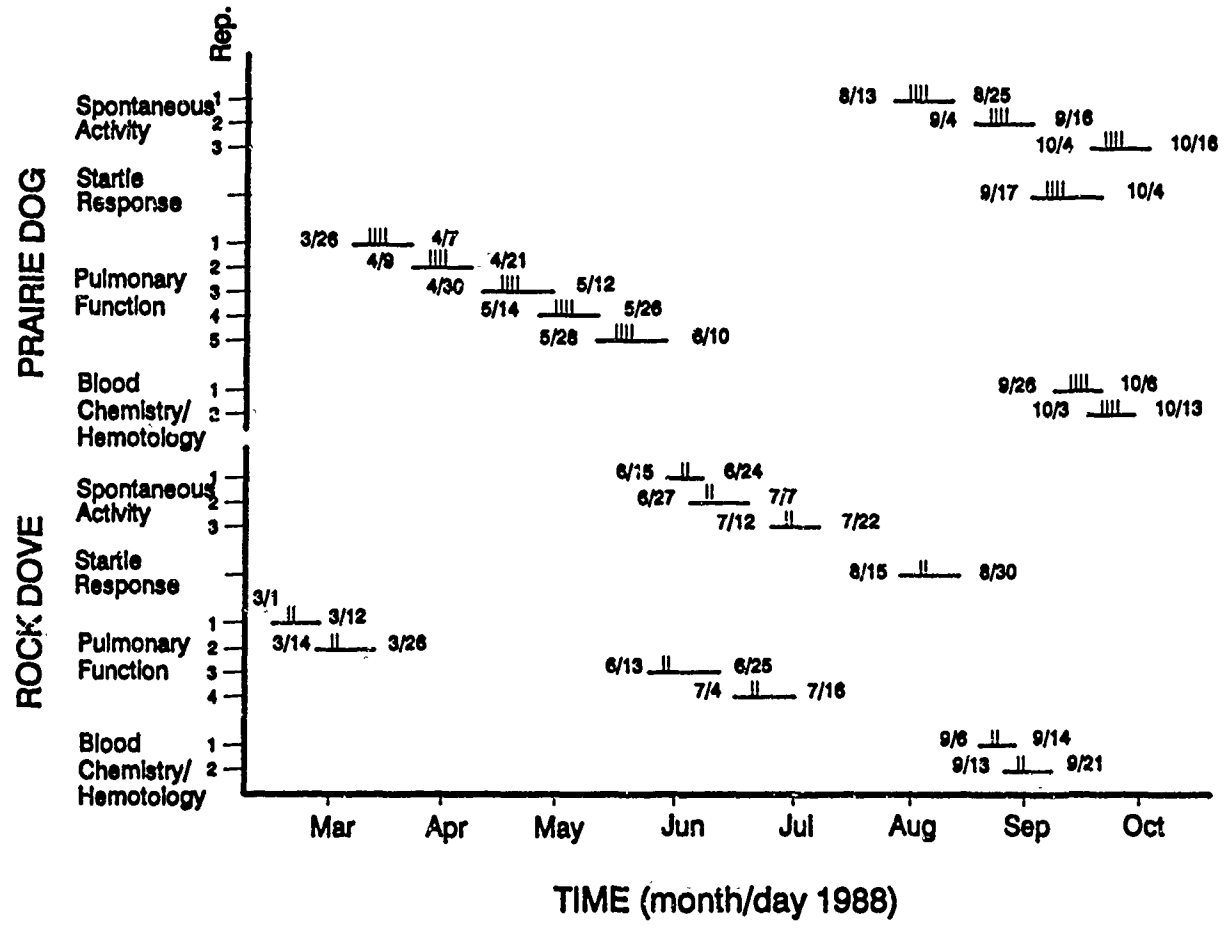


Figure 3. Schematic illustration of the research timelines for the actual conduct of the 8 behavioral-physiological studies. (Note.-- Dates shown refer to the first and last day of each replication; upright lines (|) refer to the Exposure Days.)

studies covered a 7.5-month period -- March to November 1988. Of course, preparations (equipment procurement, animal capture/ quarantine, etc.), pilot tests, and data analyses encompassed a much longer period (i.e., roughly July 1987 to June 1989).

III. GENERAL METHODS

A. Animals/Birds

The conduct of all studies adhered to provisions set forth in the "Guide for the Care and Use of Laboratory Animals" prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, Department of Health, Education, and Welfare, 1978). All animal cages, handling procedures, and anesthetizations conformed with the provisions of the Animal Welfare Act of 1966 plus amendments (Public Laws 89-554, 91-579, and 94-279) in force at the time of the research.

1. Black-tailed Prairie Dogs

Appendix A presents detailed descriptions of animal care procedures used for maintenance, care, and handling of prairie dogs and rock doves during Task 3 Studies. Animal handling procedures were essentially the same as those described for Task 2 (Shumake et al., 1989).

All prairie dogs were captured at Buckley Air National Guard Base, Aurora, Colorado. A total of 206 animals were obtained during 2 separate week-long capture sessions in early February (n = 110) and December 1987 (n = 96). Altogether, 96 prairie dogs (48 males, 48 females) were used in Task 3, plus approximately 30 that participated in pilot studies. Of these, 13 and 83 prairie dogs were from the February and December Captures, respectively.

Upon arrival at DWRC, the prairie dogs were sexed and placed in separate male and female communal areas (approximately 5.7 X 3.45 X 3.6 m each) within a brick building (Building 90C) located on the grounds of the Denver Federal Center. Wood shavings (approximately 4 cm deep) covered the floor. Animals were fed Purina Rabbit Checkers ad libitum, with fresh cabbage provided 3 times per week; water was available ad libitum in several large "poultry-type" watering dispensers. Building 90C was isolated from the main research area (Building 16) for purposes of biosecurity; it had adequate temperature control ($18^{o} \pm 5^{o}$ C), and light:dark control was well maintained (12:12 h, 0600-1759 h and 1800-0559 h, respectively). The 14-day quarantine periods were April 15 - 29, 1987 and December 20, 1987 - January 3, 1988. After health checks (Veterinary Services, APHIS, USDA), animals were officially released from quarantine; however, the animals were held under these same conditions until the time of specific studies.

Upon selection for participation in 1 of the studies, designated prairie dogs were transported to Building 16 -- the main research site. Here, the prairie dogs underwent 5+ days of acclimation prior to participation in any study. During acclimation and non-test portions of their stay in Building 16, prairie dogs were held singly or as same-sex pairs in stainless steel cages (61 X 62.5 X 41 cm) within Room 157S (see Figure 5). Throughout the time at Building 16, animals were fed the diet cited for quarantine. All of the Building 16 animal-holding areas were temperature ($23^{o} \pm 2^{o}$ C) and light:dark (12:12 h, 0600-1759 h = light and 1800-0559 h = dark) controlled.

During all non-test portions of the Startle Response, Pulmonary Function, and Blood Chemistry/Hematology Studies, animals were housed in Room 157S. These prairie dogs were held individually in stainless steel cages (61 X 62.5 X 41 cm). Prairie dogs involved in the Spontaneous Activity Study, however, were acclimatized in Room 157S, but were moved to Room 168B (see Figure 5) for continuous activity recordings. Spontaneous activity measurements involved housing prairie dogs individually in special Plexiglas cages (43.2 X 43.2 X 31.7 cm) during conduct of this Study.

2. Rock Doves

Rock doves selected for Task 3 Studies were drawn from birds that had been purchased from a local supplier on 2 separate occasions. That is, a pool of birds existed that were part of (1) a shipment of 122 doves purchased on January 7, 1987 (i.e., the remainder of unused birds from Task 2; Shumake et al., 1989) and (2) a shipment of 100 doves purchased on April 26, 1988. All birds were captured in the Greater Metropolitan Denver Area with cannon nets after pre-baiting a feeding site with cracked corn (see Grubb, 1988).

Birds obtained in 1988 were checked and held in wire mesh outdoor aviary cages (3.0 X 1.5 X 1.8 m). Up to 30 doves were held per cage, and fed ad libitum Purina Pigeon Checkers, cracked corn, grit, and water. After several weeks in this outdoor aviary, the doves were moved to an indoor quarantine facility. This was an 11.5 m-diameter steel Butler building, with heat and light provided. During quarantine, birds were maintained on Purina Pigeon Checkers and water ad libitum in this facility on a 12:12 h forward light:dark cycle for 14 days (May 25 - June 8, 1988). (Note.-- The quarantine dates for doves obtained on January 7, 1987 were April 9 - May 7, 1987).

Upon completion of quarantine, the rock doves were held in the same Butler building until selected for 1 of the Task 3 Studies. Transport and acclimation to Building 16 was identical to that described for prairie dogs, except that doves were held in galvanized wire mesh cages (51 X 27 X 38 cm) within Room 160 (see Figure 5). The birds were fed the same diet as mentioned for the Quarantine Period. Doves participating in the Spontaneous

Activity Study were housed individually in the special Plexiglas cages (43.2 X 43.2 X 31.7 cm) in Room 168B.

Altogether, 96 rock doves (49 males and 47 females) were used in Task 3, plus approximately 30 birds for pilot studies. Composition of birds from the 1987 and 1988 shipments was 39 and 57, respectively.

B. Inhalation-exposure Systems

Two separate inhalation systems were used to expose animals to either RP/BR aerosol or equivalent durations of filtered air. The Modified RP/BR Extruder and Inhalation Chamber System and the Filtered-air Inhalation Chamber System were described in the Task 1 and 2 Reports (Sterner et al., 1988; Shumake et al., 1989).

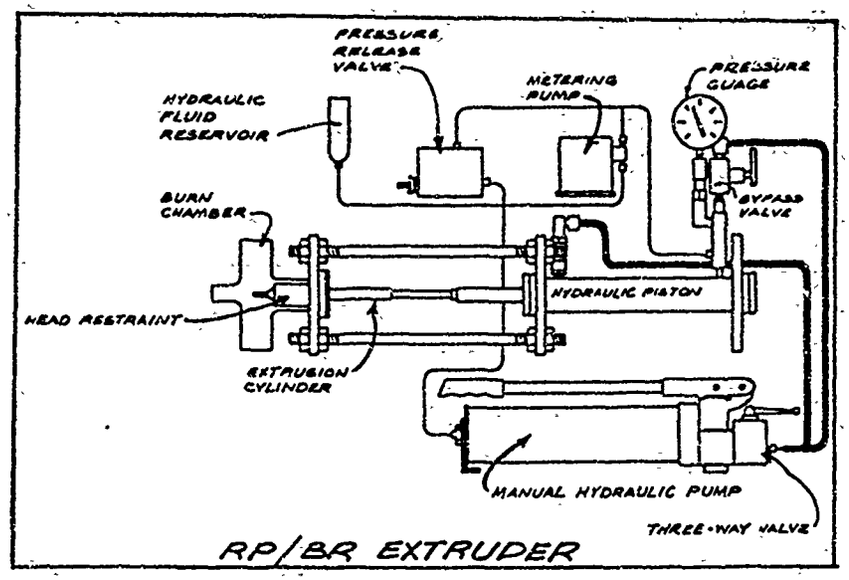
Essentially, each system was constructed of identical materials and components. Each had independent closed-air supplies with separate air-filtration, air-humidification and air-movement equipment. Negative air pressure produced by individual ceiling vents (approx. 15-room air exchanges/h) within each system-housing room (i.e., Rooms 158 and 159, see Figure 5) prevented any inadvertent smoke contamination of the animal-holding areas.

1. Modified RP/BR Extruder and Inhalation Chamber System

Figure 4 illustrates the Modified RP/BR Extruder and Inhalation Chamber System. The insert of Figure 4 is a detailed drawing of the RP/BR extruder equipment. This System is similar to that described by Holmberg et al. (1985) and Aranyi (1983a, 1983b, 1984, and 1986). Operation of the RP/BR-aerosol System involved 4 elements: (a) Formulation of RP/BR Product, (b) RP/BR Extruder/Generator Subsystem, (c) Inhalation Chamber Subsystem, and (d) Air-movement/-condition/-filtration Subsystem (see Appendix B).

2. Filtered-air Inhalation Chamber System

Figure 5 is a technical illustration of the Filtered-air Inhalation Chamber System; this is the same System described by Shumake et al. (1989). The insert of Figure 5 shows the general floor plan of the DWRC Laboratory (Building 16), with locations of animal-housing areas, inhalation systems, and behavioral-physiological test rooms noted. The Filtered-air System was used to expose control groups of animals to roughly equivalent durations of filtered air. This System was constructed to duplicate properties of the Modified RP/BR Extruder and Inhalation Chamber System, but without the generation of RP/BR aerosol. A detailed description of the System is provided in Appendix B.



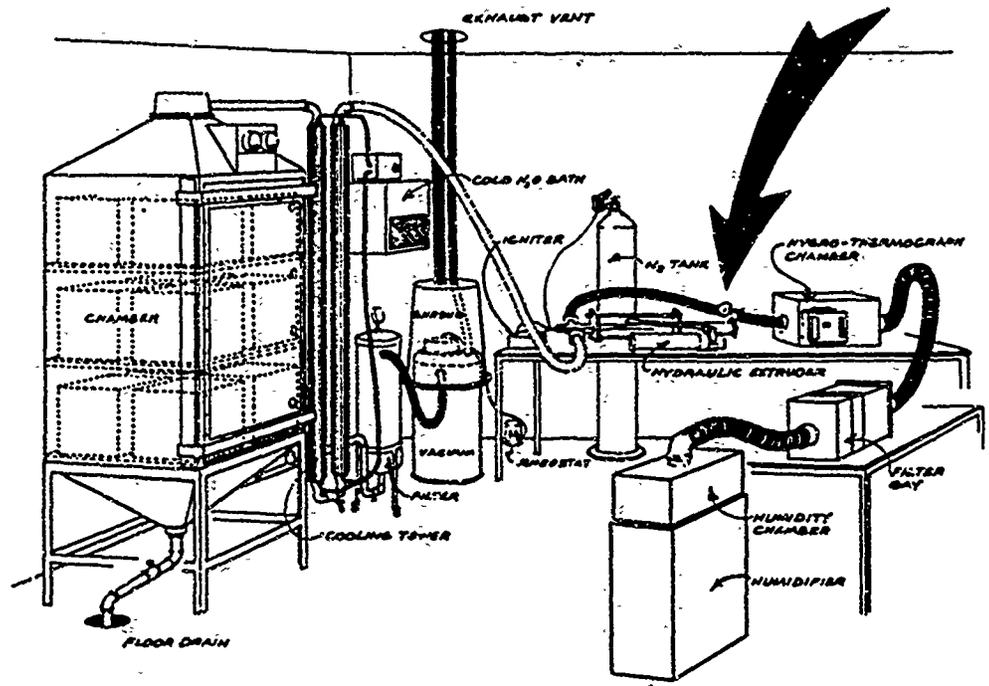
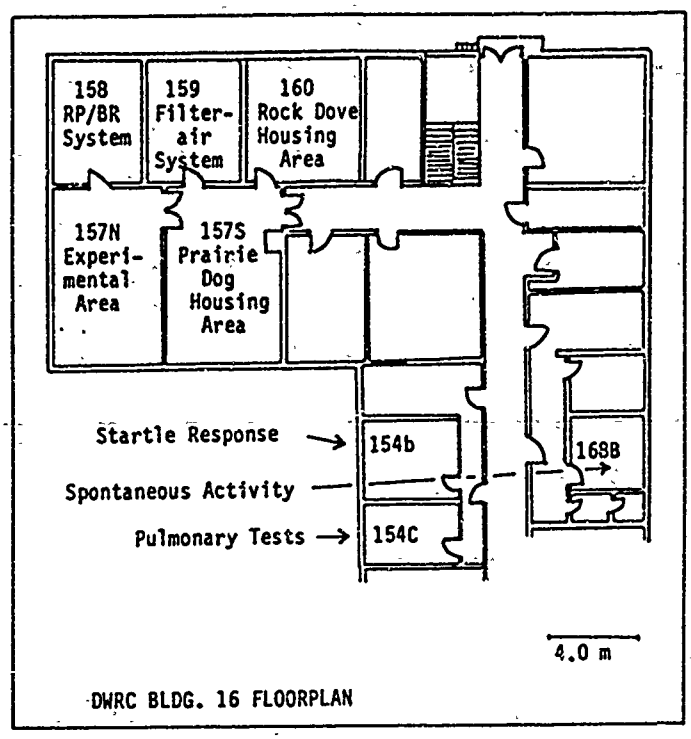


Figure 4. Technical illustration of the Modified RP/BR Extruder and Inhalation Chamber System, with schematic diagram of the RP/BR extruder/generator shown in the insert. (Note.-- Components of the System are scaled relative to the perspective, i.e., 1 cm equals 0.236 m, but the locations of some components have been drawn to improve the visual display.)



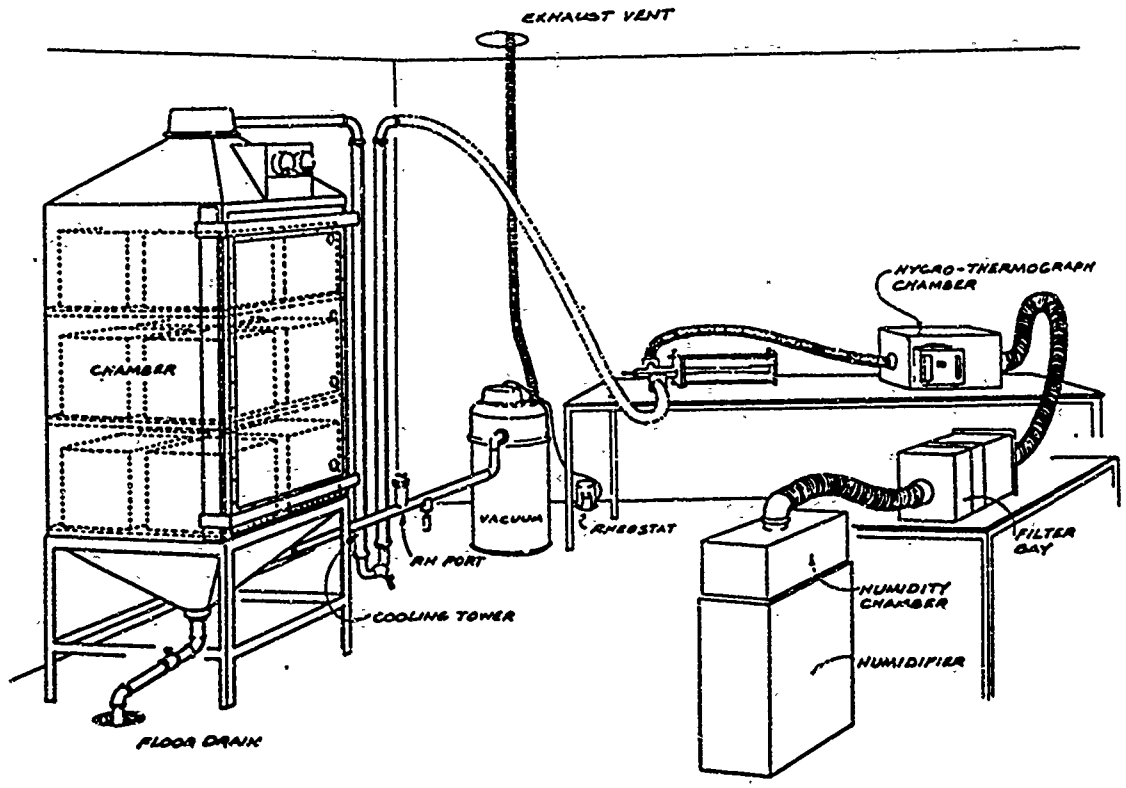


Figure 5. Technical illustration of the Filtered-air Inhalation Chamber System, with the floorplan for the research areas of the DWRC Laboratory shown as an insert. (Note.-- Components of the System are scaled relative to the perspective, i.e., 1 cm equals 0.236 m, but the locations of some components have been drawn to improve the display.)

C. RP/BR Aerosol and Filtered-air Monitoring

Characterization of the chamber atmosphere present for each RP/BR-aerosol or filtered-air exposure during the behavioral-physiological studies was accomplished using techniques also described in the Task 1 and 2 Reports (see Sterner et al., 1988; Shumake et al., 1989). These measurements provided for assessments of the RP/BR-aerosol dosages presented to the experimental animals. Table 1 lists these chamber atmosphere measurements and respective analytical methods; whereas, Figure 6 illustrates the chamber monitoring schemes associated with the Modified RP/BR Extruder and Inhalation Chamber System and the Filtered-air Inhalation Chamber System during each exposure session.

Sampling of in-chamber conditions differed for the 2 chamber Systems. Checks of the Modified RP/BR Extruder and Inhalation Chamber System atmosphere involved 7 sets of variables: (1) aerosol mass, (2) phosphoric acid (H_3PO_4) titration, (3) aerosol opacity, (4) aerosol particle size, (5) respiratory gases, (6) contaminate gases and (7) temperature/humidity. Checks of the Filtered-air Inhalation System atmosphere involved 5 sets of variables: (1) aerosol mass, (2) phosphoric acid, (3) respiratory gases, (4) contaminant gases, and (5) temperature/humidity; no opacity or particle size readings were taken for the Filtered-air System.

1. Aerosol Mass

Aerosol mass 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). Aerosol mass of both RP/BR aerosol and filtered air was measured gravimetrically.

2. Phosphoric Acid (H_3PO_4) Titration

Titration analysis was used to determine the amount of H_3PO_4 contained on each filter-disc. Following gravimetric analysis, individual filter discs were deposited into covered plastic Petri dishes (Miles Laboratories Inc., Naperville, IL). The petri dishes were then stored in a ventilated cabinet for between 48 and 168 h to allow for complete hydrolysis of the acids. Titration analysis involved the use of a Radiometer DTS-800 Multi-titration System (Radiometer America Inc., Cleveland, OH). Upon removal of the filter from storage, H_3PO_4 acid residue from each disc was extracted using 60 mℓ of boiled deionized water in a 400 mℓ glass beaker and agitated with a magnetic stir bar for 10 min. Subsequently, a 20 mℓ sample of the extracted solution was used for titration analysis with 0.1N or 0.01N NaOH. The titrator was programmed to calculate mg of H_3PO_4 in total extracted sample by inflection-point titration.

Table 1. List of variables, plus respective analytical techniques, used to characterize in-chamber conditions during the RP/BR or filtered-air exposures of the behavioral-physiological studies.

Variable	Technique
Aerosol Mass	Gravimetric Analysis
Phosphoric Acid (H_3PO_4)	Titration analysis
Aerosol Opacity [a]	ORNL Infrared Detector
Aerosol Particle Size [b]	QCM Cascade Impactor
Respiratory Gases [b]	
Oxygen (O_2)	Gastec Analyzer Tube
Carbon Dioxide (CO_2)	Gastec Analyzer Tube
Contaminant Gases [b]	
Carbon Monoxide (CO)	Gastec Analyzer Tube
Phosphine (PH_3)	Gastec Analyzer Tube
Hexane (C_6H_{14})	Gastec Analyzer Tube
Temperature/Humidity	
Temperature	Digital Thermometer
Relative Humidity	Wet-/Dry-bulb Thermometer

[a] Although aerosol opacity charts and digital counts of aerosol density were obtained for each RP/BR burn, these data were not summarized for analysis.

[b] These measures were taken during alternate day RP/BR burns, however, these were omitted from most filtered-air exposures.

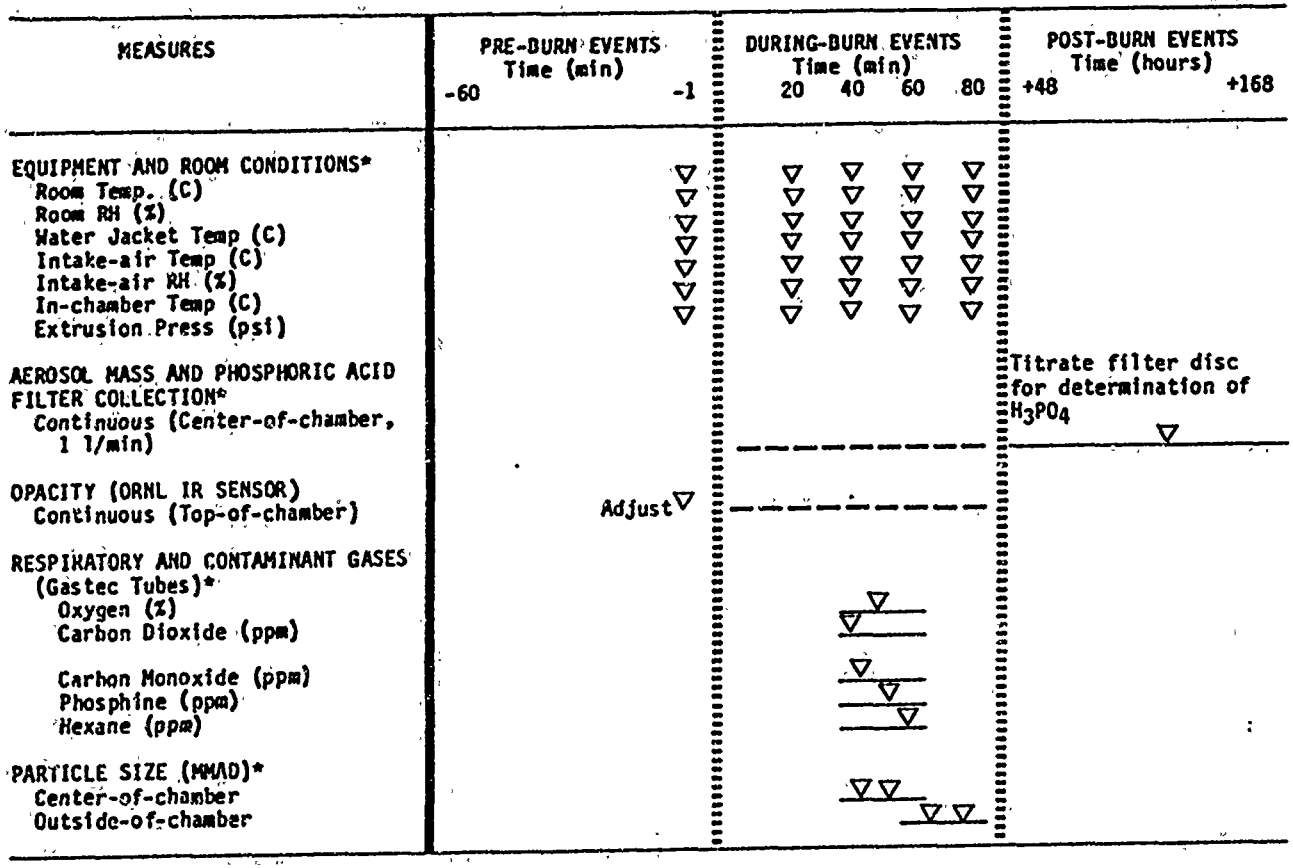


Figure 6. Schematic illustration showing the sampling schedule used to monitor RP/BR and filtered-air atmospheres on selected days. (Note.-- "Arrows" refer to discrete-type measurements taken at fixed times; "Arrows above solid lines" refer to discrete-type measurements taken at fixed, but variable, times within the period shown by the line; and "dashed lines" refer to the continuous sampling of aerosol or opacity.) Measurements of Equipment And Room Conditions and Aerosol Mass And Phosphoric Acid Filter Collection were collected for every RP/BR and filtered-air exposure; whereas, measurements marked with an asterisk (*) were collected during alternate-days of exposures for RP/BR-aerosol Groups and intermittently during the exposures for Filtered-air Groups.

3. Aerosol Opacity

The density of RP/BR aerosol within the inhalation chamber was monitored continuously during the burns using an ORNL Aerosol Sensor (Higgins, Gayle and Stokely, 1978; Holmberg et al., 1985). This sensor consisted of an infrared light-emitting diode mounted beside, but optically separated from, a photo-transistor. An analog record of the mV output from the transistor was plotted with a chart recorder.

4. Aerosol Particle Sizes

Particle sizes were derived from aerosol sample measurements using a Piezo-electric Quartz-Crystal-Micro-Balance (QCM) Cascade Impactor (California Measurements Inc., Sierra Madre, CA). A 10-sec (300 μl) sample of aerosol or air for each respective measurement was circulated a minimum of 90 sec within the impactor column. Injections of aerosol or air were drawn into the stack of matched frequency quartz crystal oscillator pairs using the high concentration slide valve. Actual determinations of MMAD for each sample were completed using a graphical procedure Chuan (1986).

5. Respiratory Gases

Oxygen (O_2) and carbon dioxide (CO_2) levels within the inhalation chamber were measured using the Gastec Gas Detection System (Gastec Inc., Newark, CA) -- a standard industrial-hygiene-type analyzer tube and pump system (see Appendix B). Actual percent O_2 and ppm CO_2 were corrected for atmospheric pressure at 1646 m (5400 ft) elevation based upon the following formula:

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

6. Contaminant Gases

Determination of the amounts of CO, phosphine (PH_3), and hexane (C_6H_{14})were performed using procedures similar to those described for respiratory gases (see Appendix B).

7. Temperature/Humidity

In-chamber temperatures were recorded at successive 20-min intervals throughout each exposure from a VWR Digital Thermometer (Van Waters and Rogers, Denver, CO).

A standard wet-/dry-bulb thermometer was used to measure in-chamber RH; the RH was determined using standard charts corrected for altitude (Department of Commerce, 1965).

D. RP/BR-aerosol and Filtered-air Exposure Conditions

Descriptions of the chamber atmospheres present during RP/BR-aerosol and filtered-air exposures are crucial to assessing the doses inhaled by test animals. Of course, precise estimates of the doses of RP/BR aerosol inhaled by individual prairie dogs and rock doves are impossible; unknown factors (e.g., respiration rate, ventilatory exchange volume) affect dose delivery. Instead, whole-body exposure studies, such as the current ones, rely on accurate characterization of the inhaled atmospheres for dosage information. These characterization data only provide approximate, group-type dosage estimates. The following is a description of current approaches used to characterize special aspects of exposure and air quality for the behavioral-physiological studies (Task 3).

1. RP/BR-aerosol Exposure

Figure 7 presents 2 representative tracings of the in-chamber opacity charts obtained using the ORNL infrared sensor during Task 3 RP/BR burns. The top tracing represents the opacity of aerosol associated with an RP/BR burn at a target concentration of 4.0 mg/ℓ (180 m extrusion setting with 250 ℓ/min air flow); whereas, the bottom tracing represents the opacity associated with a target concentration of 1.0 mg/ℓ (50 m extrusion setting with 250 ℓ/min air flow).

Note that the opacity chart for each RP/BR concentration follows a 3-phase pattern during the 80-min exposure trials. These phases can best be described as: (1) Chamber-fill Period -- an approximately 10-20 min period of increasing RP/BR-aerosol concentration during which the chamber is filled with aerosol. (2) Steady-state Concentration Period -- an approximately 40-50 min period of relatively asymptotic maximal aerosol concentration. (3) Chamber-vent Period -- an approximately 20-min period of decreasing aerosol concentration during which the RP/BR product is extinguished and the chamber is vented of aerosol. The total dose of aerosol inhaled by each animal is influenced largely by this 3-phase RP/BR-aerosol concentration pattern.

2. Estimation of Steady-state Aerosol Mass Concentration

Because the Steady-state Concentration Period (Figure 7) was the main dose-delivery portion of the aerosol-exposure cycle, an estimate of this "asymptotic, maximal, steady-state concentration" was derived for a representative number of PR/BR burns in each behavioral-physiological study. The procedure used was the same as that reported for Task 2 (Shumake et al., 1989), and a detailed description of the procedure (plus validation data) is contained in Appendix C.

Briefly, precise planimeter tracings were made of numerous ORNL infrared opacity charts for each Task 3 Study. The planimetric area under the curve of a 36 to 40 min period for maximum stability

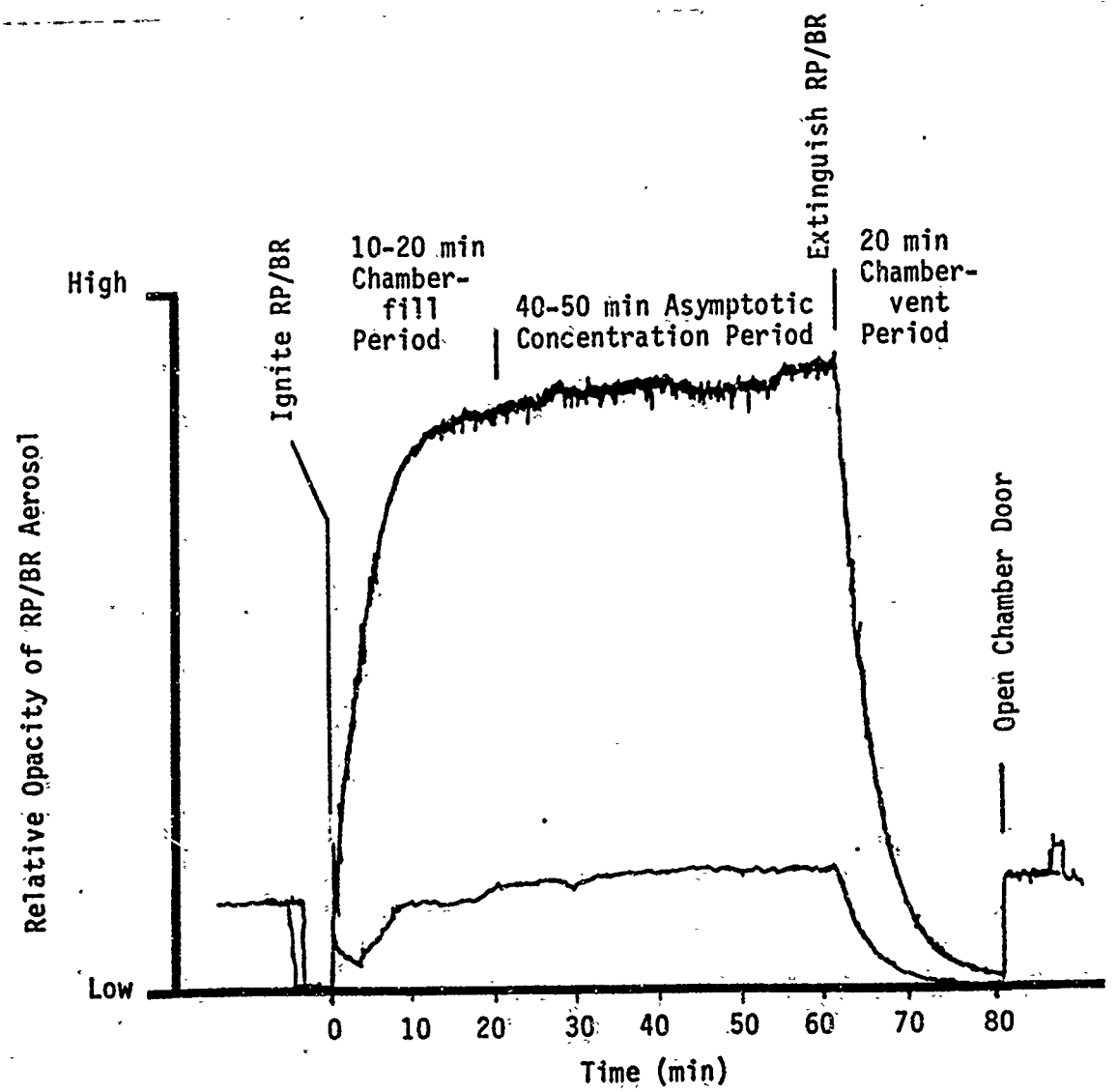


Figure 7. Two tracings of the ORNL infrared sensor recordings for representative RP/BR burns at the 4.0 (180 m pump setting; top) and 1.0 mg/ℓ (50 m setting; bottom) target concentrations at a 250 ℓ/min air flow rate. (Note.-- The 36 to 40 min portion of the Steady-state Concentration Period used to derive planimetric estimates of aerosol mass differed for specific burns due to occasional flameouts or other unstable RP/BR burn conditions.)

(usually from 24 to 60 min) and the total 80-min opacity recording area under the curve was determined. The fractional proportion of these 2 separate areas was multiplied by the total aerosol mass (mg) collected for the respective burn. This product was then divided by the number of min of 1 ℓ/min sampling under the steady-state portion of the curve. These estimates, along with other in-chamber aerosol, particle size, respiratory gas, and contaminate gas data were used to characterize the atmospheric conditions presented to prairie dogs and rock doves during RP/BR-aerosol exposures of each study (i.e., dosimetry conditions). Appendix E presents tables of all chamber atmosphere measurements for each of the 8 studies.

3. Prior RP/BR-aerosol and Air Quality Data for the RP/BR-aerosol and Filtered-air Chamber Systems

Aerosol uniformity (i.e., ≤ 20% maximal heterogeneity among cage sites) and air quality were found to be acceptable or correctable via certain RP/BR-burn restrictions, in Tasks 1 and 2 (Sterner et al., 1988; Shumake et al., 1989). The following points summarize the restrictions derived from these earlier studies:

(1) Cage Site 12 was excluded from use during animal exposure trials due to diluted concentrations of RP/BR aerosol near the Gastec Tube Sampling Port (see Sterner et al., 1988).

(2) High CO values were avoided by restricting Task 3 target concentrations to ≤ 4.0 mg/ℓ (i.e., 180 μm extrusion pump setting) and imposing multiple-exposure schedules for the current species (see Sterner et al., 1988).

(3) Task 3 target concentrations were restricted to ≤ 4.0 mg/ℓ to prevent high C_6H_{14} readings. Despite some high C_6H_{14} measurements during Task 2 Studies, results of an independent aerosol characterization for the System verified that all aerosol and respiratory/contaminate gas values were satisfactory (see Moneyhun, Moody, Jenkins, 1988, in Appendix C of Shumake et al., 1989).

IV. STUDIES OF RP/BR-AEROSOL EFFECTS UPON SPONTANEOUS ACTIVITY IN BLACK-TAILED PRAIRIE DOGS AND ROCK DOVES

Measurements of activity are commonly used to indicate performance decrements in animals exposed to sub-lethal doses of toxic substances (e.g., Boche and Quilligan, 1960; Finger, 1972; Stinson and Loosli, 1979). Two basic types of activity are generally recognized in these animal studies: locomotor and spontaneous. Locomotor activity refers to exertive-type exercise (e.g., running wheel, treadmill, maze running); whereas, spontaneous activity refers to the small, home-cage, non-exertive movements of animals (e.g., walking, grooming, scratching). Although both

types of activity are useful indices of toxicosis, locomotor and spontaneous variables probably reflect different underlying etiologies and require different theoretical explanations.

Changes in locomotor activity often have been viewed as reflecting direct or indirect biochemical and metabolic reactions to the uptake of toxic or pollutant substances (Brown, 1988). Specific chemical substances are viewed to produce either localized toxic metabolites or O_2 deficits within muscle tissue. This action, in turn, inhibits or stimulates locomotor activity due to increased or decreased phosphorylation within the muscles. Concurrently, the pain associated with oxygen debt or muscular tetany could also decrease locomotor activity. Cortical centers involved in locomotor changes would typically involve toxicant-caused effects in structures such as the pons, cerebellum or motor cortex (Gross, 1963a, b, c).

Spontaneous activity has traditionally been associated with "arousal models" of animal behavior (Grossman, 1967). Circulating metabolites from specific pesticides, aerosols, and contaminants are viewed as inhibiting or stimulating arousal. The Ascending Reticular Activating System (Magoun and Rhines, 1947, 1947; French, 1960; Lindsley, 1960), a system of diffuse cortical pathways projecting from the reticular formation of the brain stem anterior through hypothalamic and thalamic areas to various cortical projection areas, can alter the sleep-wakefulness, inattention-attention, and hypo-hyper activity of animals. Additionally, numerous data exist which demonstrate that grooming and preening movements of animals and birds are often altered by specific contaminants, drugs, and hormones (e.g., Alfano and Petit, 1981; Isaacson, 1982; Segal, Sullivan, Kuczenski, and Mandell, 1971).

Regarding the specific effects of RP/BR-aerosol, Preache, as reported in Aranyi (1984), observed increased locomotor activity in laboratory rats following inhalation-chamber exposure. Measurements involved the use of a "figure-8 maze" (see Norton, Culver, and Mullenix, 1975a). Groups of rats were exposed to multiple exposures (4 to 16) of 0.75 to 1.2 mg/ℓ RP/BR-aerosol concentrations. Separate 20-min measurements of rat movements through 8 different infrared detectors placed throughout the maze were made immediately after the final inhalation exposure and 14 days later (i.e., recovery). The locomotor activity of all RP/BR-aerosol groups was elevated compared to control animals for these 2 test periods. Generally, 30-40 percent greater activity was noted. The most pronounced increase in locomotor activity was reported for rats given 4 weeks of 4 consecutive daily 2.25 h exposures to aerosol concentrations of 0.75, 1.0, and 1.2 mg/ℓ.

The current studies seek to assess potential <u>immediate (≤ 3 h out-of-chamber)</u> and <u>acute effects (≤ 6 days post-exposure)</u> of sub-lethal RP/BR-aerosol exposure upon the restricted, home-cage (spontaneous) activity of black-tailed prairie dogs and rock doves. The mild irritation from the deposition of H_3PO_4 on soft tissue at the lower concentration (1.0 mg/ℓ) is predicted to lead to greater grooming (prairie dogs) and preening (rock doves) movements immediately post exposure. Conversely, it is

hypothesized that 4- and 2-successive, 80-min exposures to approximately 4.0 mg/ℓ concentrations of RP/BR aerosol (relative to equivalent exposures to 0.0 and 1.0 mg/ℓ aerosol) should cause acute decrements in the spontaneous activity of both species. The general malaise caused by RP/BR-aerosol (H_3PO_4) upon respiratory pathways and mucosal tissue is expected to yield temporary (acute) lethargy during the Post-exposure Phase of the research paradigm. These hypotheses are counter to the results of Preache (see Aranyi, 1984); the 23-h spontaneous activity of prairie dogs and rock doves in the current studies is expected to be affected differently than 20-min locomotor activity of laboratory rats.

A. Effects of RP/BR-aerosol upon the Spontaneous Activity of Black-tailed Prairie Dogs

1. Methods

a. Black-tailed Prairie Dogs

Twenty-four black-tailed prairie dogs (12 males, 12 females) were used in the Spontaneous Activity Study. As mentioned, all animals were caught at Buckley Air National Guard Base, Aurora, CO during the February and December 1987 Captures. Composition of prairie dogs in this study from respective Captures were: February 1987--7 (3 males, 4 females) and December 1987 -- 17 (9 males, 8 females). Because the study was conducted in 3 replications between August 13 and October 17, 1988 (see Figure 3), 29 and 71 percent of the animals were maintained in captivity for roughly 19 and 9 months, respectively, prior to measurements.

b. Group Assignments

Following 7-20 days acclimation to housing conditions in Building 16, the 8 prairie dogs within each of the 3 replications were rank ordered by body weight within sex (i.e., 4 males and 4 females per replication). The weight ranges of the males and females were 1109 to 1371 g and 958 to 1363 g, respectively, at the time of assignment. Animals were then assigned "quasi randomly" in sets of 3, (heaviest to lightest) to 1 of the 3 RP/BR-aerosol Groups (0.0, 1.0, and 4.0 mg/ℓ) within each replication.

The term "quasi randomly" refers to the constraints imposed on actual assignments of prairie dogs within replications. Only 8 activity devices were available; hence, to balance the animals and sexes among the RP/BR-aerosol Groups, unequal numbers of animals had to be assigned within these Groups for each replication. Specifically, Replication 1 involved 2 (1 male, 1 female), 3 (1 male, 2 females), and 3 (2 males, 1 female) prairie dogs assigned to the 0.0, 1.0, and 4.0 mg/ℓ RP/BR-aerosol Groups, respectively. Replication 2 involved 3 (2 males, 1 female), 2 (1 male, 1 female), and 3 (1 male, 2

females) animals assigned to the 0.0, 1.0, and 4.0 mg/ℓ Groups, respectively. Replication 3 involved 3 (2 males, 1 female), 3 (2 males, 1 female) and 2 (1 male, 1 female) animals assigned to the 0.0, 1.0, and 4.0 mg/ℓ RP/BR-aerosol Groups, respectively.

c. Opto-varimex Activity System

The Opto-varimex Activity System consists of 8 infrared-type movement detection units (Columbus Instruments International Corp., Columbus, OH), with auxiliary control and peripheral devices adapted to provide for automatic data storage and transmission (AEON Electronics, Denver, CO). Figure 8 presents a detailed technical illustration of the System as configured during the current study, whereas, the insert is a schematic illustration of the hardware and software comprising the System.

As shown, 8 Opto-varimex Activity Units form the core of the System. Each unit consists of a rectangular base (51.1 X 9.5 X 69.2 cm) that contains a large open area (43.2 X 44.4 cm) for insertion of a Plexiglas animal-housing box (42.2 X 43.2 X 31.7 cm). Along 2 adjacent edges of the base are located 15 small infrared sources (2.81 cm center-to-center distance); and, along the opposite sides of the base are aligned 15 infrared detectors. Supplying 110 Vac to the units causes 15 intersecting beams of 940 nm light to be established in a grid-like pattern across the open area (animal-housing box). Interruptions of these "standing beams" by the animal's body are tallied as spontaneous activity.

Two types of beam interruptions are discriminated: horizontal and ambulatory activity. Horizontal breaks of the light beams generate 3 msec logic pulses every time that a horizontal beam in the X or Y axis is interrupted; this count allegedly reflects all movements of an animal (e.g., grooming, scratching, walking). Ambulatory interruptions of the beams provide 3 msec logic pulses only after a "new" beam in the X or Y axis is broken; thus, this ambulatory measure reflects only lateral movements of an animal -- no output results from repeated (e.g., grooming and scratching) interruptions of the same beam.

System hardware provides for the automatic, continuous acquisition of activity measurements from each Opto-varimex Unit. Six main auxiliary control and peripheral components make up this hardware: (1) Northstar Horizon II computer with 2 floppy disk drives (Northstar Computers, Inc., Berkeley, CA), (2) Hazeltine 1500 Video Display Terminal

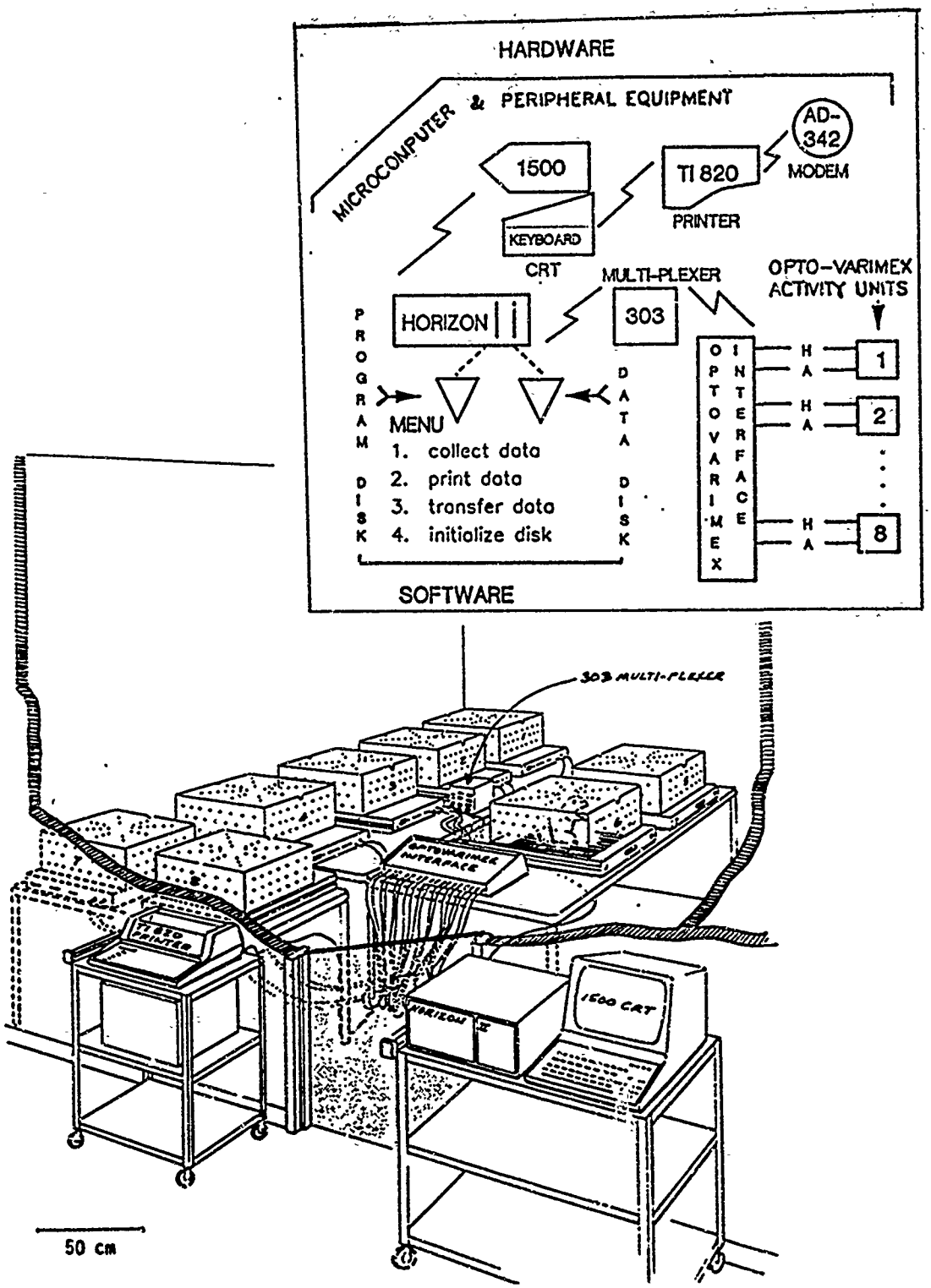


Figure 8. Technical illustration of the Opto-varimex Activity System as arranged for data collections in Room 168B, with a schematic diagram of the hardware and software elements shown in the insert.

(Hazeltine Corp., Greenlawn, NY), (3) TI820-RO Printer (Texas Instruments, Inc., Houston, TX), (4) AD-342 Modem (Anderson-Jacobson, Inc., San Jose, CA), (5) Scientech 303 Octaport multiplexer/demultiplexer (Scientech, Inc., Boulder, CO), and (6) Digital Group Opto-varimex Interface (Aeon Electronics, Denver, CO). Except for the Digital Group Opto-varimex Interface, the components were adapted from a previously described system for monitoring the continuous food intake of rodents (Sterner, 1982).

All hardware and software functions of the Opto-varimex Activity System are governed by the Horizon II Computer, with activity counts during 46 continuous, 30-min daily periods (i.e., 23 h) automatically stored on a floppy diskette. The Hazeltine 1500 Terminal (with keyboard) provides the communications link with the System. Hard copy printouts of daily activity files are obtained via the TI 820-RO Printer; whereas, automatic transmission of activity files to other file management or commercial data analysis systems (e.g., SAS) is accomplished via the AD-342 Modem.

System software is a modified set of the menu-driven programs reported by Sterner (1982). Four options allow for initializing data diskettes for prescribed time intervals (i.e., 15 sec, 1, 5, 10, 20, or 30 min), collecting/storing activity counts onto data diskettes, printing stored activity files with the TI 820-RO Printer, and transmitting stored files via the AD-342 Modem to other data management systems.

d. Procedures

All activity measurements were conducted in Room 168B (see Figure 5); and, the Opto-varimex Units were the housing cages for the animals throughout each replication of the study. Light was provided by 4 overhead fluorescent fixtures; a 12:12 h light-dark schedule (light = 0600-1759 h and dark = 1800-0559 h) was maintained throughout the study. Modal (minimum-maximum) temperature and RH for Room 168B across the 3 replications of the study were 20 C° (19-24) and 33 (21-88) percent, respectively.

Prior to each replication, an "accuracy test" was performed with the Opto-varimex System. Using the "1 min data sampling time," a pencil was passed along the inside edge of 2 adjacent sides of each Opto-varimex base to activate all infrared sensors. This procedure was repeated 10 times for each of the 8 units during 10, 1-min periods. A printout of these data was then obtained. Horizontal and ambulatory counts were expected to equal 30 (i.e., 15 per sensor panel) per min per unit. If the modal count for the 10 tests was 30, the unit was judged to be accurate; if the modal count was not equal to 30 for 10 tests, the unit was repaired and the prior data files checked and corrected/deleted as appropriate.

Between 5-10 days prior to the start of the Pre-exposure Phase, the 8 designated prairie dogs were moved from the main prairie dog housing area (Room 157S) and assigned randomly to one of the 8 Opto-varimex Units in Room 168B. No activity measurements were collected during this "acclimation period," but animals were fed and watered, and the activity cages were cleaned, every morning between 0800-0859 h.

Except for the Scientech 303 Octaport and Digital Group Opto-varimex Interface, all control and peripheral devices of the Opto-varimex system were located outside of Room 168B. No visual isolation of animals was provided among units. Ad libitum Purina Rabbit Chow Performance Blend (Purina Mills, St. Louis, MO) was provided in a semi-circular-shaped cup (6.5 cm dia. X 5 cm depth) located inside each housing box on the right-front Plexiglas panel. Ad libitum water was provided in 2 plastic bottles (200 and 100 mℓ graduated in mℓ) with rodent-lick spouts. The bottles were clamped to the outside left-front of each housing box, with the spouts protruding through 2-cm diameter holes drilled in the Plexiglas. Both the food cup and water spouts were positioned above the infrared sensors in the base of each unit.

As indicated, the Spontaneous Activity Study with prairie dogs was accomplished in 3 replications between August 13 and October 12, 1988 (see Figure 3). Each replication of 8 animals consisted of a consecutive 2-day Pre-exposure, 4-day Exposure, and 6-day Post-exposure Phase. During the Exposure Phase, respective animals were exposed to target concentrations of either 1.0, 4.0 or 0.0 (filtered air) mg/ℓ RP/BR aerosol for approximately 80 min daily.

Procedures for the collection of horizontal and ambulatory activity, body weight (g), food intake (g), and water intake (mℓ) were essentially the same throughout the Pre-exposure, Exposure, and Post-exposure Phases. Each day involved a standard 23 h activity period (i.e., approx. 0900-0759 h) and a 1-h maintenance period (i.e., approx. 0800-0859 h). Horizontal and ambulatory counts were recorded during 46 consecutive 30-min segments of each day's 23-h activity period using the Opto-varimex System. The daily body weight and food intake of each prairie dog was determined during the 1-h maintenance period using a Mettler 3600 Electronic Balance (Mettler Instrument Corp., Hightstown, NJ). The daily water intake of each animal was assessed by recording the change in water levels (miniscus) of each graduated water bottle between successive daily maintenance periods.

The 1 h maintenance period (0800-0859 h) consisted of the following sequence of events. Upon completion of the prior day's activity collection, a printout of that data file was obtained. The test room (Room 168B) was entered. The water level of each water bottle was recorded. Next, sets of 4 animals each were removed from the animal-housing boxes, placed in large cans with perforated lids, weighed (weight recorded), and confined to these cans for about 20

min. Food cups were now weighed (weight recorded), cleaned, re-filled, and re-weighed (weight recorded). Each housing box was cleaned and drop paper changed. The prairie dogs were replaced in their respective boxes, and the procedure repeated for the remaining 4 prairie dogs. Finally, the water bottles of all animals were re-filled, the miniscus within each bottle checked, and the water level (mℓ) recorded for the next day. The activity "data collection program" was then re-started for another 23 h period.

On Exposure Days, procedures differed slightly. The maintenance procedures remained the same (0800-0859 h), and at approximately 0900 h, the activity "data collection program" was re-started. However, at the times that respective animals were to be placed in the RP/BR-aerosol or Filtered-air Chamber, food and water measurements were recorded. The respective animals were then removed from their housing boxes, weighed, and transported to the inhalation test areas (see Figure 5). Next, animal identifications were verified via implanted transponders, and each prairie dog was placed into a randomly designated cage (not Cage Site 12) in the respective inhalation chamber. After all animals were loaded into the chamber, the door was closed and the 80-min exposure was conducted. Upon completion of the exposure session, animals were removed, re-identified, re-weighed and returned to their Opto-varimex housing boxes. After 2 h had elapsed, these animals were again re-weighed and their food and water intake recorded. Because of the standard exposure sequence (i.e., 1.0, 4.0 and 0.0 mg/ℓ), the time of day that respective animals were out of the Opto-varimex System for exposure trials was generally the same. Typically, prairie dogs in the 1.0, 4.0 and 0.0 mg/ℓ RP/BR-aerosol Groups were out of the activity cages (i.e., in chamber) between 0830-1100, 1130-1400, and 1430-1700 h, respectively.

e. Experimental Designs and Data Analyses

Atmospheric conditions within the inhalation chambers during Exposure Days (see Appendix E) and mortality of prairie dogs during the study were summarized using descriptive statistics (i.e., frequencies, medians, ranges).

Evaluations of the potential immediate and acute effects of RP/BR-aerosol exposure upon spontaneous activity involved a total of 4 separate ANOVAs. Where missing data occurred, these ANOVAs were computed using the General Linear Model (i.e., PROC GLM Program; SAS Institute, Inc., 1985); otherwise, balanced data sets were analyzed using the PROC ANOVA Program (SAS Institute, Inc., 1985). Throughout all ANOVAS, significant terms were assessed using Type

III sums of squares and post-hocDuncan Multiple Range Tests (Waller and Duncan, 1969).* The 0.05 level of significance was used to test all ANOVA terms and Duncan Comparisons.

(1) Immediate Effects.-- Two ANOVAs were computed to assess the immediate consequences of RP/BR exposure and chamber confinement upon the prairie dogs. Total horizontal and ambulatory activity counts for the 2-h period immediately following each exposure were analyzed separately to assess relative differences among the 3 RP/BR-aerosol Groups during the Exposure Phase. Each of these variables were analyzed as balanced 3 (RP/BR-aerosol Concentration) X 2 (Sex) X 4 (Day) ANOVAs, where Day was a repeated measures factor (Winer, 1971).

(2) Acute Effects.-- Two ANOVAs were computed to assess acute effects of RP/BR-aerosol exposure upon prairie dogs activity. Mean 30-min horizontal and ambulatory counts during Pre-exposure versus Post-exposure Days were analyzed separately as 3 (RP/BR-aerosol Concentration) X 2 (Sex) X 8 (Day) X 2 (Light On/Off) ANOVAs, where Day and Light were considered repeated measures factors (Winer, 1971). Data were averaged for the "light on" (22, 30-min sessions) and "light off" (24, 30-min sessions) portions of the daily schedule in order to derive the dependent variables. Missing data occurred on Day P-1 of Replication 2 and Days Pre-1 and Pre-2 of Replication 3.

2. Results and Discussion

As stated, presentation and description of the inhalation chamber measurements that characterized the exposures of prairie dogs to RP/BR-aerosol and filtered-air are contained in Appendix E (see Table E1).

a. Mortality/Clinical Symptomatology

No mortality occurred for the 24 prairie dogs used in the Spontaneous Activity Study. This confirms results of the Toxicity Range-finding Studies which indicated that a schedule of 4 successive daily 80-min exposures to $\leq$ 6.0 mg/ℓ RP/BR aerosol is below the "lethal threshold" for this species (Shumake et al., 1989).

* Although the use of Type III sums of squares and Duncan Tests have been questioned for unbalanced designs (PROC GLM), 2 considerations affect use of these procedures here: (1) in most cases data for only a single animal/bird were omitted per cell of respective designs (i.e., the loss of only 1 animal was unlikely to affect results based on the use of Type III sums of squares--Type III should be used with the PROC ANOVA/balanced design and Type IV should be used with the PROC GLM/unbalanced design--or the less conservative Duncan Multiple Range Test) and (2) the exploratory nature of the research justified the use of less stringent post-hoc procedures (i.e., accepting the alternative hypothesis when false a limited number of times was believed justified in our attempts to discover possible symptomatological/behavioral/physiological effects worthy of further study; see Petrinovich and Hardyck, 1969).

Although systematic measurements of vocalizations were not recorded, the vocalizations of most prairie dogs exposed to 4.0 mg/ℓ RP/BR aerosol became weak, hoarse, or raspy after 1 or 2 days of the 4-day exposure schedule. This also confirms results of Shumake et al. (1989) and suggests that extended inhalation of 4.0 mg/ℓ concentrations of RP/BR aerosol could disrupt alarm, territorial, and mating communications in this species.

b. Spontaneous Activity (Horizontal/Ambulatory)

(1) Immediate Effects.-- Results of the ANOVAs to assess "immediate, 2-h post-exposure effects" associated with the 4 successive chamber confinements yielded disparate results for the 2 activity variables. The ANOVA for horizontal activity produced no significant terms; whereas, the ANOVA for ambulatory activity yielded a significant Concentration X Session interaction.

The absence of significant effects for the 2-h, out-of-chamber horizontal activity counts implies that 4 daily 80-min exposures to 1.0 and 4.0 mg/ℓ concentrations of RP/BR aerosol had no immediate effects upon grooming, scratching, and shaking activity. Thus, the hypothesis that the potential irritation caused by phosphoric acids on the fur of RP/BR-aerosol-exposed prairie dogs would lead to increased grooming immediately after exposure is unsupported. This result involves both pro and con interpretations. No effect of exposure upon grooming-type activity can be viewed as indicative of the benign nature of the aerosol. Conversely, continued grooming could imply that prairie dogs would ingest considerable H_3PO_4 post exposure -- high oral ingestion.

The Concentration X Session interaction ($F = 2.26$, $df = 6/54$, $p \leq 0.05$) for the immediate, out-of-chamber ambulatory counts is graphed in Figure 9. Post-hoc Duncan Range Tests revealed the following pattern of mean differences: (a) means for Days E-1 and E-2 of the 4.0 mg/ℓ RP/BR-aerosol Group were significantly less than all other means, (b) means for Days E-3 and E-4 of the 0.0 mg/ℓ Group were significantly greater than all other means, and (c) the means for Day E-2 of the 4.0 mg/ℓ and Day E-4 of the 0.0 mg/ℓ Groups were less than and greater than all other means, respectively. Thus, counter to the impression conveyed in Figure 9, results indicate that the interaction is probably due to the disparity between the low counts for the 4.0 mg/ℓ-exposed animals following the first 2 exposures and the elevated (nearly asymptotic) counts of the control animals (0.0 mg/ℓ) following the last 2 exposures.

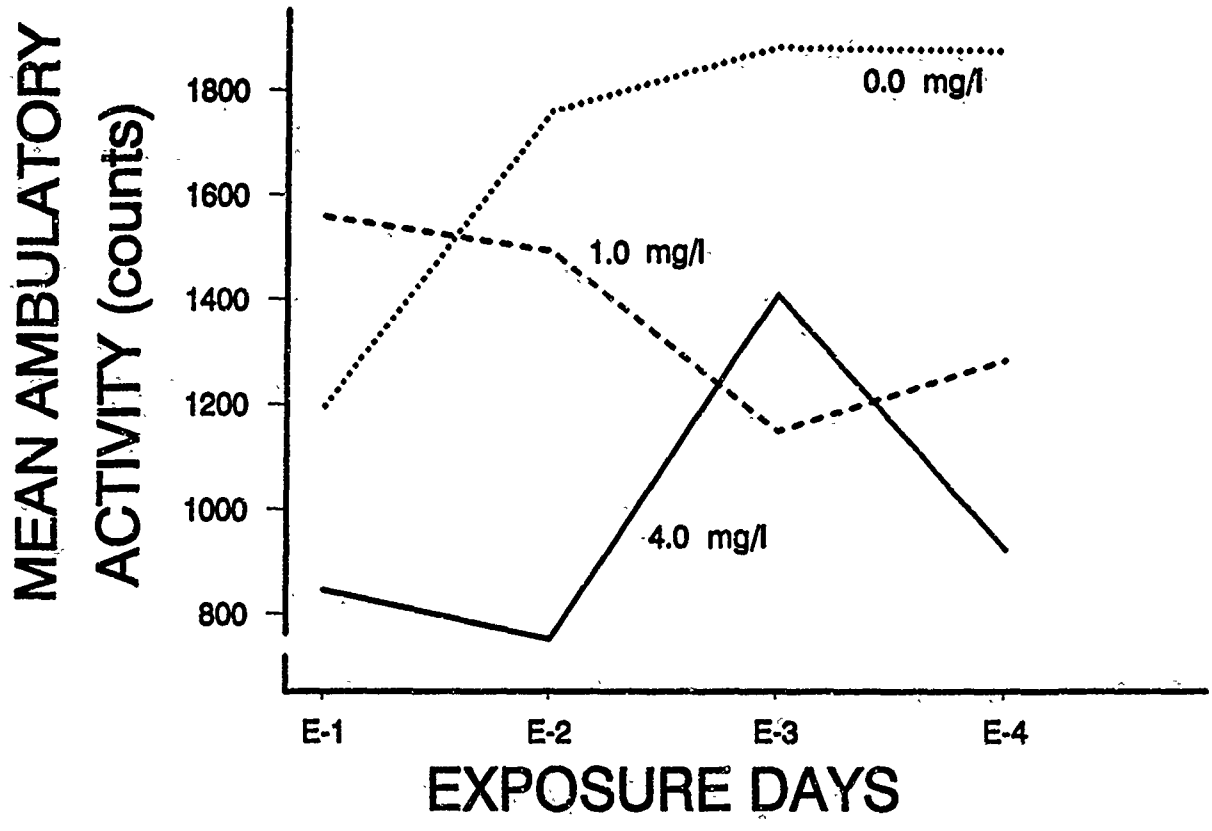


Figure 9. Graph of the mean ambulatory activity counts for prairie dogs in the 0.0 (Filtered-air), 1.0, and 4.0 mg/ℓ RP/BR-aerosol Groups during the 2 h immediately after chamber confinement on each of the 4 Exposure Days (E-1 to E-4) -- the Concentration X Session interaction effect.

The intersection and crossover of the other means are insignificant -- a result reflective of the high within- and between-group variances in the ambulatory counts for the 0.0, 1.0, and 4.0 mg/ℓ Groups.

This interaction suggests that: (a) initial exposures to 4.0 mg/ℓ RP/BR aerosol are associated with lower home-cage, restricted, lateral movements (lethargy) of black-tailed prairie dogs and (b) control exposures to filtered air (0.0 mg/ℓ) are associated with a temporary suppression of this home-cage activity which is accommodated quickly and leads to hyperactivity (walking, jumping, etc.) after the third and fourth chamber confinements. It could also be argued that the results for the 1.0 and 4.0 mg/ℓ groups also reflect a chamber-confinement/activity-suppression effect. That is, immediate post-exposure ambulation may involve a component due to chamber-confinement stress superimposed upon the aerosol-inhalation effects. Animals accommodate to chamber-confinement stress, but a residual inactivity effect (relative to that of filtered-air-exposed animals) remains evident for RP/BR aerosol Groups. Of course, in the absence of appropriate baseline data, this interpretation is only tentative. The appropriate design to isolate this effect would require preliminary (baseline) monitoring of 2 h post-confinement activity for all Groups.

Essentially, this finding is contrary to the earlier report by Preache (see Aranyi, 1984) which noted increased locomotor activity in laboratory rats immediately after RP/BR-aerosol exposure. The current interaction shows that exposure to RP/BR aerosol (4.0 mg/ℓ) causes immediate post-exposure lethargy, not hyperactivity, in prairie dogs. The basis for this discrepancy cannot be inferred; any number of factors (e.g., type of activity, species differences, aerosol concentrations) could have produced the divergent results for the 2 studies. It is obvious, however, that the reduced lateral activity observed in the current study was not due to the prairie dogs' pre-occupation with grooming -- no differences in horizontal counts were found. In retrospect, additional "control groups" would also have been beneficial -- a "positive-type control" (e.g., sub-lethal CO exposures) and a "no chamber-confinement control" (e.g., equivalently-handled, non-inhalation-chamber, novel cage exposures) would have allowed more definitive statements about the nature of the current effect.

(2) Acute Effects.-- Results of the Pre- versus Post-exposure ANOVAs of mean daily (23 h) spontaneous activity for the Light On/Off periods yielded few significant effects. Only the Light On/Off main effects for the horizontal

(F = 36.16, df = 1/116, $p \leq 0.0001$) and ambulatory activity variables (F = 30.12, df = 1/116, $p \leq 0.001$) were significant. No "concentration, day or sex terms" were significant.

Regarding the Light On/Off main effect, this is viewed as validative evidence for the Opto-varimex measurements in the current study. Mean horizontal counts were 774.23 versus 395.75 for Light On versus Off periods, respectively, across the 2 Phases of the study. Mean ambulatory counts were 387.24 versus 190.63 for Light On versus Off portions of the circadian cycle, respectively. For a diurnal species such as the black-tailed prairie dog, this pattern of means gives validation to the activity readings and confirmation that no acute effects of RP/BR-aerosol exposure upon spontaneous activity, beyond the immediate out-of-chamber effects of the Exposure Phase, occurred. This species appears to quickly recover typical movements following even 4 daily 80-min exposures to concentrations of 4.0 mg/ℓ RP/BR aerosol. Again, these results are somewhat counter to those reported by Preache (see Aranyi 1984) for laboratory rats -- no short-term (≤ 6 days) increased activity was observed for prairie dogs subsequent to RP/BR-aerosol inhalation.

B. Effects of RP/BR-aerosol upon the Spontaneous Activity of Rock Doves

1. Methods

a. Rock Doves

Twenty-four wild-caught rock doves (11 males, 13 females) were purchased from a local supplier as part of 2 main shipments (see III. General Methods, A. Animals) received in January 1987 and April 1988. Composition of doves in the Spontaneous Activity Study from these respective shipments were: January 1987 -- 17 (4 males, 13 females) and April 1988 -- 7 (7 males, 0 females). The 3 replications of the Study encompassed the period between June 15 and July 21, 1988 (see Figure 3). Thus, 71 and 29 percent of the birds were maintained in captivity for approximately 18 and 2 months, respectively, prior to measurements.

b. Group Assignments

Rock doves were acclimatized to housing conditions in Building 16 and assigned to groups identical to prairie dogs. Doves were rank ordered by weight, then "quasi randomly" assigned to 0.0, 1.0, and 4.0 mg/ℓ RP/BR-aerosol groups in sets of 3 (lightest to heaviest). Weight ranges of birds at the time of assignments were: 257 to 387 g for males and 293 to 354 g for females.

Assignments of doves by weight to the 3 RP/BR-aerosol Groups were made using the procedure described for prairie dogs. Again, the availability of only 8 Opto-varimex units required the assignment of unequal numbers of doves to RP/BR-aerosol Groups within replications. Specifically, Replication 1 involved 2 (1 male, 1 female), 3 (1 male, 2 females), and 3 (2 males, 1 female) doves assigned to the 0.0, 1.0, and 4.0 mg/ℓ Groups, respectively. Replication 2 consisted of 3 (2 males, 1 female), 2 (1 male, 1 female), and 3 (0 males, 3 females) assigned to these same respective Groups. Replication 3 involved 3 (1 male, 2 females), 3 (2 males, 1 female), 2 (1 male, 1 female).

It should be noted here that the 3 females comprising the 4.0 mg/ℓ Group in Replication 2 was caused by 1 mis-sexed male bird based on the cloacal examination technique (Miller and Wagner, 1955). Conduct of laparotomies on all birds at completion of the Study led to identification of the unbalanced design for the Sex Factor.

c. Opto-varimex Activity System

Measurements of spontaneous, home-cage activity (horizontal and ambulatory) were accomplished using the same Opto-varimex Activity System described for prairie dogs (see Figure 8).

d. Procedures

Procedures were basically identical to those described for prairie dogs. Main exceptions were: (a) modal (minimum-maximum) temperature and RH for Room 168B across the 3 replications of this Study were 21° C (16-23.5°) and 50 percent (34-90), respectively; (b) doves were fed Purina Pigeon Checkers (Purina Mills, St. Louis, MO) ad libitum whenever birds were in the Opto-varimex Activity System boxes; (c) water was available ad libitum whenever birds were in the Opto-varimex boxes, but was provided in a 100-mℓ graduated (mℓ) drinking tube which was affixed to the front left (outside) of each activity box, with an open drinking reservoir protruding into each box through a 2-cm diameter hole drilled in the Plexiglas; (d) the Spontaneous Activity Study with rock doves was accomplished in 3 replications between June 15 and July 21, 1988 (see Figure 3); and, (e) no immediate, out-of-chamber food intake, water intake, and body weight measurements were collected for rock doves (see Appendix D); however, immediate (2 h out-of-chamber) measurements of horizontal and ambulatory activity during the Exposure Phase were analyzed.

e. Experimental Designs and Data Analyses

Characterization of inhalation chamber conditions during the Exposure Phase (see Appendix E) and mortality of rock doves during the study were summarized using frequencies, medians, or ranges.

Evaluations of the potential immediate and acute effects of RP/BR-aerosol exposure upon the activity of the birds involved 4 ANOVAs. Missing data necessitated use of the General Linear Model (i.e., PROC GLM Program using Type III sums of squares; SAS Institute, Inc., 1985). Significant terms of ANOVAs were further delineated using post-hoc Duncan Multiple Range Tests (Waller and Duncan, 1969); and, the 0.05 level of significance was used to test all ANOVA terms and Duncan Comparisons.*

(1) Immediate Effects.-- Analyses of immediate effects within 2 h of RP/BR-aerosol exposure upon spontaneous activity involved 2 separate ANOVAs. The actual 2-h out-of-chamber horizontal and ambulatory activity counts of each dove were analyzed as unbalanced 3 (Concentration) X 2 (Sex) X 2 (Day) ANOVAs, where Day was treated as a repeated measures factor (Winer, 1971).

(2) Acute Effects.-- Two ANOVAs were computed to assess the acute effects of RP/BR-aerosol exposure upon rock dove activity.

Mean 30-min horizontal and ambulatory activity counts for the Light On and Off portions between the 2 Pre-exposure and 6 Post-exposure Days were analyzed as 3 (Concentration) X 2 X (Sex) X 8 (Day) X 2 (Light On/Off) ANOVAs, where Day and Light were considered repeated measures factors (Winer, 1971). Data were averaged for the "light on" (22, 30-min sessions) and "light off" (24, 30-min sessions) portions of the daily schedule to derive the dependent variables. Data were unbalanced due to the previously mentioned mis-sexed male rock dove in Replication 2; and, the death of 1 male dove in the 4.0 mg/ℓ Group during Replication 1.

2. Results and Discussion

a. Mortality/Clinical Symptomatology

One male rock dove was found dead in its activity chamber on the morning of the 4th Post-exposure Day (P-4) of Replication 3. This dove was in the 4.0 mg/ℓ RP/BR-aerosol Group.

*See footnote on Page 27.

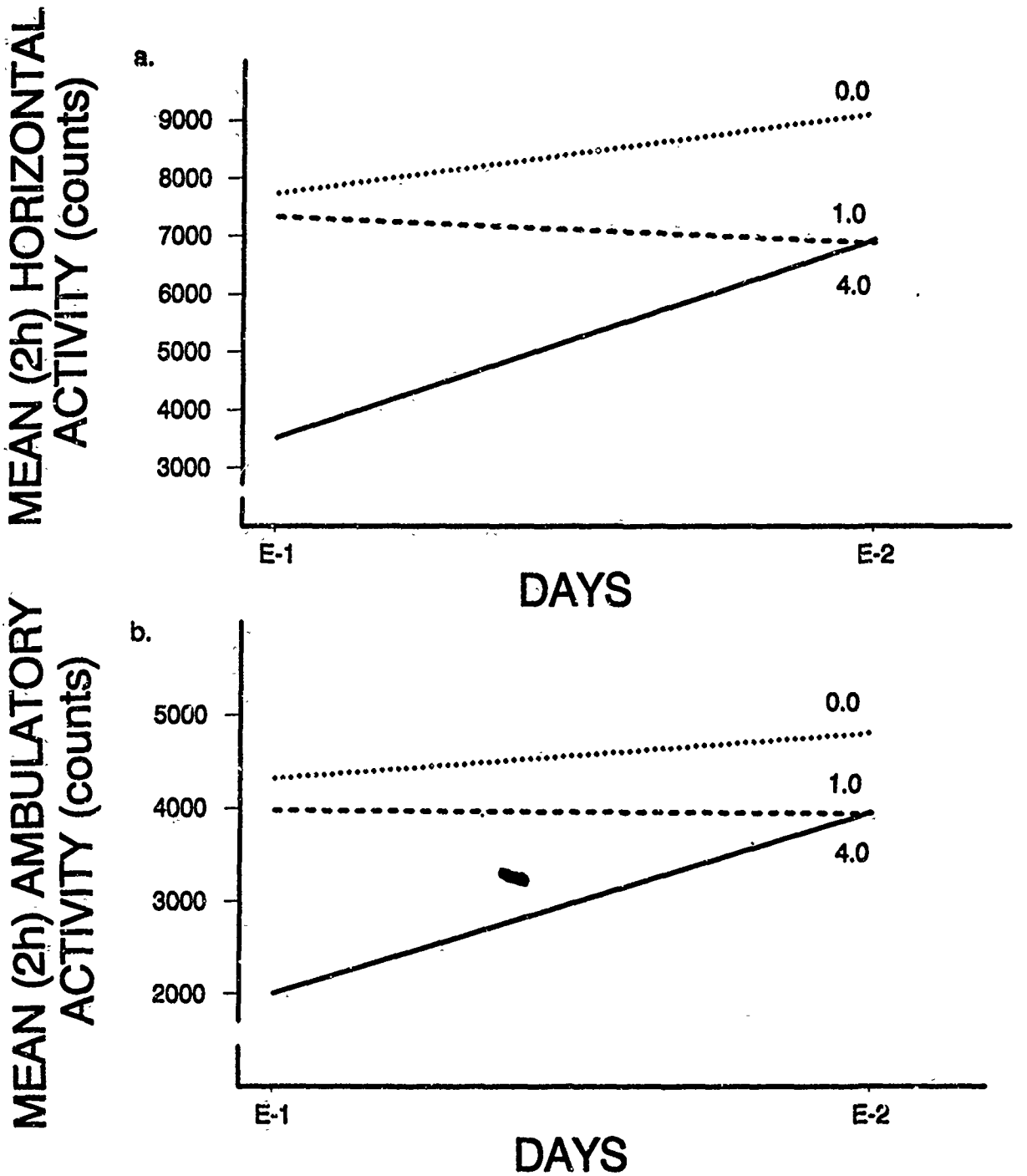
Minimal overt symptoms preceded the death; the bird showed "listing-forward posture" and "molt-like" ruffed feathers approximately 24 h prior to death.

Regarding symptoms in surviving doves, several of the birds in the 4.0 mg/ℓ condition displayed "parted beak" during Post-exposure. This symptom was reported by Shumake et al. (1989) during the Toxicity Range-finding Studies with this species. Congestion and other overt symptoms were practically non-existent.

b. Spontaneous Activity (Horizontal/Ambulatory)

(1) Immediate Effects.-- Mean horizontal and ambulatory activity counts for the 2 immediate out-of-chamber sessions (i.e., Day E-1 and E-2) yielded identical results. That is, PROC GLM ANOVAs of both variables yielded significant Concentration X Session interactions (horizontal activity: $F = 3.98$, $df = 2/18$, $p \leq 0.01$ and ambulatory activity: $F = 4.51$, $df = 2/17$, $p \leq 0.026$) and Session main effects (horizontal $F = 6.63$, $df = 1/18$, $p \leq 0.01$ and ambulatory: $F = 7.02$, $df = 1/18$, $p \leq 0.017$). Because of the similarity of statistical effects for these variables, interpretations are presented jointly.

Figures 10a and 10b present graphs of the mean 2-h, out-of-chamber horizontal and ambulatory activity counts for the 0.0 (filtered-air), 1.0, and 4.0 mg/ℓ RP/BR-aerosol Groups on Days E-1 and E-2, respectively (i.e., plots of the Concentration X Session interactions). Post-hoc Duncan Multiple Range tests for mean horizontal (preening-type) counts indicated that doves in the 4.0 mg/ℓ-aerosol Group made significantly fewer horizontal movements immediately following exposure than doves in any of the remaining Concentration Groups and Sessions. Similar Duncan Tests for mean ambulatory (lateral movement-type) counts revealed that (a) rock doves exposed to 4.0 mg/ℓ aerosol made fewer average out-of-chamber lateral movements than all other doves, (b) doves in the 0.0 mg/ℓ (Filtered-air) Group made greater mean ambulatory movements immediately after exposure (i.e., chamber confinement) than all other doves, with the average activity for the 2-h period on the second Exposure Day (E-2) significantly elevated relative to that on Day E-1.



Figures 10a and 10b. Plots of mean 2 h immediately out-of-chamber horizontal (top) and ambulatory (bottom) activity for rock doves in the 0.0 (Filtered-air), 1.0, and 4.0 mg/ℓ Groups for the 2 Exposure Days (E-1 and E-2) -- the Concentration X Session interactions.

The Session main effects both showed the same pattern of mean activity; namely, that mean horizontal and ambulatory activity was lower for Day E-1 than E-2. Mean (± SD) horizontal-type counts for the 2-h sessions were 6221 (± 3150) and 7666 (± 3189) for E-1 and E-2, respectively; whereas, mean (± SD) 2-h ambulatory counts were 3439 (± 1993) and 4251 (± 2101) for these respective Days (i.e., E-1 and E-2). These effects indicate that a general chamber-confinement stress caused significantly greater out-of-chamber preening, wing-flapping, strutting movements immediately after the second exposure session (E-2) as compared to the 2 h period after the first chamber-confinement session.

Obviously, the hypothesis predicting increased out-of-chamber preening behavior of doves following RP/BR-aerosol exposure (i.e., horizontal activity) is again refuted. Results for rock doves clearly show that exposure to a 4.0 mg/ℓ RP/BR-aerosol for 1, 80-min bout probably suppresses immediate attempts by the birds to preen material from their feathers. As for the Prairie Dog Study, interpretation of the RP/BR-smoke caused suppression is only tentative; a design involving preliminary baseline measurements of doves handled and confined during the Pre-exposure Phase is needed to unequivocally conclude that preening was decreased due to RP/BR aerosol exposure. At the same time, the hypothesis that exposure to 4.0 mg/ℓ aerosol concentrations would be associated with less ambulatory movement and lethargy is again substantiated.

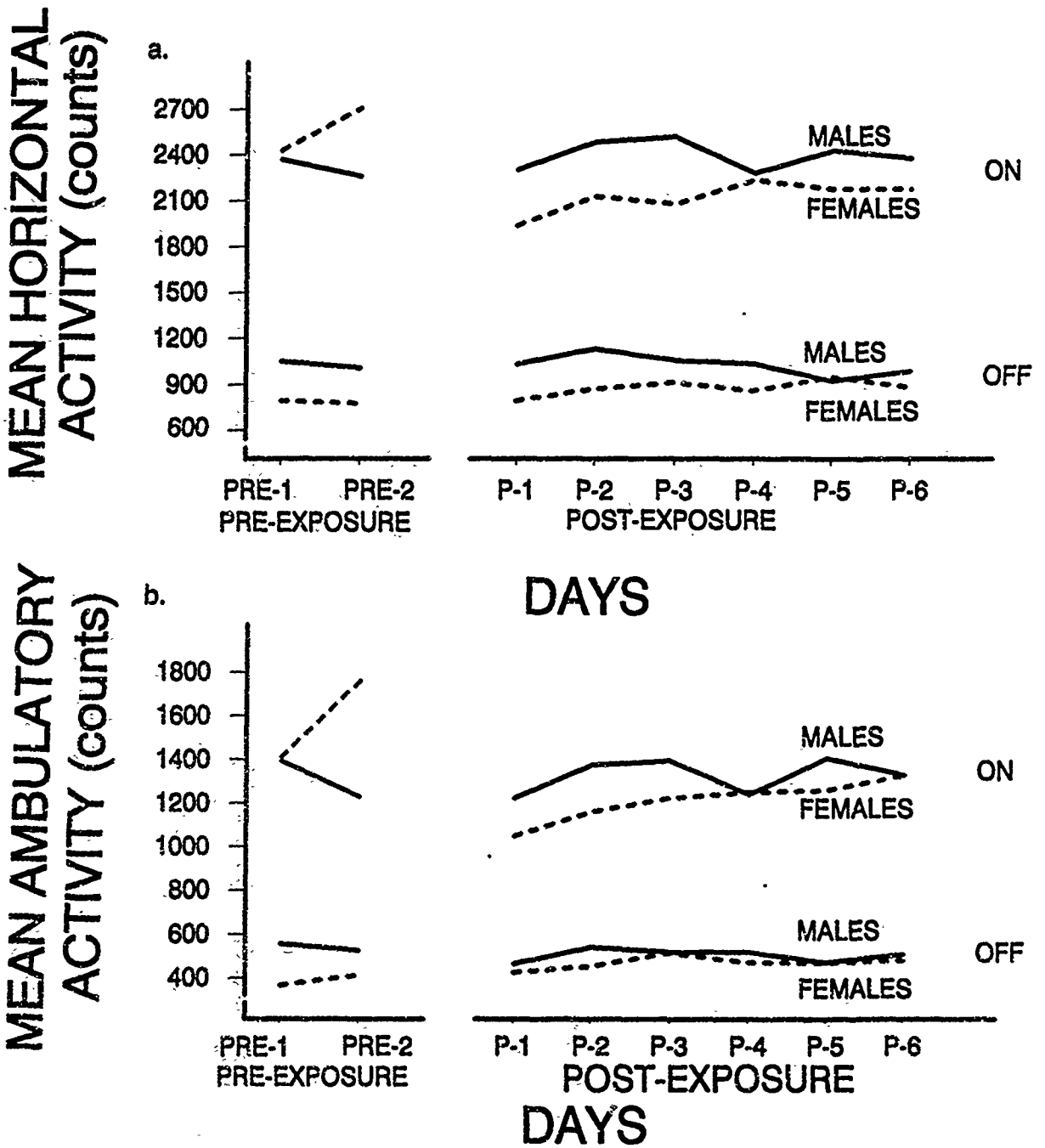
Thus, it appears that (a) exposure to a single 4.0 mg/ℓ RP/BR aerosol causes an immediate and initial 1-period reduction in 2 h out-of-chamber horizontal activity (i.e., preening/wing-flapping behavior) of doves (b) exposure to a single 4.0 mg/ℓ concentration of RP/BR-aerosol is associated with an immediate and initial 2 h out-of-chamber decreased ambulatory activity (i.e., walking/strutting behavior), (c) both horizontal and ambulatory activity are unaffected by RP/BR aerosol on the second exposure day (E-2), and (d) exposure to filtered air (0.0 mg/ℓ aerosol) causes greater 2-h, out-of-chamber movements relative to either 1.0 or 4.0 mg/ℓ aerosol, with greater activity especially displayed following the second Exposure Day (E-2) chamber-confinement session. Together, these "immediate post-exposure measurements of results for spontaneous activity in wild-caught doves were opposite those reported by Preache for locomotor, exploratory-type activity of laboratory rats (Aranyi, 1984). Analogous to observations for prairie dogs, the current Concentration X Session interactions imply that relatively high concentration exposures of RP/BR aerosol produce brief periods of hypoactivity in doves (not hyperactivity) immediately after the initial exposure session. This concurrence for 2 diverse species (prairie dogs and rock doves) implies that the

discrepancy between the present results and those of Preache probably reflect basic differences in the 2 types of behaviors -- not species differences. Home-cage, restlessness is not the same as "figure-8-maze," exploration; but, why these different species would become lethargic in a small confined space, yet wander about in a maze, following RP/BR-aerosol inhalation is inexplicable.

(2) Acute effects.-- The Pre- versus Post-exposure Phase ANOVAs of mean daily "light on and off" activity yielded a relatively complex set of results. The Sex X Day X Light On/Off ($F = 2.34$, $df = 7/124$, $p \leq 0.03$) and Sex X Day interaction ($F = 2.07$, $df = 7/124$, $p \leq 0.05$), plus the Light On/Off main effect ($F = 150.81$, $df = 1/18$, $p \leq 0.0001$) were significant sources of variance for horizontal-type activity; whereas, the Sex X Day X Light On/Off interaction ($F = 2.12$, $df = 7/117$, $p \leq 0.05$) and Light On/Off main effects ($F = 680.71$, $df = 1/17$, $p \leq 0.0001$) were significant for ambulatory activity.

Figures 11a and 11b illustrate the Sex X Day X Light On/Off interactions for the mean daily "light on and off" horizontal and ambulatory activity counts for male and female doves across the 2 Pre-exposure (Pre-1 and Pre-2) and 6 Post-exposure Days (P-1, P-2, P-3, P-4, P-5, and P-6) of the Study. In the absence of significant Concentration-related effects, the Sex X Day X Light On/Off interactions for both types of activity are discussed jointly.

In general, data indicate that male and female birds differed in their daily diurnal and nocturnal home-cage activity, but these effects were induced by generalized chamber-confinement stressors, rather than RP/BR-aerosol exposures, per se. Post-hoc Duncan Multiple Range Tests indicated a cumbersome, but relatively interpretable, pattern of mean differences. Two key sets of mean comparison results for horizontal and ambulatory activity were: (a) Day P-1 diurnal means for female doves were significantly less than all other diurnal sub-group means and (b) all diurnal versus nocturnal sub-group comparisons were significantly different from each other (i.e., the Light On/Off effect). Similar to results for prairie dogs, the Light On/Off main effects for both horizontal and ambulatory activity of the rock doves were considered validations of the Opto-varimex measurements. Again, rock doves are a diurnal species. Mean horizontal



Figures 11a and 11b. Plots of the mean daily (11 h Light On and 12 h Light Off) horizontal (top) and ambulatory (bottom) activity counts for male and female rock doves during the light "on" and "off" portions of the circadian schedule across the 8 days (Pre-exposure and Post-exposure Phases) of the Spontaneous Activity Study -- the Sex X Day X Light On/Off interactions.

counts were 2300 versus 925 for Light On versus Off Periods across the 2 Phases, respectively. Mean ambulatory counts were 1304 versus 471 for Light On versus Off portions of the circadian cycle, respectively.

Together the aforementioned findings indicate that a general chamber-confinement stress operated to lower both the horizontal and ambulatory activity of female doves during the diurnal portion (Light On) of the light cycle for about 24 h after the last Exposure Day (i.e., Post-exposure Day P-1). Without Concentration-related effects, results strongly suggest that 2 successive daily 80-min sessions of either 1.0 and 4.0 mg/ℓ RP/BR-aerosol exposure produce no measurable acute effects upon the spontaneous activity of rock doves. The obtained evidence implies that RP/.BR-aerosol inhalation causes a number of significant dose-related shifts in restlessness for the birds, but these effects are limited to the 2-h period immediately following chamber confinement and are dissipated during the 20-plus h periods following actual aerosol inhalation.

C. Summary and Conclusions (Spontaneous Activity Studies)

Essentially, the current activity studies show that 4 and 2 successive daily 80-min exposures to 1.0 and 4.0 mg/ℓ target concentrations of RP/BR aerosol are associated with only short-duration, often subtle, effects upon the restricted, home-cage activity of prairie dogs and rock doves, respectively. Nevertheless, implications of the findings for horizontal activity (e.g., grooming, preening) to the oral ingestion of H_3PO_4 and BR as a secondary route of RP/BR-aerosol toxicity remain potentially significant. Detailed review of the (1) RP/BR-aerosol characterization data (see Appendix E), (2) mortality and clinical symptomatology data, (3) clinical toxicology indices of body weight, food consumption, and water consumption (see Appendix D), and (4) immediate and acute spontaneous activity data yield a number of general conclusions. Summarizations of these follow:

1. Controlled RP/BR-aerosol Exposures

Evaluations of the RP/BR-aerosol characterization measurements show that all groups of prairie dogs and rock doves were administered target concentrations of 1.0 and 4.0 mg/ℓ having acceptable uniformity. The Modified RP/BR Extruder and Inhalation Chamber System, plus the Filtered-air Inhalation Chamber System, functioned well. There is every reason to conclude that the activity effects observed are representative of prairie dogs and rock doves exposed to well-controlled, non-contaminant (i.e., no PH^3 or C_6H_{14}, with ≤ 24 ppm CO), sufficient-air quality (i.e., ≥ 16% O_2 and < 1% CO_2), and respirable-aerosol atmospheres (i.e., MMAD particles of ≤ 0.85 μm).

2. Mortality and Clinical Symptomatology

Results of mortality and symptomatology data confirm results of the Toxicity Range-finding Studies (see Shumake et al., 1989).

No prairie dogs died during the course of the Study. Thus, selection of the 4 successive daily 80-min exposure schedule for prairie dogs appears to be sub-lethal for this species. the most troublesome symptom (ecologically) concerns the raspy and hoarse bark displayed by a number of animals exposed to 4.0 mg/ℓ RP/BR aerosol. The present observations further confirm the work of Shumake et al. (1989), and imply that repeated "field exposures" of this species to prolonged, high concentrations (4.0 mg/ℓ steady state) of RP/BR smoke could alter/decrease vocalization and interfere with communication systems of these rodents.

One male rock dove died approximately 3 days after 2 successive daily 80-min exposures to 4.0 mg/ℓ RP/BR aerosol. Although antecedent respiratory and postural symptoms were difficult to discern, the lethally-dosed bird displayed slight congestion and a forward-listing posture at least 24 h prior to death. This indicated that the exposure schedule used with doves was close to the lethal/sub-lethal threshold, and probably relatively more severe than that chosen for prairie dogs.

3. Acute Decreases in Body Weight, Food Consumption and Water Consumption

Analyses of auxiliary measurements to corroborate RP/BR-aerosol-induced, sub-lethal toxicosis effects upon consummatory and body weight variables in the 2 species are presented in Appendix D. These confirm (i.e., water consumption and body weight) and extend (i.e., food consumption) findings of Shumake et al. (1989). Results demonstrate that the current exposure schedules produce acute decreases in body weight, food and water intake of both prairie dogs and rock doves -- with recovery generally complete by 3 days post exposure.

4. Immediate and Acute Spontaneous Activity Effects

a. Black-tailed Prairie Dogs

No immediate, out-of-chamber (2 h) effects during the Exposure Phase were noted for horizontal activity (i.e., agitated, grooming, shaking behavior) of prairie dogs; however, an important Concentration X Session interaction occurred for the immediate ambulatory activity measurements (i.e., walking, jumping behavior) during this Phase. Immediate, out-of-chamber ambulation scores of the prairie dogs exposed to 4.0 mg/ℓ RP/BR aerosol were significantly lower than those of other groups following the first 2 exposure sessions. This index of greater "lethargy" for the 4.0 mg/ℓ RP/BR-aerosol

Groups is counter to a prior report of increased "figure-8 maze" activity for laboratory rats following RP/BR-aerosol exposures (see Aranyi, 1984). In contrast, no acute effects (≤ 6 days port-exposure) of RP/BR-aerosol upon black-tailed prairie dogs were found.

b. Rock Doves

Examination of the immediate, out-of-chamber (2 h) spontaneous activity measurements for rock doves reveals that birds in the 4.0 mg/ℓ RP/BR-aerosol Group showed lower horizontal (i.e., agitated, preening, wing flapping, head bowing behavior) and ambulatory activity (i.e., walking, strutting behavior) following the first exposure. Again, these results contrast with the earlier report by Preache (Aranyi, 1984) concerning increased activity following RP/BR-smoke exposures in laboratory rats using the "figure-8 maze."

As was the case with prairie dogs, no acute (≤ 6 days post-exposure) RP/BR-aerosol concentration-related effects were found for the spontaneous activity measurements in rock doves. Several significant interactions showed that a general chamber confinement effect occurred. Specifically, decreased horizontal and ambulatory activity of female doves relative to males was noted during the diurnal portion of the light schedule; however, neither effect was attributable to RP/BR-aerosol exposure, per se.

V. STUDIES OF RP/BR-AEROSOL EFFECTS UPON STARTLE RESPONSES IN BLACK-TAILED PRAIRIE DOGS AND ROCK DOVES

A startle response is an abrupt, short-latency, reaction of an animal to a sudden, intense stimulus (Hoffman and Fleshler, 1963; Bullock, 1984). Startle reactions are commonly assumed to be indices of neural and sensory function (e.g., Hoffman and Ison, 1980; Wecker, Ison, and Foss, 1985) and have been routinely studied in humans, rats, and mice. It is believed that forms of the reaction can be elicited in all mammalian species (Davis, 1984). Stitt, Hoffman, Marsh, and Schwarz (1976) have demonstrated startle reactions in pigeons. Whereas most mammalian studies have involved acoustical, air blast, or electrical shock stimuli, most avian research has involved the use of intense visual stimuli using electronic photoflash equipment. For both mammals and birds, startle response amplitudes and latencies can be modified using brief prepulse stimuli of the same or a different modality (Hoffman, 1984, Stitt et al., 1976). Additionally, many other stimulus variables (e.g., background noise, stimulus intensity, frequency of stimulation) affecting the mammalian startle response are also effective in altering the startle reactions of pigeons (Hoffman and Searle, 1965; Stitt, Hoffman, Marsh, and Boskoff, 1974; Stitt et al., 1976) supporting the thesis of similar underlying sensory-motor mechanisms in both classes of vertebrates.

Numerous reports demonstrate that startle responses of rodents and birds can be altered by pharmacological and toxicological agents (e.g., Ison, 1984; Kellogg, Tervo, Ison, Parisi, and Miller, 1980; Buelke-Sam, Kimmel, Adams, Nelson, Vorhees, Wright, St. Omer, Korol, Butcher, Geyer, Holson, Kutscher, and Wayner, 1985; Davis, 1980; Ruppert, Dean, and Reiter, 1984; Squibb and Tilson, 1982). Noteworthy here is a report by Lock, Dalbey, Gayle and Schmoyer (1985). These authors showed that 2 h of exposing albino rats to ≥ 2.0 mg/ℓ concentrations of diesel-fuel aerosol increased startle reaction time and latency-to-peak amplitude, but decreased force of the acoustic startle response. Their data suggest that these startle response parameters should be sensitive indicators of sub-lethal effects of RP/BR-aerosol exposure in both prairie dogs and rock doves. Although no published accounts of prairie dog startle reactions exist, measures of peak amplitude and average response amplitude are expected to decrease, whereas time-to-peak amplitude is expected to increase following extensive RP/BR-aerosol exposures due to possible interference with oxygen consumption and/or other central nervous system effects. The same changes are also expected to characterize the effects of RP/BR-aerosol exposure on the visual startle responses of rock doves. Based upon previous drug studies (e.g., Davis, 1980; Ison, 1984), a short acoustic pre-pulse stimulus is predicted to cause reduced inhibition of startle response amplitude and average response, and to cause increased response latency, after RP/BR smoke exposure in both species.

A. Effects of RP/BR-aerosol upon the Startle Responses of Black-tailed Prairie Dogs

1. Methods

a. Black-tailed Prairie Dogs

A total of 24 prairie dogs was used in the Startle Response Study. The 12 males and 12 females were first rank-ordered by body weights. Males ranged from 1099 to 1387 g ($\bar{x}$ = 1216.6, SD = 96.6 g) and females ranged from 826 to 1189 g ($\bar{x}$ = 981.9, SD = 103.9 g). Next, 4 weight classes were developed for each sex by assigning the 3 lowest weight prairie dogs to "light" group, the next 3 to the "medium" group, the next 3 to the "medium heavy" group, and the last 3 to the "heavy" group. Finally, 1 male and 1 female prairie dog from each of the 4 weight classes (i.e., 4 males, 4 females) were randomly assigned to either the 0.0, 1.0, or 4.0 mg/ℓ RP/BR-aerosol groups.

b. Equipment

The Startle Response System (SR-LAB) used to measure RP/BR-aerosol-caused effects in paririe dogs and rock doves was manufactured by San Diego Instruments, Inc., San Diego, CA. The System was designed to test 2 animals simultaneously utilizing dual startle chambers within dual isolation cabinets. Each chamber had separate stimulus generators.

A dedicated minicomputer controlled stimulus events and data acquisition. Figure 12 presents a schematic drawing of the SR-LAB System. The following is a detailed description of the 4 major components of the System (i.e., Control Unit, Startle Chamber, Isolation Cabinet, and Startle Stimulus Generators).

The Control Unit controls the 2 startle chambers simultaneously and consists of an American Research Corporation (Monterey Park, CA) Model 10 Computer (ARC-10) with a factory-installed I/O board, 2 half-height 5.25 in floppy disk drives, a 30-M byte hard disk drive, and a Zenith (Glenview, IL) Model ZVM-1220A monochrome video monitor. A program named TEST EXECUTION automatically controls stimuli, monitors, and records responses. All software operations are menu-driven. A Panasonic NLQ Model KPX 1091 Printer (Secaucus, NJ) is included with the Control Unit to print copies of recorded data.

In operating the SR-LAB System, the Control Unit prompts the user for animal and test session file labels. Based upon user-provided stimulus, response, and data-capture configurations, the SR-LAB TEST EXECUTION Program now controls the System. Each animal's averaged responses are immediately displayed following a given stimulus presentation (i.e., after each trial) and all data are automatically recorded on the hard disk. Data are recorded for up to a 250 msec interval -- the "response window" -- after a stimulus is presented. These data include: the peak voltage (response amplitude), latency-to-peak voltage, and the average voltage across the "response window".

The SR-LAB software also allows elimination of those response sequences where animal movements obscured startle measurements. This data-reduction software (SRRED) allows the user to apply other scoring parameters to the measurements before data are analyzed (e.g., eliminating responses that are "too long" in terms of latency or "too low" in terms of amplitude).

Both of the identical Startle Chambers were constructed of transparent acrylic. Each chamber had inside dimension of 33 X 35 X 40 cm, with a grid floor of 0.63 cm diameter stainless steel rods spaced at 1.70 cm center-to-center distances. This spacing allowed fecal material to pass through the grid, but also ensured prairie dog foot contact on multiple rods when electrical foot-shock stimuli were delivered. Separate 5.0 X 5.0 X 1.8 cm accelerometer sensors were attached on the top outside center of each chamber to detect startle responses.

Each chamber rested on 4 (No. 9 Size) solid rubber bottle stoppers located at each of the bottom corners so as to maintain stability. The stoppers were affixed to both the chambers and the cabinet floors with 2.2 cm diameter pieces of

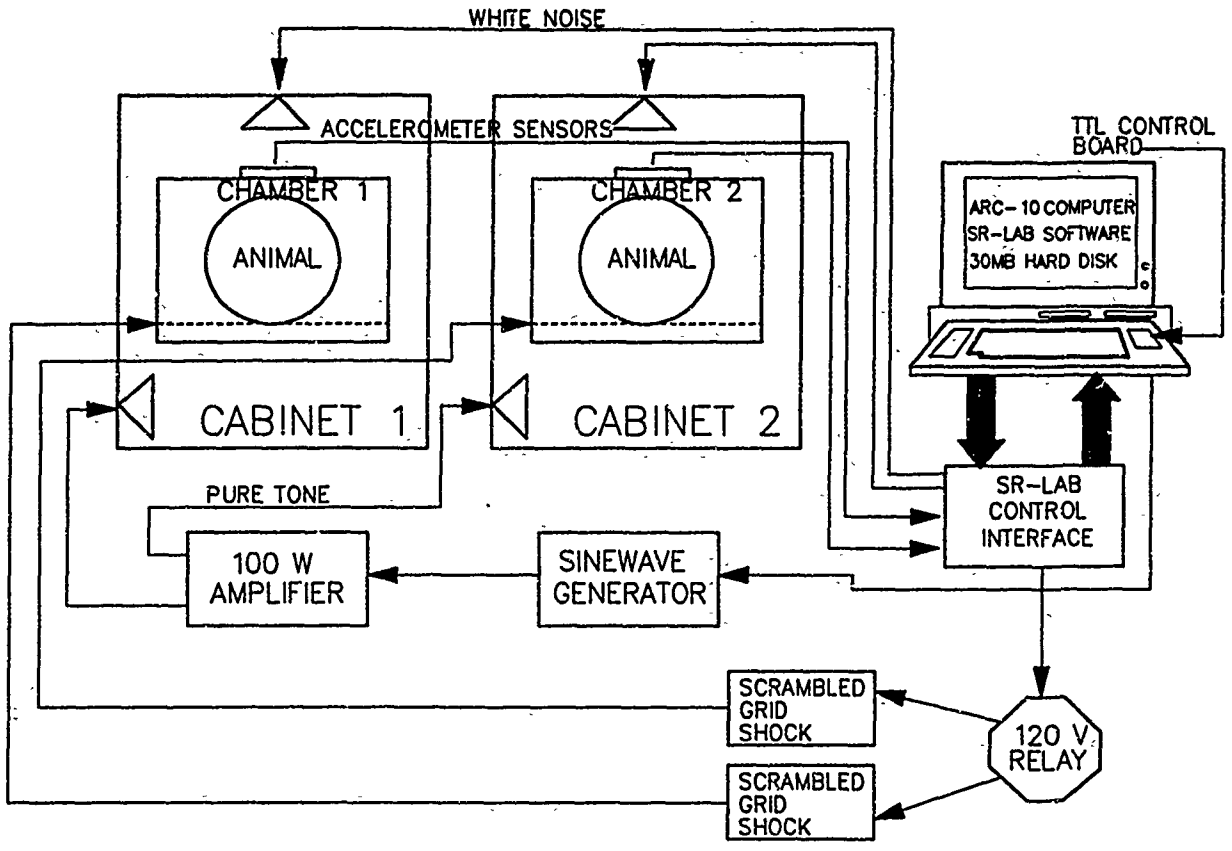


Figure 12. Block diagram of the SR-LAB San Diego Instruments Startle Response System. Two animals (prairie dogs or rock doves) are given a series of stimulus presentations over brief (12 to 14 min) sessions. Startle responses are detected by the accelerometer sensors; the animal response information in terms of mV changes over each msec of recording is stored on the ARC-10 computer 30 MB hard disk. All stimulus presentation parameters including duration, intensity, frequency, and inter-trial intervals are controlled by the computer. White noise pre-pulses, pure tone pulses at 7.8 and 15 kHz, and brief, mild scrambled grid foot shocks were the startle stimuli used in the prairie dog study. White noise pre-pulses and electronic photoflashes were stimuli used in the rock dove study.

Velcro tape. This arrangement made the chamber fairly stable for the elimination of response artifacts, but still sensitive for the detection of rapid, short "ballistic-type" startle response movements of the prairie dogs.

The dimensions of each Isolation Cabinet were 38.1 X 40.6 X 58.4 cm. Both were constructed of plywood and laboratory-grade laminated plastic. The cabinets served to isolate each animal from extraneous sounds and vibrations. Both were separately equipped with ventilation fans, lights (15 W bulbs), and "birds-eye" viewing lenses. The cabinets were also each equipped with high frequency white noise generators and a set of 2 speakers (Realistic Model 40-1377) for transduction of white noise pre-pulses and pure tone startle stimuli. Outputs for the 120 VAC relays that controlled the shock or photoflash stimuli were located on the cabinet control panels.

The Startle Stimulus Generators consisted of electrical shock sources and a tone generator/amplifier. Scrambled shock pulses were delivered to the grid floors of each chamber via cables from separate shock sources. This ensured independence of shock intensities for the 2 chambers and trial-to-trial, foot-shock stability. Shock bar grids in Chamber 1 were connected by an 18-conductor cable to a Model E13-08 Coulbourne Grid Shocker (Lehigh Valley, PA), with the output set at 1.5 mA. Shock bar grids in Chamber 2 were connected by an 18-conductor cable to a Summit Engineering (Boulder, CO) Model SE-MIS Matched Impedance AC Shocker - grid Scrambler with the rheostat set to deliver 200 VAC. Voltage and amperage checks were performed before each alternate pair of prairie dogs were tested during each session.

A custom-built tone generator/amplifier (San Diego Instruments, Inc., San Diego, CA) was used to produce sine wave tones over a frequency range of 961 Hz to 31.25 kHz. It consists of 5 interconnected modules (Coulbourne Instruments, Lehigh Valley, PA): 2, 5 VDC to 12 VDC logic converters, a digital-analog converter, a voltage controlled oscillator, and a programmable amplifier. Frequency, duration, and amplitude levels of pure tones produced by the generator were programmed and controlled using the ARC-10 minicomputer's TTL-control board. Further amplification of the pure tone signals was achieved with the Realistic MPA-90 Solid State Amplifier (Tandy Corp., Fort Worth, TX). Pure tone signals from the Coulbourne Amplifier were fed to the MPA-90 which can achieve 100 W output. Realistic Model 40-1377 Speakers were used to transduce the pure tone sound bursts within each SR-LAB Isolation Cabinet.

c. Procedures

The exposure schedule for the Startle Response Study with prairie dogs was outlined earlier (II. EXPERIMENTAL APPROACH). Three groups of 4 males and 4 females each were assigned to receive 4 successive, 80-min exposures of either 0.0 (filtered air), 1.0, or 4.0 mg/ℓ RP/BR-aerosol target concentrations. Animals were monitored for peak amplitude (mV), latency-to-peak (msec), and average response voltage (mV) on 2 days of the Pre-exposure Phase (Pre-1 and Pre-2), within 2 h of the 4 RP/BR-aerosol or filtered-air exposures during the Exposure Phase (E-1, E-2, E-3, and E-4), and on 4 days of the Post-exposure Phase (P-1, P-2, P-11, and P-12) as indicated in Figure 2.

Within-session measurements involved 2 animals being tested simultaneously for startle responses. Each session consisted of multiple stimulus presentations (trials) over a 14 min period -- a series of mixed, variable interval presentations of brief mild shocks (i.e., 45 msec at 1.5 mA or 200 VAC; Chamber 1 vs Chamber 2, respectively) and brief pure tone pulses (i.e., 5 msec at 7.8 or 15 kHz at 109 to 112 dB). The mean inter-trial interval was 30 sec.

The timed events and recording intervals for each of 5 types of trials are depicted in Table 2. Each trial consisted of a startle stimulus event(s) followed by a 190 msec recording interval. For the foot-shock stimuli, recording began concurrently with stimulus onset; for the pure tone stimuli, recording began 20 msec after onset. Latencies-to-peak response times were therefore not directly comparable between shock and tone stimuli (i.e., without a 20 msec adjustment); shock and tone trial data were, however, handled by separate analyses. Several "no stimulus (control) trials" were imposed to assess changes in all 3 response measures. These involved measurement and recording of the peak amplitude, latency, and average response variables at various times without a stimulus being presented, and then using these base measurements as reference values for assessing startle response values to the shock and tone stimuli.

Trials were presented to each prairie dog during either of 2 types of sessions -- TONSHOK or TONSHOK1 (Table 3). Both types generated a sequence of variable time intervals between trials. In each session, foot shock and tone trials were also interspersed with no stimuli (control) trials. The 7.8 kHz and 15 kHz order of presentation of tone stimuli was reversed for the 2 session types, otherwise the 2 stimulus presentation

Table 2. The stimulus events and durations used to evaluate startle responses in prairie dogs to 5 types of trials.

Trial Type	Stimulus Event	Stimulus Duration (msec)	Lapsed Interval Before Recording (msec)	Recording Interval (msec)
Shock	Foot Shock	45	0	0-190
Prepulse Shock	Noise Pulse + Shock	40 45	40 0	0-190
No Shock	--	--	--	0-190
15 kHz Tone	High Tone	5	15	0-190
7.8 kHz Tone	Low Tone	5	15	0-190

Table 3. Trial type sequences in each of 2 sessions used for alternate prairie dog pairs run concurrently.

Trial Number	Session Name [a]			
	TONSHOK		TONSHOK1	
	Trial Type	Inter-trial Interval (sec)	Trial Type	Inter-trial Interval (sec)
1	Shock [b]	240 (acclimation) [c]	Shock	240 (acclimation)
2	15 kHz Tone [d]	30	7.8 kHz Tone	30
3	Prepulse Shock [e]	25	Prepulse Shock	25
4	7.8 kHz Tone [f]	30	15 kHz Tone	30
5	No Shock	35	No Shock	35
6	Shock	30	Shock	30
7	15 kHz Tone	25	7.8 kHz Tone	25
8	Prepulse Shock	30	Prepulse Shock	30
9	7.8 kHz Tone	35	15 kHz Tone	35
10	No Shock	30	No Shock	30
11	Shock	25	Shock	25
12	15 kHz Tone	30	7.8 kHz Tone	30
13	Prepulse Shock	35	Prepulse Shock	35
14	7.8 kHz Tone	30	15 kHz Tone	30
15	No Shock	25	No Shock	25
16	Shock	30	Shock	30
17	15 kHz Tone	35	7.8 kHz Tone	35
18	Prepulse Shock	30	Prepulse Shock	30
19	7.8 kHz tone	25	15 kHz Tone	25
20	No Shock	30	No Shock	30

[a] Two males and 2 females from each of 3 RP/BR-aerosol concentration groups (n = 8 animals/ea) consistently received TONSHOK sessions; the other 4 animals consistently received the TONSHOK1 sessions.

[b] For Chamber 1, the shock intensity was set to read 1.5 mA using the Coulbourn Instruments Model E13-08 Animal Test Cage Grid-Floor Shocker. For Chamber 2, the shock intensity was set to read 200 VAC using the Summit Engineering SE-MIS Matched Impedance AC Shocker-grid Scrambler. Stimulus intensities were re-checked and re-adjusted for every other tested pair of animals during all sessions.

[c] Background readings with Bruel and Kjaer Type 4135 Impulse Sound Level Meter and Type 4135 condenser Microphone indicated background white noise of 73.3 ± .5 dB for Chamber 1 and 73.3 ± .9 dB for Chamber 2. Background chamber illumination was achieved using a 15 watt (General Electric, Soft White) bulb in each cabinet. Using a Salford Electrical Instruments Exposure Photometer, illumination levels in both chambers were measured to be 1.10 to 1.15 log Foot Lamberts.

[d] Intensities of the 15 kHz tone pulse startle stimulus were 112.0 ± 1.0 dB for Chamber 1 and 109.3 ± 2.3 dB for Chamber 2.

[e] White noise prepulse stimuli were 80-81 dB for Chamber 1 and 76-77 dB for Chamber 2.

[f] Intensities of the 7.8 kHz tone pulse startle stimulus were 109.5 ± 1.5 dB for Chamber 1 and 110.5 ± 0.5 dB for Chamber 2.

orders were the same. Because variable intervals occurred between the different trial types, the animals were assumed to have been unable to anticipate when the next startle stimulus was due to occur. As indicated in Table 3, each trial type was presented to pre-assigned same-sex pairs of prairie dogs 4 times per session. Two males and 2 females from each of the main RP/BR-aerosol concentration groups received TONSHOK Sessions and the other pairs of animals received TONSHOK1 Sessions. After all animals had been tested for startle responses during a given session (day), the TONSHOK session was again run with no animal in either chamber. This was done to evaluate the potential for stimulus artifacts that could bias the recorded startle responses.

d. Experimental Designs and Data Analyses

Separate univariate ANOVAs were computed on the startle response means of peak amplitude, latency-to-peak, and average response voltage variables using the PROC ANOVA Program and Type III sums of squares (SAS Institute, Inc., 1985) to determine significant effects. The means for each animal and variable were calculated and analyzed across the Pre-exposure, Exposure, and Post-Exposure Sessions (i.e., Pre-1, Pre-2, E-1, E-2, E-3, E-4, P-1, P-2, P-11, and P-12). Electrical shock and acoustical startle responses were analyzed separately. Thus, 3 ANOVAs (i.e., one for each dependent measure) were computed for the measurements obtained using shock and 3 ANOVAs were computed for the measurements obtained using pure tone stimuli. Each ANOVA involved a 3 (RP/BR-aerosol Concentration) X 2 (Sex) X 3 (Trial Type) X 10 (Session) design, with Trial Type and Session treated as repeated measures factors (Winer, 1971). Significant ANOVA terms were further evaluated using post-hoc Duncan Multiple Range Tests (Waller and Duncan, 1969).*

To evaluate the potential problems of interfering stimulus artifacts, the trials run with empty chambers were analyzed separately. The mean session data for each measure were analyzed in a 5 (Trial Type) X 10 (Session) ANOVA design, with repeated measurements on the Session factor.

2. Results and Discussion

Measures of RP/BR aerosol quantity (e.g., concentrations and mass), quality (e.g., particle sizes, respiratory and contaminant gases present) and exposure chamber conditions (e.g., duration, temperature) are listed as a summary table in Appendix E.

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*See footnote on Page 27.

a. Mortality/Clinical Symptomatology

No prairie dog died during the Startle Response Study. There were, in fact, only 2 instances of animals showing clinical signs of RP/BR exposure effects throughout the 18-day test. One male in the 1.0 mg/ℓ Group exhibited a distorted, raspy bark after the first exposure session. Another male in the 4.0 mg/ℓ group exhibited labored breathing and wheezing when handled after the fourth exposure session.

b. Startle Response Decrements

The 3 analyses for stimulus artifact effects with empty chambers yielded no significant main or interaction terms. Actual startle response data from the prairie dogs were thus regarded as being free of stimulus artifact effects. Results are presented separately for the electrical shock and acoustical startle response data.

(1) Electrical Foot-shock Stimuli.-- The "no shock", "prepulse shock", and "shock" trial data were first screened for spurious movement responses during the 20 msec baseline period prior to shock onset. Only 7 responses or < 0.3 percent of the data had to be omitted by this process. Although, other data-selection criteria could have been applied to the data sets (e.g., selections of specific latency and peak amplitude values), these criteria were not applied before analysis due to the relatively unknown response topography of prairie dog startle responses. Data were then averaged for each animal for each session by calculating mean values for each trial type and each response measure. These means formed the data sets for each ANOVA.

The peak amplitude measure produced a significant Sex X Trial Type effect ($F = 4.50$, $df = 2/36$, $p < .018$) and a significant Trial Type effect ($F = 12.34$, $df = 2/36$, $p < .0001$). The Sex X Trial Type interaction is shown in Figure 13. Whereas, the females produced significantly greater responses on both "prepulse shock" and "shock" trials ($p < .05$, Duncan's Test), the males did not show reliable responding to foot-shock in these pair-wise comparisons. The main effect for Trial Type simply confirmed this effect, with mean response amplitudes for "no shock", "prepulse shock" and "shock" trials of 47.3, 66.2, and 72.9 mV, respectively.

The latency-to-peak data for electrical foot-shock yielded a significant Concentration X Trial Type interaction ($F = 4.35$, $df = 4/36$, $p < .0057$). Post-hoc Duncan mean comparisons revealed that the mean "no shock" and "shock" trial latencies for the 0.0 mg/ℓ and 1.0 mg/ℓ RP/BR-aerosol groups, respectively, were greater than mean latencies for the remaining 7 cell combinations of the design.

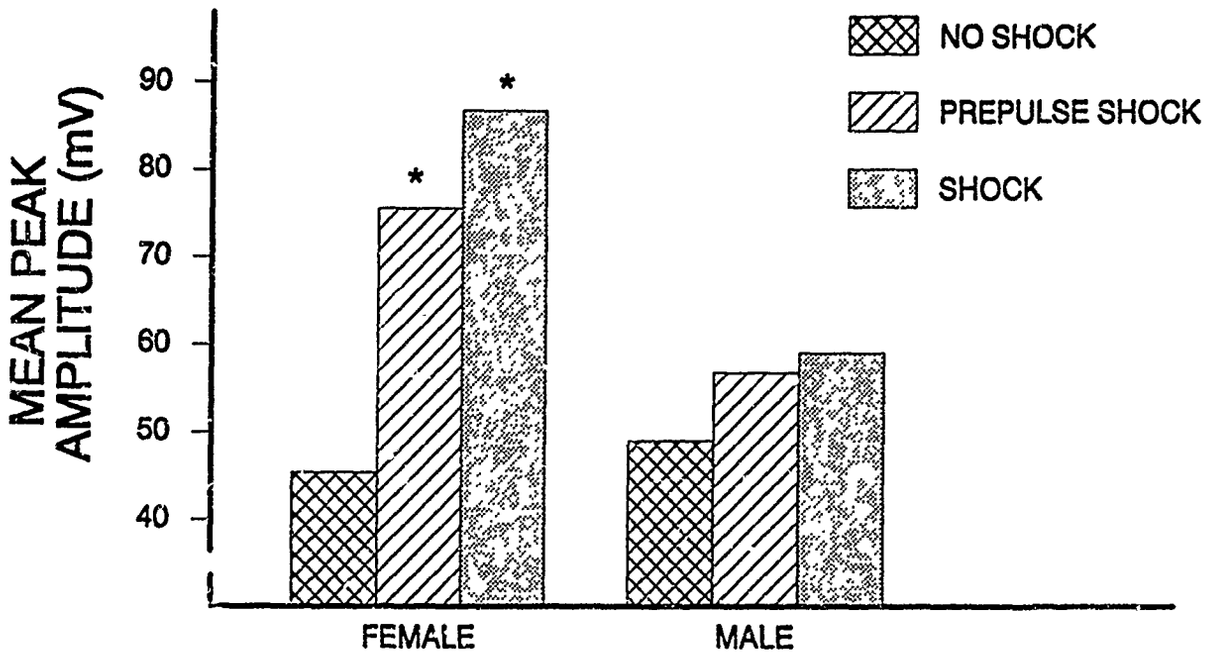

Figure 13. The Sex X Trial Type interaction for the peak amplitude measure of startle response in prairie dogs. All animals were presented with each type of stimulus (NO SHOCK, PREPULSE SHOCK, SHOCK) 4 times during each of 10 Sessions. As indicated by the asterisks, PREPULSE SHOCK and SHOCK stimuli elicited significantly ($p < 0.05$) greater amplitude responses than the NO SHOCK (control) stimuli in the females.

Figure 14 depicts this Concentration X Trial Type interaction for the latency-to-peak variable. Under the null hypothesis that RP/BR-aerosol has no effect on startle response, latencies for the RP/BR-aerosol-exposed groups should have been no different from those of the 0.0 mg/ℓ group. As predicted, a significantly longer mean latency value for "no shock" versus either "prepulse shock" or "shock" was indicated in the 0.0 mg/ℓ group. The two RP/BR-aerosol-exposed groups (1.0 and 4.0 mg/ℓ target concentrations), on the other hand, did not duplicate this latency-to-peak pattern among the 3 types of trials indicating possible disruption of the latency pattern by RP/BR-aerosol exposures. However, a conservative interpretation is that weaker stimulus control was achieved overall in the RP/BR aerosol groups, but this was not directly related to RP/BR-aerosol effects since no Session main or interaction effects were found to be significant.

The average response voltage measure yielded results similar to the mean peak amplitude measure for electric foot shock. A significant Sex X Trial Type interaction effect ($F = 4.25$, $df = 2/36$, $p < 0.022$) occurred. The interaction is displayed as a bar graph in Figure 15. Females again showed stronger average startle responses to the "prepulse shock" and "shock" stimuli compared with the "no shock" (control) trials; whereas, average voltages for males across "shock", "prepulse shock", and "no shock" trials were not different.

As expected, there was an overall main effect for Trial Type ($F = 13.04$, $df = 2/36$, $p < 0 .0001$) with the "shock" and "preshock" trials producing greater average response values than the "no shock" trials.

The Session main effect was also significant ($F = 2.76$, $df = 9/162$, $p < 0.0049$). Mean values for the average response voltages are plotted across the 10 Sessions in Figure 16. The general trend is for the average response values to gradually habituate between Sessions Pre-1 and P-12; however, the average response for the first session that the animals were confined to the chamber (E-1) is significantly less than the Pre-exposure Sessions (Pre-1 and Pre-2). On the next day, (E-2), average response performance was non-significantly different from the pre-exposure sessions. The Day effect thus indicated that the initial session of chamber confinement in some way reduced or interfered with the average amplitudes of all animals and that animals immediately recovered from these reduced response amplitudes after the second exposure session (E-2).

(2) Acoustical Stimuli.-- The same screening process for spurious movement responses was performed on the acoustical data to remove data affected by animal movement responses not related to the tone stimuli. Likewise, data were then averaged for each animal for each session by calculating the mean values for each of the 3 trial types ("7.8 kHz", "15.0 kHz", and "no

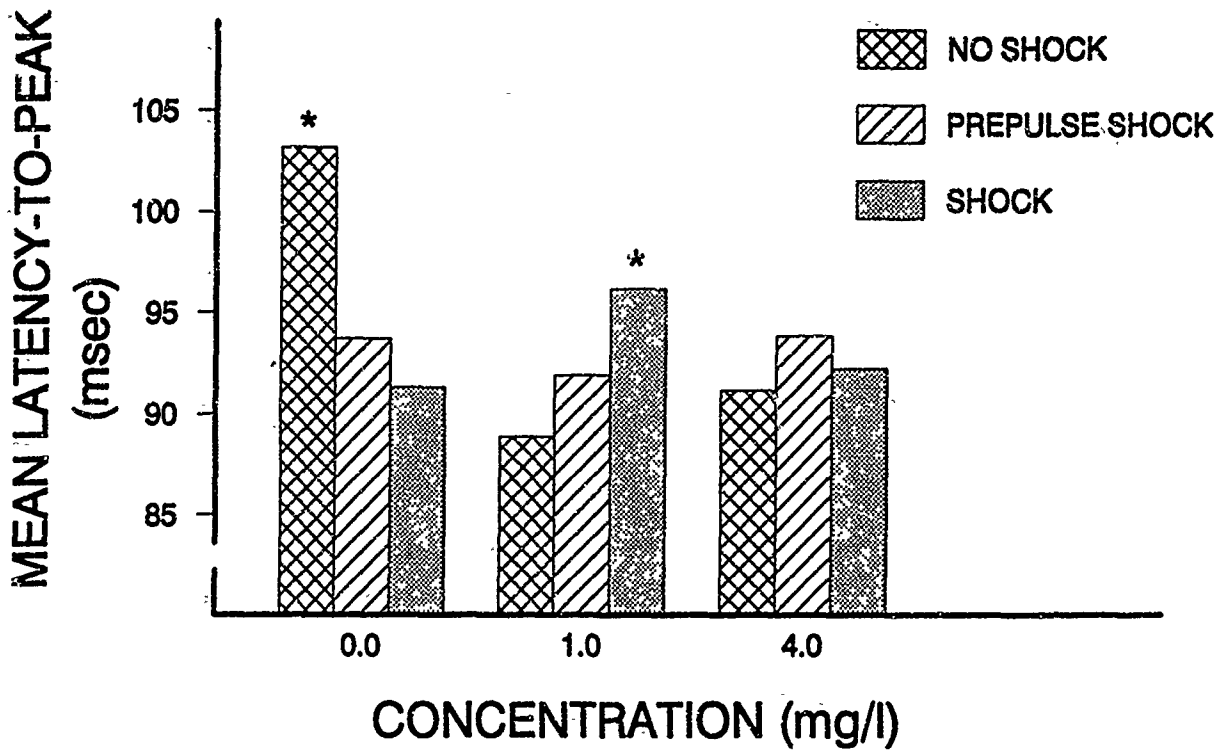

Figure 14. Mean latency-to-peak (msec) of startle responses in prairie dogs as a function of concentration of RP/BR smoke and startle stimuli. As indicated (*), the mean response latencies for the 0.0 mg/ℓ concentration NO SHOCK stimulus and the 1.0 mg/ℓ concentration SHOCK stimulus were significantly longer than all other mean response latencies.

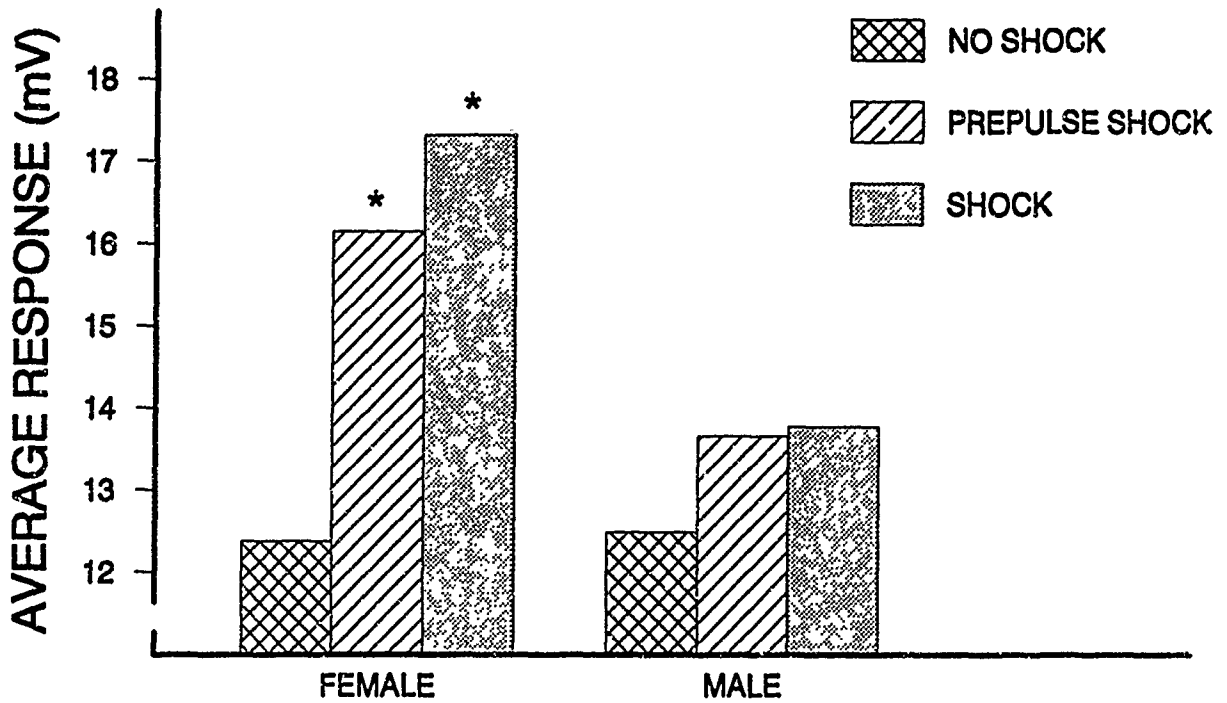

Figure 15. The Sex X Trial Type interaction for the average response amplitude (mV) measure of startle response in prairie dogs. As indicated (*), PREPULSE SHOCK and SHOCK stimuli elicited significantly ($p < 0.05$) greater average response amplitudes than the NO SHOCK (control) stimuli in the females.

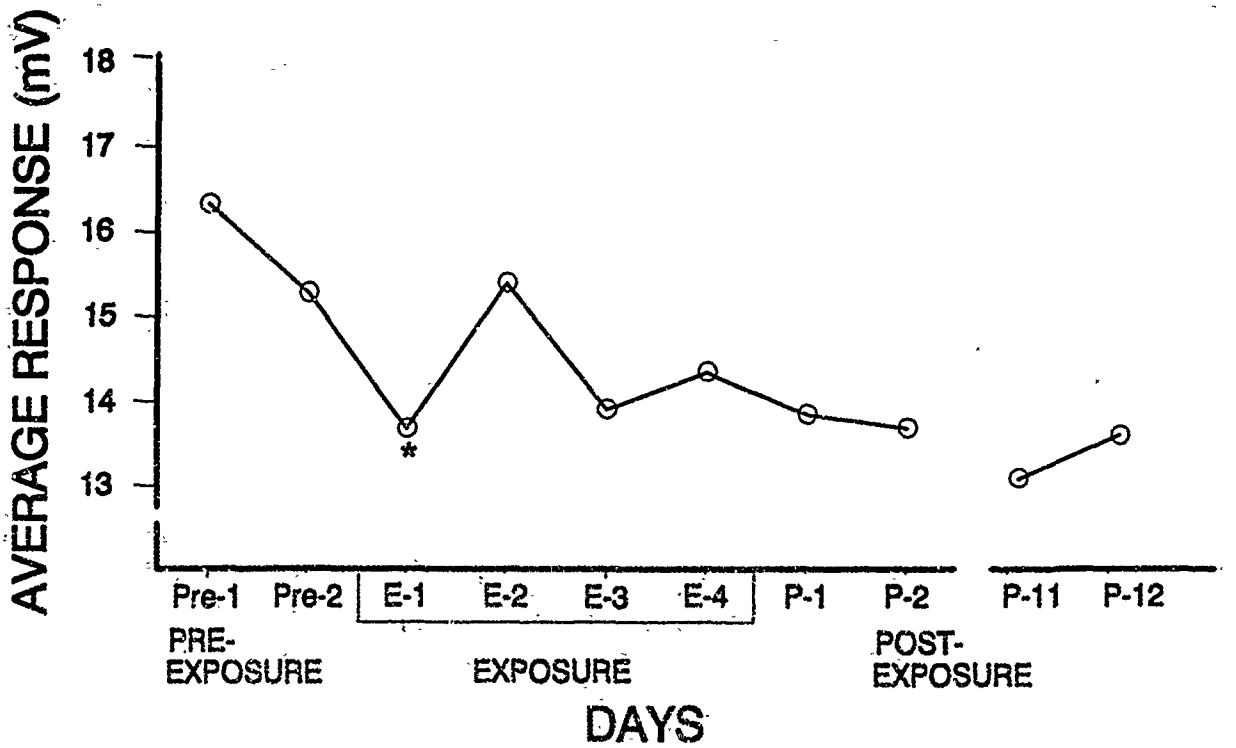

Figure 16. Average response amplitude as a function of Sessions. A significant decrease (*, $p < 0.05$) in this measure occurred on the first Exposure Day (E-1), followed by a strong rebound of average response, however, on the second Exposure Day (E-2), and then average response habituation through the last Post-exposure Day (P-12).

shock") and each of the 3 response measures (peak amplitude, latency-to-peak, and average response voltage).

Only 2 significant effects were obtained for the 3 ANOVAs, and these involved Session main effects for both the peak amplitude ($F = 2.03$, $df = 9/162$, $p < 0.0392$) and the average response ($F = 3.48$, $df = 9/162$, $p < 0.0006$) measures.

Figures 17 and 18 illustrate these main effects across Sessions; similar adaptation effects to tone stimuli by the prairie dogs are evident for the peak amplitude and average response measures to tone, respectively. In both cases, the first Pre-exposure Session (Pre-1) is significantly different from the means for all other sessions. These effects indicated that prairie dogs responded maximally to the tone stimuli on this initial session and then were essentially non-responsive to the 2 acoustical stimuli in all later sessions. In contrast to the electrical foot-shock stimuli, the "7.8" and "15.0 kHz tone" trial responses were not significantly different from the "no shock" (control) trial responses over the 10 sessions in this study. These tone burst frequencies at 108 to 113 dB levels were therefore found to be ineffective in producing reliable startle responses in prairie dogs.

In summary, only female prairie dogs exhibited reliable responses to electrical-foot shock. Neither males nor females showed reliable startle responses to the 7.8 and 15.0 KHz pure tone stimuli over the 10 sessions. Habituation to these acoustical stimuli was apparently quite rapid. The latency-to-peak response measure gave some indication that the 1.0 and 4.0 mg/ℓ target concentration levels of RP/BR smoke affected the prairie dog startle response to foot shock. This effect, in the form of a significant RP/BR Concentration X Trial Type interaction, however, was suspected of being an anomalous finding. There were, for example, no significant Session main or interaction effects which would have indicated that the above 2-way effect occurred only during or immediately after the smoke exposures. Thus, without further confirmatory data, we conclude that no changes occurred in any of the 3 startle response measures after exposure.

B. Effects of RP/BR-aerosol upon the Startle Responses of Rock Doves

1. Methods

a. Rock Doves

A total of 24 animals (12 of each sex) was used to assess RP/BR-aerosol effects upon the visual startle responses of rock doves. Doves of each sex were initially rank-ordered by body weight. Males ranged from 303 to 382 g ($x = 327.2$; $SD = 20.2$) and females ranged from 293 to 389 g ($x = 327.4$;

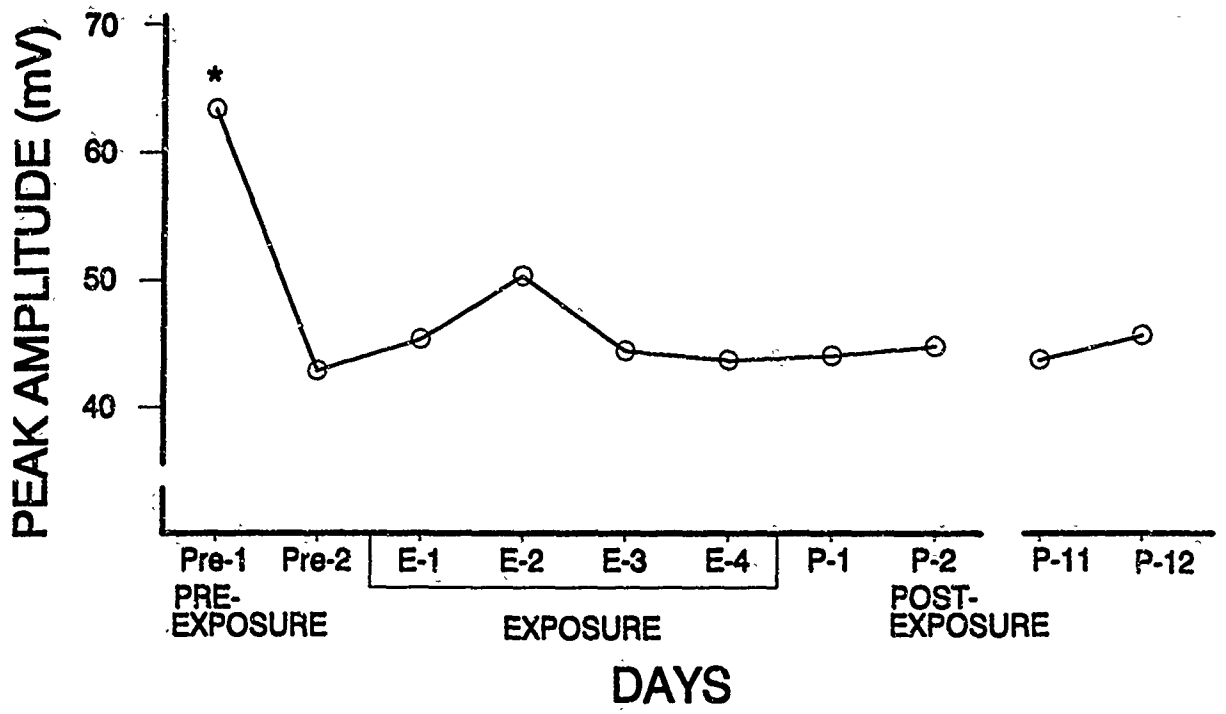

Figure 17. Peak amplitude of response to tone as a function of Sessions. The peak amplitude response was significantly stronger (*, $p < 0.05$) on the first Pre-exposure Day (Pre-1), followed by immediate habituation to the pure tone stimuli on Days Pre-2 through P-12.

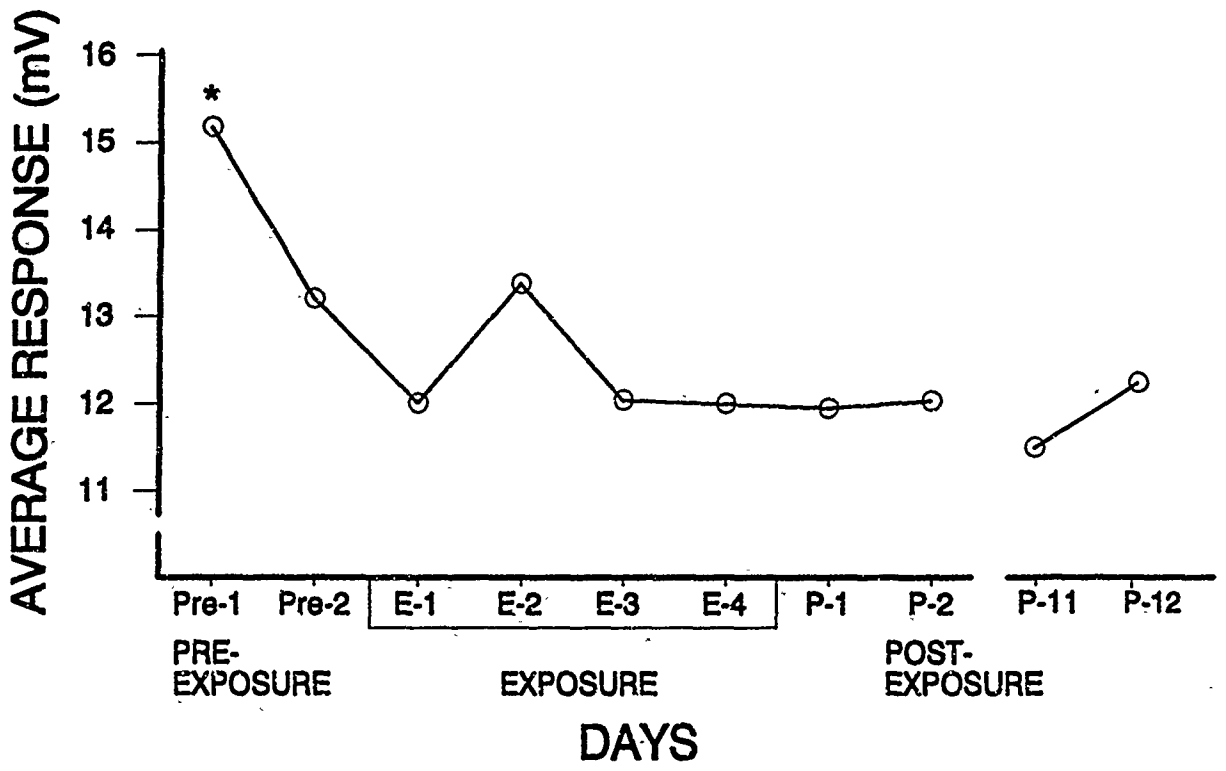

Figure 18. Average response amplitude to tone as a function of Sessions. The average response measure was significantly greater (*, $p < 0.05$) on the first Pre-exposure Day (Pre-1) followed by rapid habituation to the pure tone stimuli on Days Pre-2 through P-12.

SD = 24.7). Next, 4 weight classes were developed for each sex by assigning the 3 lowest weight rock doves to the "light" group, the next 3 to the "medium" group, the next 3 to the "medium heavy" group, and the last 3 to the "heavy" group. Then, 1 male and 1 female rock dove from each of the weight classes (i.e., 4 males and 4 females) were randomly assigned to 1 of 3 RP/BR-aerosol groups (i.e., 0.0, 1.0, or 4.0 mg/ℓ).

b. Equipment

The Startle Response System used to measure RP/BR-aerosol-caused effects in rock doves was the same as that described for prairie dogs. The only difference was that the 2 scrambled grid shock units were replaced with 2 Vivitar Model 35.D thyristor electronic flash units (Vivitar Corp. Santa Monica, CA) and these were operated by the 120 VAC relay. A flash unit was placed on the top right front corner of each clear Plexiglass Startle Chamber in a fixed location to control light flash levels across trials and sessions. The light intensity levels for each photoflash unit were checked before and after each session. Batteries were replaced in the photoflash units regularly. Prepulse stimuli were generated using the internal white noise generator-amplifiers in each test cabinet. Neither the pure tone sine wave generator nor the 100 W amplifier were used for the rock dove startle response study.

The Startle Chambers were modified for rock doves. Inside dimensions were 29.1 X 29.1 X 24.3 cm, with a floor of 0.63 cm diameter stainless steel rods spaced at 1.3 cm center-to-center intervals. Affixed to the top outside center of each chamber was a 5.0 X 5.0 X 1.8 cm accelerometer sensor. Background and prepulse white noise stimuli were provided through the overhead Model 40-1377 Realistic speakers.

c. Procedures

Rock dove groups were exposed to the RP/BR aerosol or filtered air (control) over 2 daily, 80-min sessions according to the paradigm described in the Section II, EXPERIMENTAL APPROACH (see Figure 2). All animals were monitored for 3 startle response measures (peak amplitude, latency-to-peak, and average response voltage) for: (1) 2 days during the Pre-exposure Phase (Pre-1 and Pre-2), (2) within 2 h of RP/BR-aerosol or filtered-air exposure during each of the 2 days of the Exposure Phase (E-1 and E-2), and (3) during 6 days of the Post-exposure Phase (P-1, P-2, P-5, P-6, P-11, and P-12).

The stimulus events during each of the 4 types of trials presented within each session are shown in Table 4. Each trial consisted of a startle stimulus event (or events)

Table 4. The stimulus events and durations used to evaluate startle responses in rock doves to 4 types of trials.

Trial Type	Stimulus Event	Stimulus Duration (msec)	Lapsed Interval Before Recording (msec)	Recording Interval (msec)
Flash	Photo Flash	40	0	0-170
No Flash	--	--	--	0-170
Prepulse Flash	Noise Pulse + Flash	40 40	40 0	0-170
Prepulse No Flash	Noise Pulse	40	40	0-170

followed by a 170 msec recording interval (i.e., "record window").

The recording interval was always initiated simultaneously with photoflash onset, but the white noise prepulse stimulus always preceded the recording interval by 40 msec. This ensured that prepulse inhibitory effects on the startle responses to the photoflash would be measured in a comparable time interval relative to measurement of the photoflash startle responses per se. "No Flash" (Control) trials served to produce reference response values within a session. Essentially, these were ambient sensor voltage amplitude readings in the absence of a stimulus. Photoflash startle responses were thus assessed by comparison with the "No Flash" trials. "Prepulse No Flash" trials were presented to evaluate any potential startle responses to the white noise prepulse stimuli per se.

During each 9.4-min session, multiple "Flash", "No Flash" (Control), "Prepulse Flash", and "Prepulse No Flash" trials were presented to each pre-assigned pair of rock doves (see Table 5). Each session involved a series of variable time interval presentations of these trials. A mean inter-trial interval of 30 sec was again used in all sessions.

Rock doves received the trials under either the FLASHER or FLASHER1 trial sequences (Table 5). These 2 types of sequences were run on alternately assigned, same sex pairs of rock doves throughout the study. The only difference between the 2 types of sessions was the initiating trial type -- "Flash" versus "Pre-pulse Flash". These were alternated between dove pairs to counter balance within-session habituation effects that could pose a bias in the comparison of these 2 types of trials. As indicated previously, the prepulse white noise stimulus that occurred 40 msec prior to the photoflash startle stimulus was predicted to reduce peak amplitude and average response values and to increase response latency-to-peak. Variable time intervals ($\bar{x}$ = 30 sec) between trials were used to reduce the dove's ability to accurately anticipate occurrence of the next startle stimulus.

As indicated in Table 5, each trial type was presented to each rock dove 3 times per session. For each of the 3 RP/BR-aerosol concentration groups, 2 male and 2 female birds received FLASHER sessions; the alternate same sexed pairs received FLASHER1 sessions. After all birds had been tested for startle responses on a given session day, the FLASHER session was again run with no animals in either chamber. This was run to evaluate potential stimulus artifacts that could pose a possible source of biasing interference to the recorded rock dove startle responses.

Table 5. Trial-type sequence in each of 2 sessions used for alternate rock dove pairs run concurrently.

Trial Number	Session Name [a] FLASHER Trial Type	FLASHER Inter-trial Interval (sec)	FLASHER1 Trial Type	FLASHER1 Inter-trial Interval (sec)
1	Flash [b]	400 (acclimation) [c]	Prepulse Flash	400 (Acclimation)
2	No Flash	30	Prepulse No Flash	30
3	PrePulse Flash [d]	25	Flash	25
4	Prepulse No Flash	30	No Flash	30
5	Flash	35	Prepulse Flash	35
6	No Flash	30	Prepulse No Flash	30
7	Prepulse Flash	25	Flash	25
8	Prepulse No Flash	30	No Flash	30
9	Flash	35	Prepulse Flash	35
10	No Flash	30	Prepulse No Flash	30
11	Prepulse Flash	25	Flash	25
12	Prepulse No Flash	30	No Flash	30

[a] Two males and 2 females from each of the 3 RP/BR aerosol concentration groups (n = 8 animals/ea) consistently received FLASHER sessions; the other 4 animals consistently received FLASHER1 sessions.

[b] Readings on the Wein WP/500B Flash Meter were within the range from F18 to F22 for both chambers on all trials.

[c] Background readings with a Bruel and Kjaer Type 4135 Impulse Sound Level Meter and Type 4135 Condenser Microphone indicated background white noise of 72 to 73 dB (linear scale) for both chambers. Background chamber illumination was achieved using a red 7-1/2 W (General Electric, type S/CR) bulb in each cabinet. Illumination levels were measured to be -1.50 to -1.65 log Foot Lamberts for Chamber 1 and -1.30 to -1.40 log Foot Lamberts for Chamber 2 using a Salford Electrical Instruments Exposure Photometer.

[d] Prepulse stimuli ranged from 80 to 81 dB in Chamber 1 and from 76 to 77 dB in Chamber 2 (linear scale, B&K Type 4135 Sound Level Meter and Type 4135 Microphone).

d. Experimental Designs and Data Analyses

Session means of the untransformed raw data for each trial type and each dependent variable (peak amplitude, latency-to-peak, and average response) were analyzed separately for each sex. One male and 1 female rock dove were mis-sexed by the cloacal examination method when individual rock doves were initially assigned to the different treatment groups. The actual groups composition was as follows: 0.0 mg/ℓ = 3 males + 5 females; 1.0 mg/ℓ = 4 males + 4 females; and 4.0 mg/ℓ = 5 males + 3 females. The PROC GLM Program of the Statistical Analysis System (SAS Institute, Inc., 1985) was used to deal with missing data in certain cells of the design. ANOVAs were computed using Type III sums of squares for males and females separately because the computer memory requirements for inclusion of Sex as a factor in the design were prohibitive. Each of the 6 analyses was a 3 (RP/BR-aerosol Concentration Group) X 4 (Trial Type) X 10 (Session) ANOVA, where Session involved repeated measures. Post-hoc Duncan Range Tests were used to assess significant ANOVA effects.*

To evaluate the potential of stimulus artifacts in trials run with empty chambers, the mean session data sets for each measure were analyzed as a 4 (Trial Type) X 10 (Session) ANOVA design with repeated measures on the Session factor.

2. Results and Discussion

The RP/BR-smoke and filtered-air exposures were monitored in terms of quantity (e.g., mass and concentration), quality (e.g., particle sizes, respiratory and contaminant gases present) and the exposure chamber conditions (e.g., duration, temperature, humidity). Data are summarized in Appendix E.

a. Mortality/Clinical Symptomatology

One male rock dove in the 4.0 mg/ℓ RP/BR-aerosol-exposure group exhibited excessive coughing during the 400 sec acclimation period prior to the startle test session on Day 2 post exposure (P-2) and died on Day 6 post exposure (P-6). It lost 23 g of body weight (7%) between Day Pre-2 and Day P-5. Upon necropsy, this bird showed exudate in the upper beak and in the trachea -- other organs appeared normal. No other doves died or showed outward clinical signs of respiratory distress after the RP/BR smoke exposures.

*See footnote on Page 27.

b. Startle Response Decrements

The 3 analyses for stimulus artifact effects yielded 3 significant terms: Session main effects on the response amplitude and average response measures ($F = 3.38$, $df = 9/40$, $p < 0.0036$; $F = 2.17$, $df = 9.40$, $p < 0.0457$, respectively) and a Trial Type main effect on the latency-to-peak measure ($F = 3.07$, $df = 3/40$, $p < 0.0387$).

Mean separation tests on the significant Session effects for response amplitude and average response yielded the same pattern. The first post exposure session (P-1) was significantly elevated compared to all other sessions -- no other session means were significantly different from one another. For response amplitude, the P-1 mean was 49.25 mV which was higher than the other 9 means ranging from 40.04 (P-12) to 47.87 (E-2). For average response, the P-1 mean was 12.91 mV which was higher than the other 9 means ranging from 10.50 (P-12) to 12.29 (E-2). This effect represents an increased sensitivity of the sensors to the amplitude and

average response measures on session P-1 of approximately 15.4 and 12.8 percent, respectively. Thus, in comparing the RP/BR smoked-caused effects upon startle responses of rock doves using these 2 measures, the interpretive comparisons have been restricted to the other 9 sessions to avoid this source of sensor bias.

The Trial Type main effect on the latency-to-peak measure was further analyzed with Duncan mean separation tests. The mean of the "prepulse no flash" trials was found to be significantly shorter compared to the mean of the "no flash" trials -- 69.9 versus 94.7 msec, respectively. Means for "flash" and "prepulse flash" trials were not significantly different from each other (82.5 versus 80.2 msec) or from the other trial-type means. Although the standard deviations for all 4 trial types were fairly uniform and high (ranging from 20.0 to 28.9 msecs), the mean difference between the "prepulse no flash" and the "no flash" means (i.e., 24.8 msecs) is quite pronounced and represents a 35 percent latency change. With empty chambers, the prepulse white noise sounds were apparently detected by the accelerometer sensors on the latency-to-peak measure. Interpretations of the PR/BR smoke-caused effects upon rock dove startle responses using this measure were therefore limited to the 3 Trial Types not found to be significantly different from one another (i.e., "flash", "no flash", and "prepulse flash") with both chambers empty to avoid this source of sensor bias. As indicated by the ANOVAs performed on the data sets, results are presented separately for male and female rock doves.

(1) Male rock doves.-- Data for the 4 trial types ("flash", "no-flash", "prepulse flash" and "prepulse no flash") were screened for spurious movement responses during the 20 msec baseline period prior to photoflash onset. Only 2 responses for the entire data set were omitted by this method. The presence of the "no-flash" (Control) trials provided a set of continual, internal reference values for the peak amplitude, latency to peak, and average response measures so that no assumptions were necessary in regard to the "typical" startle response topography of rock doves to the photoflash stimuli. The raw data values for each dependent measure were then averaged by calculation of mean values for each animal during each session.

The peak amplitude response measure produced a significant Trial Type main effect ($F = 4.21$, $df = 3/27$, $p < 0.0145$). Duncan mean separation tests indicated that only the "flash" was significantly different from any of the other trial types. This main effect is depicted in (top panel). The "prepulse flash" combination stimulus thus suppressed mean peak amplitude almost completely when compared with the "flash" stimulus alone. The RP/BR smoke exposures did not alter this response measure in this analysis (i.e., neither the Trial Type X Session nor the Concentration X Session interaction terms were significant).

The latency-to-peak measure also produced a significant Trial Type main effect ($F = 19.31$, $df = 3/27$, $p < 0.0001$). Mean separation tests in this case revealed that both "flash" and "prepulse flash" caused reduced latencies-to-peak response when compared to "no flash" Trials. This Trial Type main effect is shown in (center panel). Unlike the empty chamber analysis previously mentioned, the "prepulse no flash" mean was not significantly different from the "no flash" mean. The weight and/or sound absorbency of the doves in the chambers apparently greatly reduced the stimulus artifact effect on this dependent measure. Nevertheless, the "prepulse no flash" data were regarded as affected by stimulus artifacts and thus no further mean comparisons were made for this trial type. Again, on this measure, RP/BR smoke exposures did not alter response latencies to the "flash" or "prepulse flash" startle stimuli.

Average response voltage also yielded a main effect of Trial Type ($F = 5.33$, $df = 3/27$, $p < 0.0052$) and the mean comparison pattern duplicated that shown for the peak amplitude measure. (bottom panel) shows that only the "flash" produced significantly higher average response voltage when compared with the 3 other stimulus trial types. No RP/BR-aerosol concentration effects were found with this analysis.

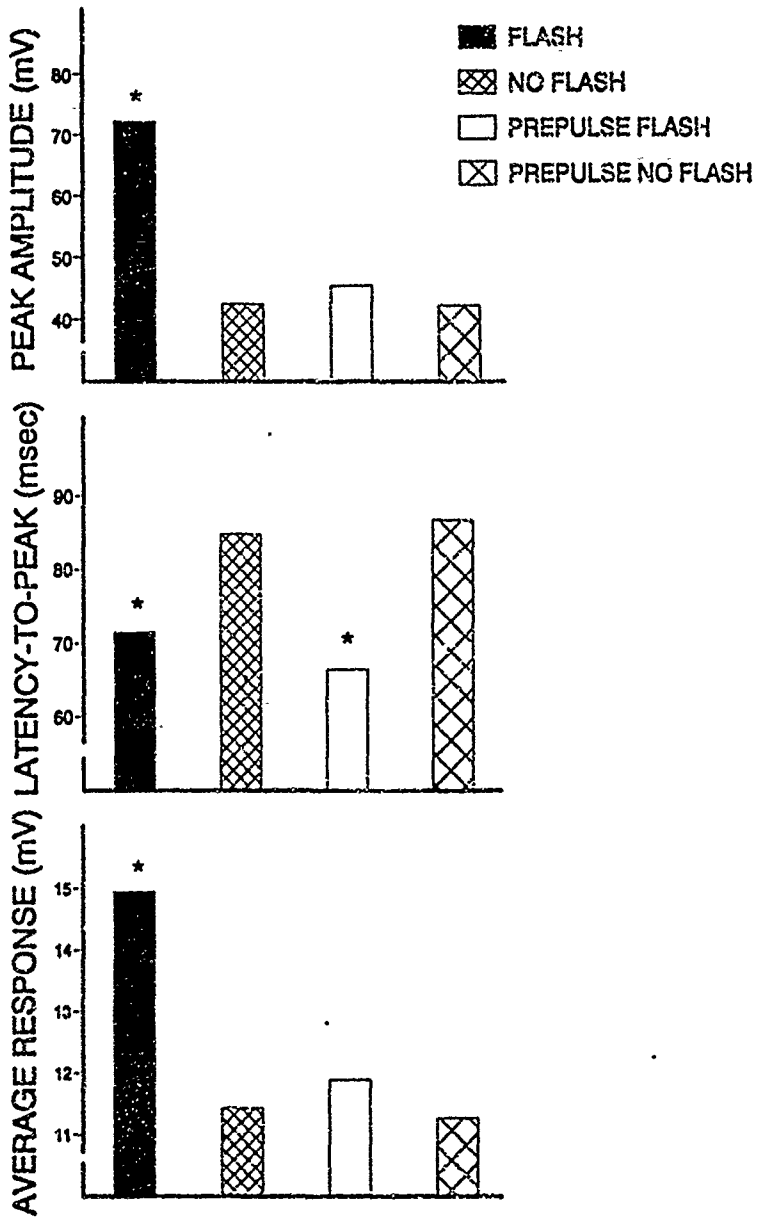

Figure 19. Significant (*, $p < 0.05$) main effects of Trial Type for the 3 response measures: peak amplitude, latency-to-peak, and average response in male rock doves. The electronic photoflash startle stimulus produced elevated peak and average amplitudes and shortened latencies compared to the control stimuli (NO FLASH and PREPULSE NO FLASH). Latencies of responses to the FLASH and PREPULSE FLASH stimuli were shorter than to the NO FLASH (Control) stimulus. The white noise prepulse reduced the peak and average startle response amplitudes when paired with the photoflash stimulus, but did not significantly alter the latency-to-peak response when paired with the photoflash stimulus.

(2) Female Rock Doves.-- Essentially, the same screening process for spurious pre-stimulus movement responses for the 20 msec baseline period was again performed on the raw data. No responses were eliminated for females by this process. Raw data values for each of the 3 dependent measures were then averaged by calculation of mean values for each dove during each of the 10 sessions.

Peak amplitude of response showed a significant main effect for Trial Type ($F = 8.45$, $df = 3/27$, $p < 0.0004$) as illustrated in Figure 20 (top panel). Mean separation analysis revealed that only the "flash" trials produced higher peak amplitude responses than the other 3 trial types -- the same pattern that was shown by male doves. Again, the prepulse white noise stimuli suppressed the female's peak amplitude response to the photoflash stimuli when they were presented 40 msec before the flash stimuli. Taken together, the separate analyses on male and female doves confirmed the predicted change in peak amplitude and indicated no effects of RP/BR-aerosol exposures for either sex.

The latency-to-peak measure produced 3 significant effects: Concentration X Trial Type X Session ($F = 1.52$, $df = 54/243$, $p < 0.0175$), Trial Type X Session ($F = 1.55$, $df = 27/243$, $p < 0.0448$), and Trial Type ($F = 19.30$, $df = 3/27$, $p < 0.0001$). The Concentration X Trial Type X Session effect can be broken down into 3, 2-way effects as shown in Figure 21. All significant effects are described excluding the "prepulse no flash" latencies due to the stimulus artifacts previously noted.

The top panel indicates the Concentration X Trial Type interaction which did not achieve significance ($p < 0.10$). Basically, latencies for both the "flash" and "prepulse flash" were consistently shorter than for the corresponding "no flash" control trials across all RP/BR-aerosol concentrations. Although Concentration did not significantly affect the degree of differences between Trial Types, the 4.0 mg/ℓ Group had the shortest latencies for "flash" and "prepulse flash" trials and the highest degree of stimulus control based on this measure. This Group possibly became more excitable after RP/BR-aerosol exposure, and their enhanced arousal generated the shortest response times after photoflash stimulation.

Figure 21 (middle panel) also indicates the extent of the significant ($p < 0.04$) Trial Type X Session interaction for latency-to-peak data. As a general trend, the female rock doves in all groups improved their startle response performance after the first exposure session (E-1). Mean latencies-to-peak for the different Trial Types were not significantly different during the Pre-1 and Pre-2 Sessions.

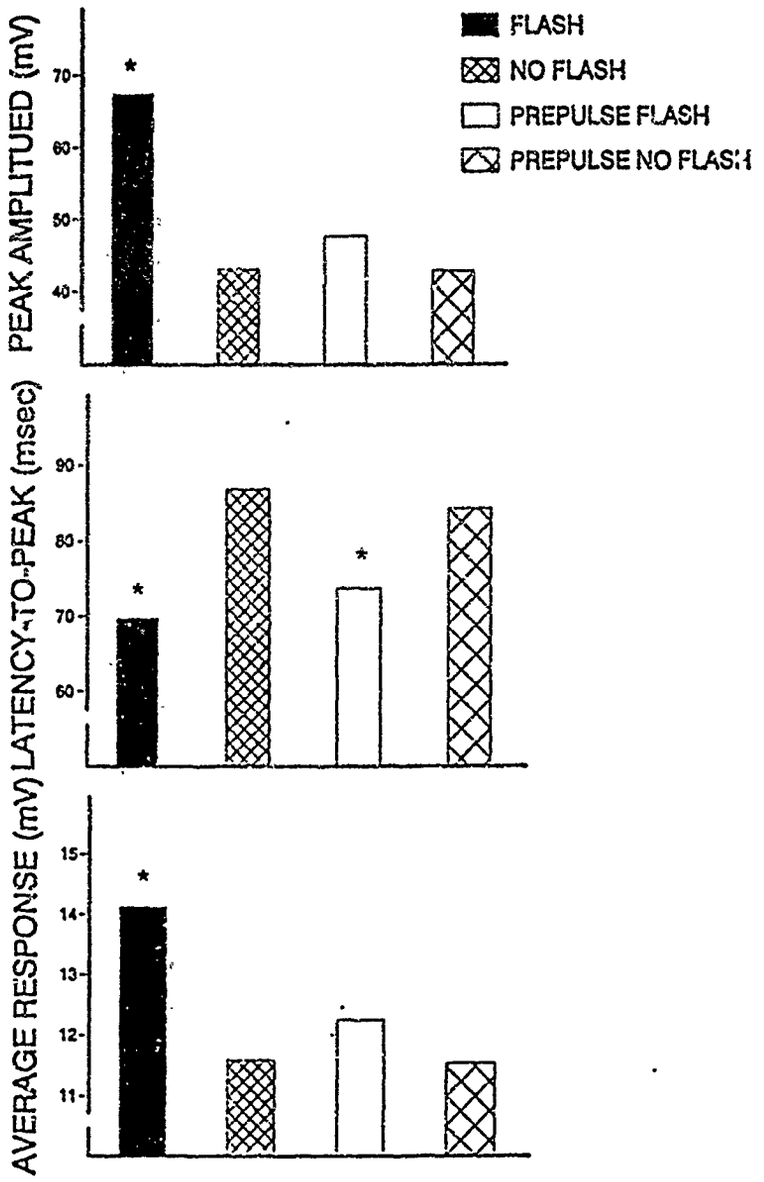

Figure 20. Significant (*, $p < 0.05$) main effects for Trial Type for the 3 response measures: peak amplitude, latency-to-peak, and average response in female rock doves. The FLASH startle stimulus generated elevated peak and average amplitudes and shortened latencies compared with the control stimuli (NO FLASH and PREPULSE NO FLASH). Latencies of responses to the FLASH and PREPULSE FLASH stimuli were shorter than to the NO FLASH control stimulus. The white noise prepulse reduced the peak and average startle response amplitudes when paired with the photoflash stimulus, but did not significantly alter the latency-to-peak response when paired with the photoflash stimulus.

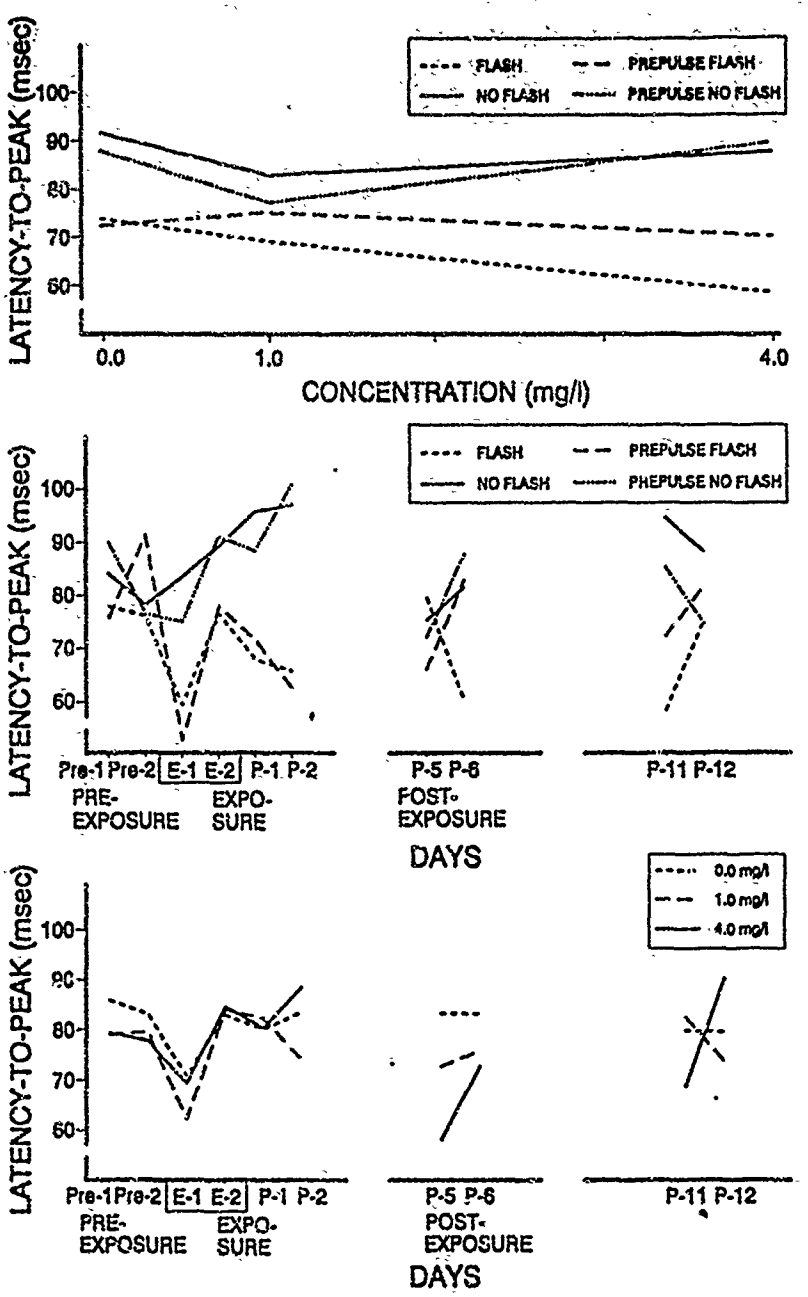

Figure 21. Three components of the Concentration X Trial Type X Session interaction for the latency-to-peak response measure in rock doves. Top panel: the Concentration X Trial Type interaction was not significant ($p < 0.10$) but there were indications of increased response differentiation to the 4 trial types at the high, (4.0 mg/ℓ) concentration level. Middle panel: the Trial Type X Session interaction was significant ($p < 0.04$) with significant depressed latencies on Days P-1, P-2, and P-11 for FLASH trials compared with NO FLASH trials. Bottom panel: the Concentration X Session interaction was not significant ($p < 0.63$). There was, however, an indication of a consistent depression of mean latencies for all 3 RP/BR-smoke groups on the first Exposure Day (E-1).

On the first session immediately after RP/BR smoke exposure (E-1), however, the "prepulse flash" trial mean (52.7 msec) was significantly less than the "flash" trial mean (59.3 msec). The only other significant difference for latencies occurred between Sessions P-1, P-2 and P-11. During each of these sessions, "flash" trial means (ranging from 58.3 to 68.0 msec) were significantly less than the respective "no flash" control trial means (ranging from 94.6 to 97.1 msec). Thus, there was a brief trend toward improved startle response performance on the initial post exposure sessions, P-1 and P-2.

One interpretation of this trend is that the female doves showed more sensitivity to the startle stimuli post exposure due to fewer interfering events (i.e., adaptation to handling and non-confinement in the exposure chambers). Chamber confinement per se apparently reduced the mean latencies to the startle stimuli, but it also generated more variance in response latencies, possibly due to increased excitability among all groups.

Figure 21 (bottom panel) illustrates the latency changes over 10 Sessions for the 3 Concentration Groups. This 2-way interaction (Concentration X Session) was not significant ($p < 0.63$). However, restricting the area under discussion to the exposure sessions, a consistent decrease in latencies for all concentration groups is shown for the first exposure session (E-1). The rationale for this heightened responsiveness after the female rock doves had been confined to the exposure chambers is uncertain. Other physiological-neurological measures of increased excitability (e.g., heart rate, respiration, EEG activity) after chamber confinement would help clarify the cause of the more rapid startle response. It is likely that the birds had heightened sensitivity to the "flash" and "prepulse flash" stimuli after confinement in the exposure chamber, but again this increased sensitivity led to not only shorter but also greater variability of startle response latencies.

The Concentration X Trial Type X Session effect is thus interpreted as being primarily due to: increased differentiation among the 4 types of trials at the 4.0 mg/ℓ concentration, decreased latencies to the "flash" stimulus immediately post exposure (P-1 and P-2), and decreased latencies to photoflash stimuli but with increased variance on the first exposure day (E-1). The latter effect was assumed to be due to increased excitability related to the initial exposure chamber confinement of the female doves.

The main effect of Trial Type ($p < 0.0001$) is included in Figure 20 (middle panel). "Flash" and "prepulse flash" trials evoked shorter response latencies than the "no flash" trials.

This was essentially the same result exhibited by male rock doves for the latency-to-peak variable.

Finally, the overall average response voltage of females only produced 1 significant effect -- Trial Type ($F = 11.22$, $df = 3/27$, $p < .0001$). The pattern of the means for the 4 trial types is shown in Figure 20 (bottom panel). As was the case with male rock doves, the females responded significantly more to the "flash" trials as compared to the other 3 trial types. Thus, the prepulse stimulus, again had the effect of suppressing the average startle response voltage to the photoflash stimulus.

In summary, both male and female rock doves showed reliable startle responses to the photoflash stimuli. The white noise prepulse stimuli were also effective in suppressing peak amplitude and average startle responses to the photoflash stimuli in both sexes. A significant Concentration X Trial Type X Session interaction effect on the latency-to-peak measure for female rock doves indicated mainly that: the birds in the 4.0 mg/ℓ group had the greatest differentiation between the 4 trial types compared with the other groups, all rock doves showed sharp decreases in mean latency-to-peak on the first exposure day, and these decreases were attributed to the photoflash-type trials. The filtered air (control) group doves showed a mean latency change pattern over the 10 sessions that was highly similar to and intermediate between the 1.0 and 4.0 mg/ℓ exposure groups. This indicated that only a subtle and weak effect was produced by the RP/BR aerosol exposures and this effect was superimposed on the much stronger initial chamber confinement effects.

C. Summary and Conclusions (Startle Response Studies)

Peak amplitude and average response measures of startle in female prairie dogs indicated reliable effects generated by brief electrical foot shock pulses. White noise prepulse inhibition of the startle responses was not generated, however, and male prairie dogs failed to show reliable responses to the foot shock levels chosen. Neither sex showed sustained-reliable startle responses to 7.8 and 15 KHz pure tone stimuli at 109-112 dB. Animals were found to be unaffected in their startle response behavior by exposures to RP/BR smoke even though a significant Concentration X Trial Type interaction effect was found for the latency-to-peak measure. This effect was interpreted as due to less stimulus control in the 2 RP/BR smoke exposure groups throughout the 10 test sessions.

Both male and female rock doves, in contrast, showed reliable responses to brief photoflash stimuli on the peak amplitude, latency-to-peak, and average response measures. In addition, all rock dove groups showed inhibition of peak amplitude and average response measures when brief white noise prepulse stimuli preceded the

photoflash stimuli by 40 msecs. Female rock doves also showed a significant RP/BR smoke Concentration X Trial Type X Session interaction effect on the latency-to-peak measure.

This 3-way effect was related to at least 3 characteristic trends in the data: (1) more differentiation in the latency values of the 4 trial types in the 4.0 mg/ℓ Group when compared with the 1.0 and 0.0 mg/ℓ Groups, (2) relatively shorter latencies for photoflash type stimuli on the first 2 days of post-exposure, and (3) a consistent decrease in latencies for all 3 RP/BR smoke concentration groups on the first day of exposure.

RP/BR-smoke effects were therefore only observed in female rock doves on the latency-to-peak measure of startle response. The main effect of the smoke exposures was to generate decreased latencies to the flash stimuli in the 4.0 mg/ℓ Group (i.e., improved stimulus control). However, this was superimposed upon a consistent initial chamber confinement effect that yielded depressed latencies to photoflash stimuli in all 3 treatment groups.

VI. STUDIES OF RP/BR-AEROSOL EFFECTS UPON PULMONARY FUNCTION AND BLOOD CHEMISTRY/HEMATOLOGY IN BLACK-TAILED PRAIRIE DOGS AND ROCK DOVES

This report describes the Exposure and Post-exposure effects of RP/BR aerosol on pulmonary function, blood chemistry, and hematology of black-tailed prairie dogs and rock doves. The rationale for the research is based on previous reports showing that RP/BR smoke causes sclerosis, irritation, and alveolar lung tissue scaring in rats (Burton et al., 1982). Such evidence suggests that sufficient exposure to RP/BR smoke could adversely impact pulmonary function, blood chemistry, and hematology. To test this hypothesis for pulmonary function, respirations per minute (Rf), oxygen consumption, mℓ/kg/hr (Vo_2), carbon dioxide production, mℓ/kg/hr (Vco_2), respiratory exchange ratio, Vco_2/Vo_2 (RER), and metabolic rate, Kcal/hr/kg (MR) were measured in each species before and after multiple exposures to 0.0, 1.0, and 4.0 mg/ℓ target concentrations of RP/BR aerosol. Similarly, the hypothesis was tested in separate studies with each species under corresponding experimental conditions by determining the effect of RP/BR aerosol on 7 blood chemistry variables (i.e., pH, O_2 partial pressure (Po_2) CO_2 partial pressure (Pco_2), total hemoglobin (Hb), oxyhemoglobin (O_2Hb), carboxyhemoglobin (COHb), and methemoglobin (MetHb)) and 3 hematology variables (i.e., packed cell volume (PCV), total white cells (WBC) and differential WBC counts).

A. Methods and Procedures

1. Prairie Dogs and Rock Doves

Twenty four (24) prairie dogs (12 males, 12 females) and 24 rock doves (14 males, 10 females) were selected for the 2 Pulmonary Function Studies. An additional 24 individuals of each species

were also selected and divided equally according to sex (i.e. 12 males, 12 females) for the 2 Blood Chemistry/Hematology Studies.

Prairie dogs were captured locally and rock doves were purchased from a local supplier. Capture, quarantine and maintenance of animals were performed according to the procedure described in III. GENERAL METHODS, A. Animals/Birds.

2. Research Designs

Two Pulmonary Function Studies and 2 Blood Chemistry/Hematology Studies were conducted. Each study involved 24 animals, with sex divided as stated previously.

The 2 Pulmonary Function Studies consisted of 4 replications of 6 animals each, whereas each of the 2 Blood Chemistry/Hematology Studies consisted of 2 replications of 12 animals each. All studies involved the sequential Pre-exposure, Exposure, and Post-exposure paradigm. Effects of RP/BR aerosol were determined by analyzing for differences in pulmonary function, blood chemistry, and hematology variables.

3. Pulmonary Function Procedures

a. Instrumentation

All respiratory measurements were obtained with a computer-controlled (IBM-XT-PC) Oxymax-85 Pulmonary Function System (Columbus Instruments, Columbus, OH). The Oxymax-85 System was installed and maintained according to the Manufacturer's instructions as stated in the Instruction Manual (Columbus Instruments International Corp., Columbus, OH). The System was equipped and programmed to measure Rf, Vo_2, Vco_2, RER, and MR of 2 animals at 2-min intervals for 2 h, with readings of each animal made during alternate min. Animals were held in adjacent Plexiglas chambers (43 X 43 X 33 cm) with a visual barrier between chambers. Measurements were conducted in Room 154C (see Figure 5) which had temperature and relative humidity (RH) monitored using a Hygrothermograph (Belfort Instruments Co., Baltimore, MD.). Temperature and RH for this room normally ranged between 21 and 25° C and 15 and 40%, respectively. All respiratory measurements were conducted in a darkened room with no operator present.

Each chamber was equipped with air-flow pumps for supplying and withdrawing air. Airflow was measured by mass flow meters and adjusted for standard temperature and pressure (STP). Approximately 50 percent of the air supplied to each cage was withdrawn every 20 min, circulated through a drying column (Drierite) and then sampled for O_2 and CO_2. The O_2 sensor was electrochemical and the CO_2 sensor was an infrared non-dispersing spectrometer. The O_2 and CO_2 measurements were

determined by the difference between ambient room air and test cage air. Respiratory rate, Vo_2, Vco_2, RER, and MR, were calculated by algorithms contained in the software. Data outputs were printed and stored on floppy disks for subsequent computer analysis.

b. Test Procedures

Testing was conducted according to the 3-phase paradigm (Pre-exposure, Exposure, and Post-exposure). A total of 8 tests were conducted with prairie dogs on designated days: 1 Pre-exposure (Day Pre-1; the day before exposure), 4 Exposure (Days E-1, E-2, E-3, and E-4; the day of exposure, after each exposure), and 3 Post-exposure days (Days P-1, P-3, and P-6 following the Exposure Phase). A total of 7 tests were conducted with rock doves. The schedule was the same as prairie dogs except there were 2 Exposure (Days E-1 and E-2) and 4 Post-exposure tests (Days P-1, P-3, P-5, and P-8).

Pilot studies indicated that animals with Vo_2 exceeding 1,000 mℓ/kg/hr during 1 of 2 successive days prior to RP/BR-aerosol exposure were not adjusting to the test situation and they were not included in the Study. After animals were selected for each Study, they were balanced according to sex, and randomly assigned to the 0.0, 1.0, and 4.0 mg/ℓ RP/BR-aerosol Groups. They were then assigned to cages 1 or 2 of the Oxymax-85 Pulmonary Function System. Animals were weighed immediately before testing and placed in the Oxymax-85 System for 2 hours. Effects of RP/BR smoke were determined by comparing shifts in Rf, Vo_2, Vco_2, RER, and MR. Although measurements were recorded for each 2-hour period, only data of the second hour was analyzed to allow equilibration of each Oxymax-85 Chamber's atmosphere and adaptation of animals.

c. Data Analyses

A separate ANOVA was performed on each variable for each species. Since there was only one animal per cell in each replication we had to ignore replications as a factor. Thus, the validity of the analyses is based on the assumption that experimental conditions remained relatively constant within each replication.

The designs were 3 (Concentration) X 2 (Sex) X 8 or 7 (Day) ANOVAs, where Day was a repeated measures factor (Winer, 1971). Data for the prairie dog study were analyzed using the PROC ANOVA Program and data for the rock dove study were analyzed using the PROC GLM Program and Type III sums of squares (SAS Institute, Inc., 1985) Prior to performing the analyses, means of the 30 observations per animal per test day were determined (i.e., alternate min measurements for the second h of each test) for each of the 8 and 7 days of

measurement. These means were used in the ANOVAs to represent a single value response for each animal per respiratory parameter per test. Significant effects were further evaluated using Duncan's Multiple Range Test (Waller and Duncan, 1969).*

4. Blood Chemistry/Hematology Procedures

a. Instrumentation

An IL 1306 Blood Gas analyzer and an IL 282 CO-Oximeter (Instrumentation Laboratory, Lexington, MA) were used to determine 7 blood chemistry variables of the animals: pH, oxygen partial pressure (Po_2), carbon dioxide partial pressure (Pco_2), hemoglobin (Hb), oxyhemoglobin (O_2Hb), carboxyhemoglobin (COHb) methemoglobin (MetHb). The blood gas analyzer was used to determine pH, Po_2, and Pco_2. The co-oximeter was used for HB, O_2HB, COHb, and MetHb. Additionally, measurements of packed cell volume (PCV), total white blood cell count (WBC), and differential WBC were determined by a local clinical veterinary laboratory using standard techniques (Colorado Veterinary Laboratory, Inc., Broomfield, CO).

b. Blood Sampling Procedures

Immediately after 0.6 mℓ of venous blood was drawn for blood chemistry/hematology assessment, any air in the syringe was expelled, the syringe was capped and then placed into a bag of crushed ice until analysis within the next 20 minutes. After hand mixing by rotating the syringe between the palms for 1 minute, a 90 $\mu\ell$ sample was aspirated directly from the syringe into the blood gas analyzer. The prairie dog blood (350 $\mu\ell$) was then aspirated into the co-oximeter. For rock doves, the remainder of the sample in the syringe after blood gas analysis was processed for analysis in the co-oximeter. Several properties of avian blood make analysis difficult in the IL 282 co-oximeter. Therefore, the following procedure was used to provide a suitable sample. Equal parts of the blood and IL 282 hemolyzing diluent (IL No. 33119-06) were placed in a plastic snap top 1.5 mℓ micro-centrifuge tube. The tube was inverted several times and then placed in an ultrasonic bath for 1 minute to insure complete hemolysis. Then the tube was centrifuged at 2,600 rpm for 10 minutes in a clinical centrifuge. The supernate (350 μl) was drawn off by aspiration as the sample was introduced into the co-oximeter.

*See footnote on Page 27.

c. Test Procedures

Separate experiments were conducted for each species. Test animals were exposed to the 3 concentrations of RP/BR aerosol in accordance with the paradigm shown in Figure 2. A total of 8 separate, 0.6 me venous blood samples were collected from each animal. The samples were collected as 4 pairs of samples on 4 separate days. One sample in a pair was for blood chemistry and the other for hematology. Each animal was weighed and a pair of blood samples drawn on the Pre-exposure day (Pre-1) just before start of the Exposure Phase, within 2 hours after the last exposure the last Exposure day (Day E-4 for prairie dogs and E-2 for rock doves), and on 2 Post-exposure days (Days P-2 and P-6).

Blood was drawn from the femoral veins of prairie dogs and ulnar veins of rock doves. Samples were obtained using 1 me syringes with a 25 ga needle. Syringes were heparinized for blood chemistry/hematology measurements but treated with EDTA for hematology assessment. Prairie dogs were immobilized with ketamine hydrochloride (50 mg/kg the first day to 38 mg the fourth day) for blood collections. The depth of anesthesia in prairie dogs would have increased if the dose of ketamine hydrochloride had not been reduced on subsequent blood draw days in this manner. Rock doves were unanesthetized and only hand restrained during blood sampling.

d. Data Analyses

Each blood chemistry and hematology variable was analyzed separately for each species using a 3 (Concentrations) X 2 (Sex) X 4 (Day) factorial ANOVA, where Day was a repeated measures factor (Winer, 1971). These ANOVAs were computed using the PROC ANOVA Program and Type III sums of squares (SAS Institute, Inc., 1985). Each study was conducted as 2 replications that involved 4 animals per RP/BR-aerosol Concentration Group. Replications were used only as a method to allow completion of the exposures and blood processing in a one day period. They were assumed to be equal. Significant effects were further evaluated using Duncan Multiple Range Tests (Waller and Duncan, 1969).*

B. Results and Discussion (Pulmonary Function)

1. Black-tailed Prairie Dogs

In evaluating the results, it should be noted that the test methods facilitated obtaining measurements while animals were unrestrained in a quiet and darkened room -- essentially free from

*See footnote on Page 27.

human disturbances. As such, animals appeared to readily adapt to experimental conditions as evidenced by their leveling of Rf and their remaining relatively passive during pulmonary function tests. To our knowledge the only other report on metabolism of prairie dogs is that of Lund and Folk (1976) in which heart rate and Vo_2 were correlated. While mean Vo_2 in that study is comparable to this one, no information was obtained on Vco_2, RER, MR, or Rf.

Appendix E presents detailed descriptions of the chamber aerosol and air quality data characterizing the exposures with prairie dogs in the Pulmonary Function Study.

a. Mortality

No prairie dogs died during the Pulmonary Function Study.

b. Pulmonary Function Effects in Prairie Dogs

Table 6 presents mean (± SD pulmonary function values for the 3 RP/BR-smoke Concentration Groups. All values appear to be in a range expected for prairie dogs or other rodent species of comparable size (Altman and Dittmer, 1974; Fowler, 1986; Rugh, 1968). Results of PROC ANOVAs for each variable revealed only 1 of 28 p values was significant, the Day effect for RER ($F = 5.81$, $df = 7/126$, $p < 0.01$). The RER was significantly suppressed on Exposure Days E-1, E-2, and E-4, but mean values returned to the Pre-exposure level by Post-exposure Day P-1 and remained at that level throughout the rest of the Post-exposure Phase (Figure 22). The lack of a significant RER Concentration X Day interaction ($F = 1.80$, $df = 14/126$, $p \geq 0.25$) shows that the 0.0 mg/ℓ RP/BR-smoke Group responded the same as the 1.0 and 4.0 mg/ℓ Groups. As such, the RER Day effect appears to be a general experimental effect unrelated to RP/BR-smoke concentration, perhaps a transient stress effect of chamber confinement or handling. The results of the RP/BR pulmonary function study revealed no mortality, evidence of toxicity, or pulmonary impairment due to RP/BR smoke. Prairie dogs appear to be very resistant to pulmonary effects resulting from multiple exposures to high concentrations of RP/BR smoke.

2. Rock Doves

Appendix E presents chamber aerosol and air quality data characterizing the exposures with rock doves in the Pulmonary Function Study.

a. Mortality

Two rock doves died during the course of the Pulmonary Function Study with this species.

Table 6. Mean (± SD) pulmonary function measurements of prairie dogs exposed to 3 concentrations of RP/BR smoke.

RP/BR-smoke Concentration (mg/ℓ) [a]	Vo_2 (mℓ/kg/h)	Vco_2 (mℓ/kg/h)	RER (Vco_2/Vo_2)	Rf (resp/min)	MR (Kcal/kg/h)
0.0	583 ± 141	564 ± 143	0.97 ± 0.1	24.2 ± 9.7	2.921 ± 0.700
1.0	575 ± 113	586 ± 117	1.02 ± 0.1	25.1 ± 9.4	3.048 ± 0.568
4.0	605 ± 143	597 ± 161	0.98 ± 0.9	25.9 ± 10.7	2.918 ± 0.736

[a] n = 8 animals/concentration; 56 observations/parameter.

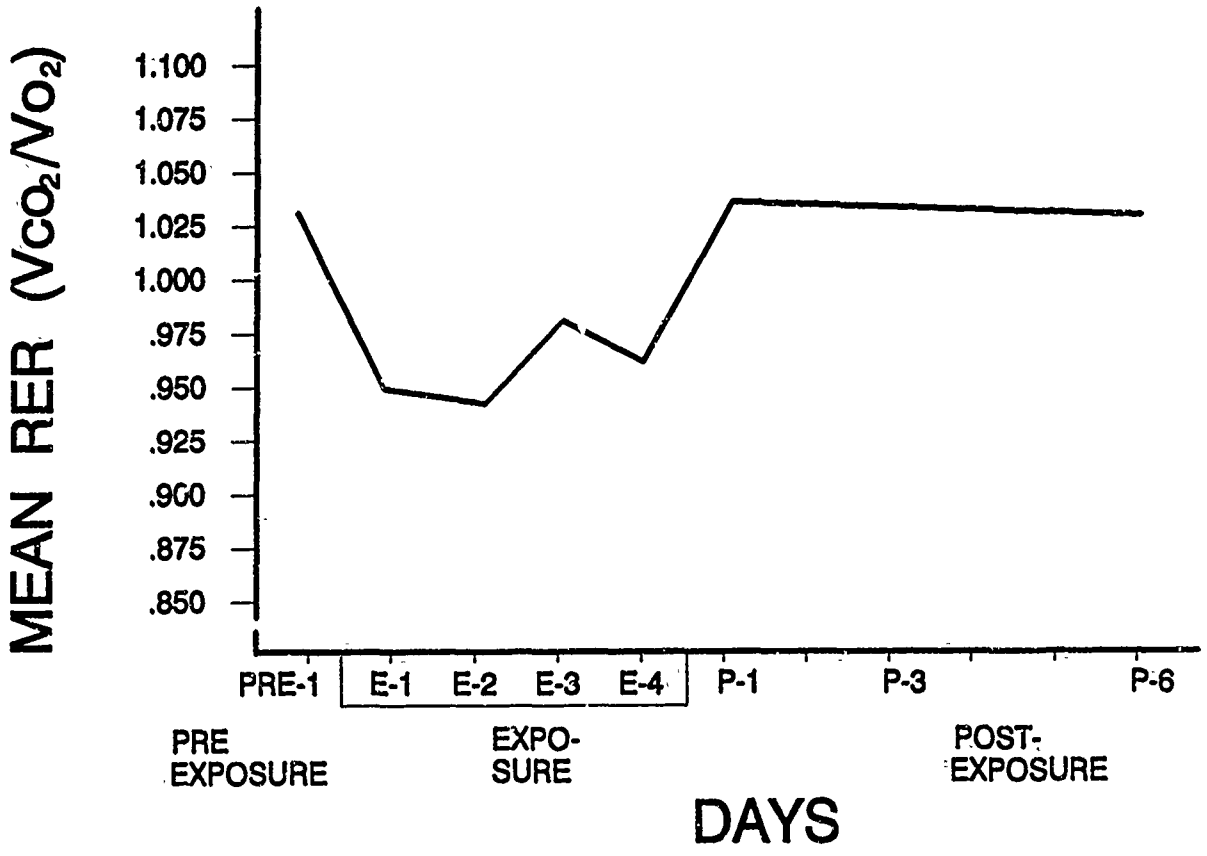

Figure 22. Mean respiratory exchange ratio (RER) of prairie dogs before, during, and after exposure to 3 Concentrations (0.0, 1.0, and 4.0 mg/ℓ) of RP/BR smoke (n = 24; 8/Concentration).

b. Pulmonary Function Effects in Rock Doves

Table 7 presents mean (± SD) pulmonary function values for the 3 RP/BR-aerosol Concentration Groups. All values appear to be typical of pigeons (Abs, 1983).

Results of PROC GLM ANOVAs showed that there were no significant effects on Rf; however, there was a significant Day effect on Vo_2 ($F = 2.60$, $df = 6/106$, $p \leq 0.02$,) and a significant Concentration X Day interaction for Vco_2 ($F = 2.31$, $df = 12/106$, $p \leq 0.01$), RER ($F = 2.36$, $df = 12/106$, $p \leq 0.01$), and MR ($F = 1.85$, $df = 12/106$, $p \leq 0.05$).

The Vo_2 Day means are shown in Figure 23, and the Concentration X Day interaction means for Vco_2, RER, and MR are depicted in Figures 24, 25, and 26, respectively. These figures show that the pattern of response across Days was similar for each variable. This is expected since RER and MR are derived from Vco_2 and Vo_2.

Beginning at Exposure Day E-2, Vo_2 significantly decreased below Pre-exposure and, except for Day P-3, remained there through the last Post-exposure Day. At 0.0 and 4.0 mg/ℓ RP/BR concentrations, Vco_2, RER and MR significantly decreased below Pre-exposure levels by Exposure Day E-2, whereas at 1.0 mg/ℓ there was no significant change in any variable until Post-exposure Day P-2 when Vco_2 and MR were elevated above all other days. While a definitive explanation for the elevated values on Day P-3 is not readily apparent, the delayed timing of their occurrence makes it unlikely to have been caused by the 1.0 mg/ℓ smoke exposure. Additionally, there was no difference in Vco_2 between 0.0 and 4.0 mg/ℓ RP/BR-smoke concentrations groups across days. This means that the Concentration X Day interaction for Vco_2 was caused by the elevated Vco_2 value at 1.0 mg/ℓ on Day P-3 (Figure 24). With RER, there was no difference between 0.0 and 4.0 mg/ℓ smoke exposures until Post-exposure Day P-8 (Figure 25). Therefore, the interaction was caused by RER at 1.0 mg/ℓ being significantly higher than at 0.0 mg/ℓ on Day P-3. Similarly, the Concentration X Day interaction for MR was caused by the significantly higher value for 1.0 mg/ℓ than for 0.0 or 4.0 mg/ℓ on Day E-1 and Days P-1 and P-3 (see Figure 26).

While the suppression of MR following exposures to 0.0 and 4.0 mg/ℓ RP/BR smoke is indicative of elevated adrenocortical function to combat nonspecific stress (Turner, 1955), the close similarity of MR at 0.0 and at 4.0 mg/ℓ show that chamber confinement or handling or both affected pulmonary function as much as the RP/BR 4.0 mg/ℓ smoke treatment in rock doves. Perhaps another type of handling effect is reflected in the significantly elevated Vco_2 and RER at 1.0 mg/ℓ on

Table 7. Mean (± SD) pulmonary function measurements of rock doves exposed to 3 concentrations of RP/BR smoke.

RP/BR Concentration (mg/ℓ)	Number of Obs. [a]	Vo_2 (mℓ/kg/h)	Vco_2 (mℓ/kg/h)	RER (Vco_2/Vo_2)	Rf (resp/min)	MR (Kcal/kg/h)
0.0	56	754 ± 126	666 ± 128	0.88 ± 0.08	23.5 ± 6.4	3.697 ± 0.624
1.0	56	872 ± 202	795 ± 205	0.91 ± 0.09	25.6 ± 7.0	3.675 ± 1.009
4.0	54	748 ± 102	666 ± 148	0.88 ± 0.1	25.8 ± 8.5	4.309 ± 0.588

[a] n = 8 animals/concentration: 56 observations/parameter at 0.0 and 1.0 mg/ℓ and 54 observations/parameter at 4.0 mg/ℓ.

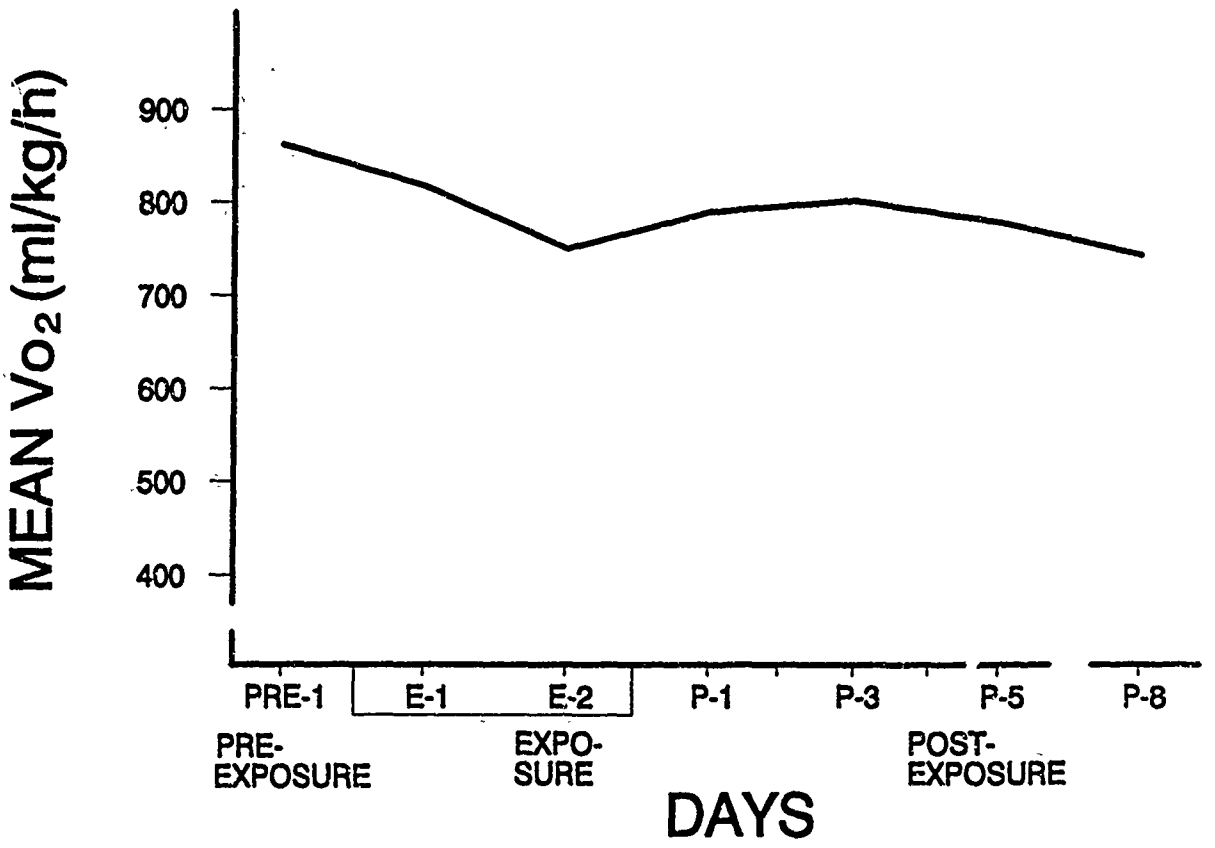

Figure 23. Mean oxygen consumption (Vo_2) of rock doves before, during, and after exposure to 3 concentrations (0.0, 1.0, and 4.0 mg/ℓ) of RP/BR smoke (n = 24; 8/concentration).

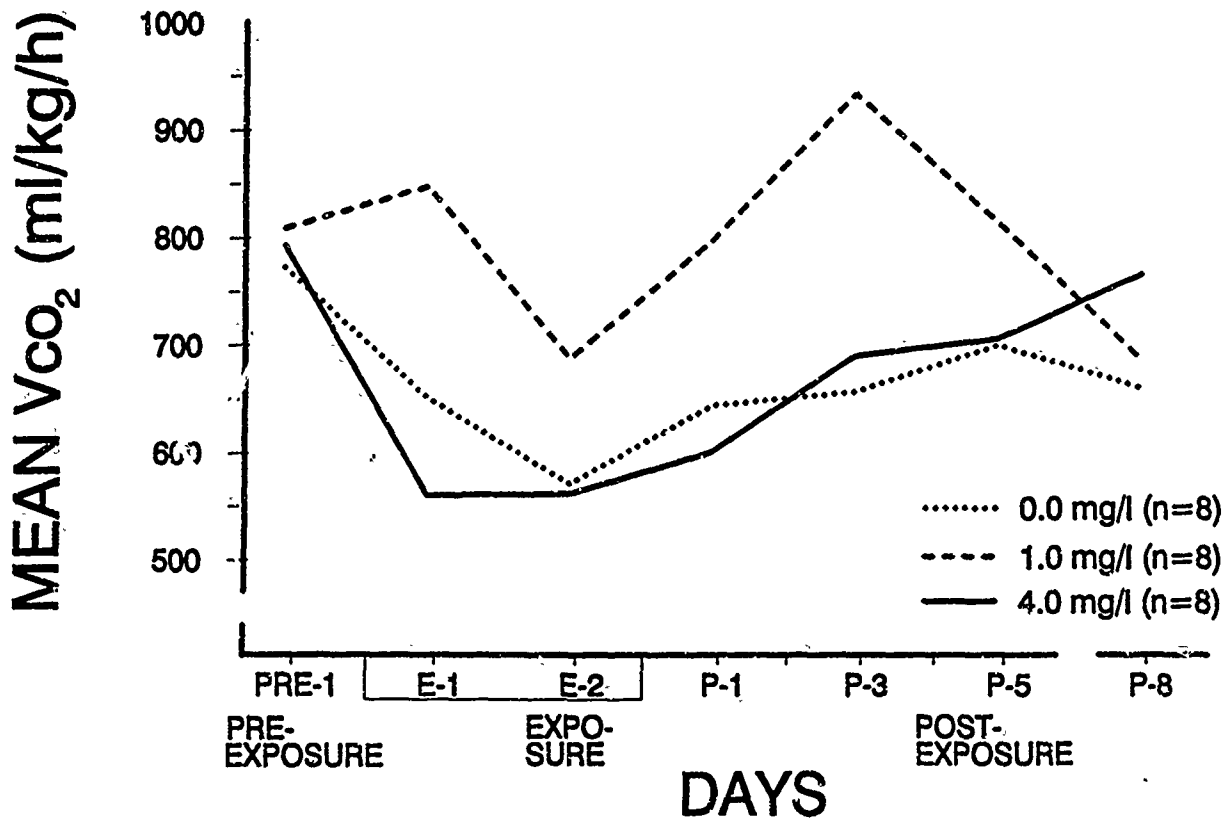

Figure 24. Carbon dioxide production (Vco_2) Concentration X Day interaction means of rock doves before, during, and after exposure to 3 concentrations of RP/BR smoke.

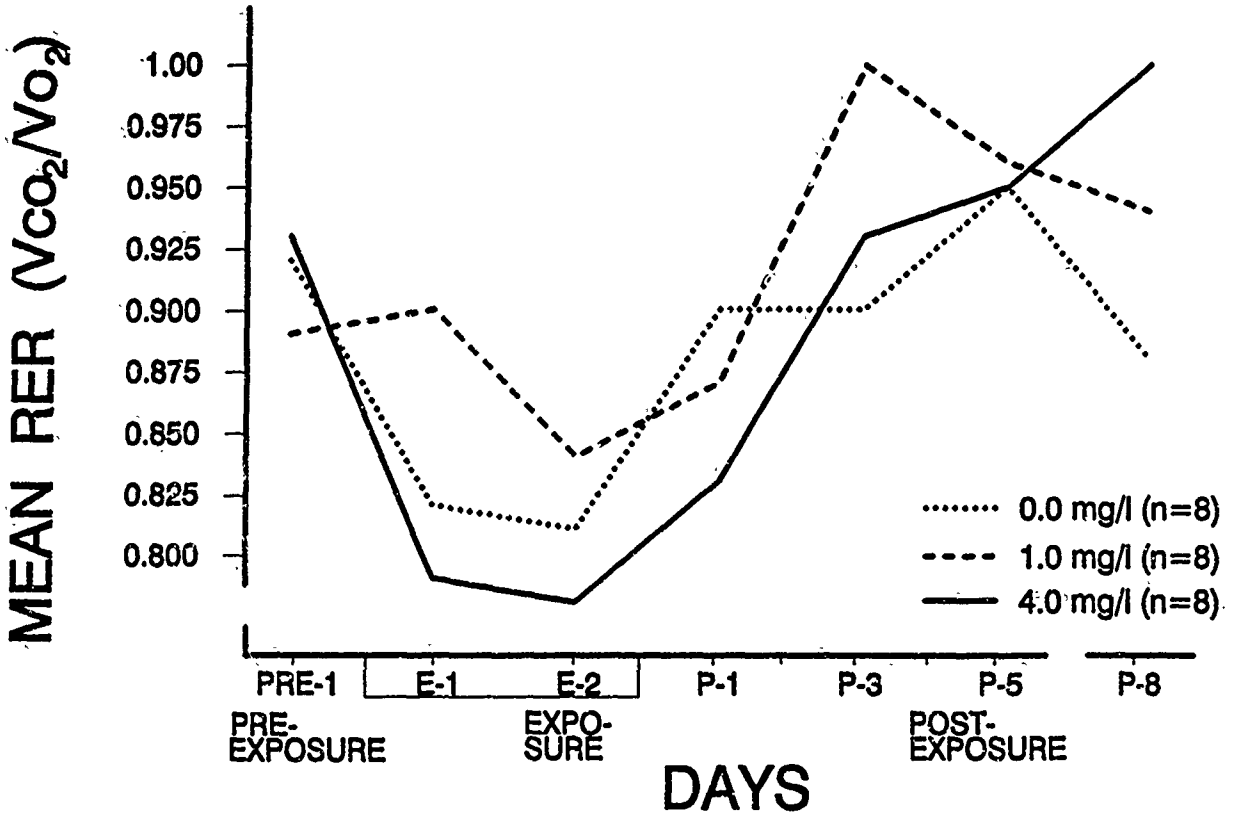

Figure 25. Respiratory exchange ratio (RER) Concentration X Day interaction means of rock doves before, during, and after exposure to 3 concentrations of RP/BR smoke.

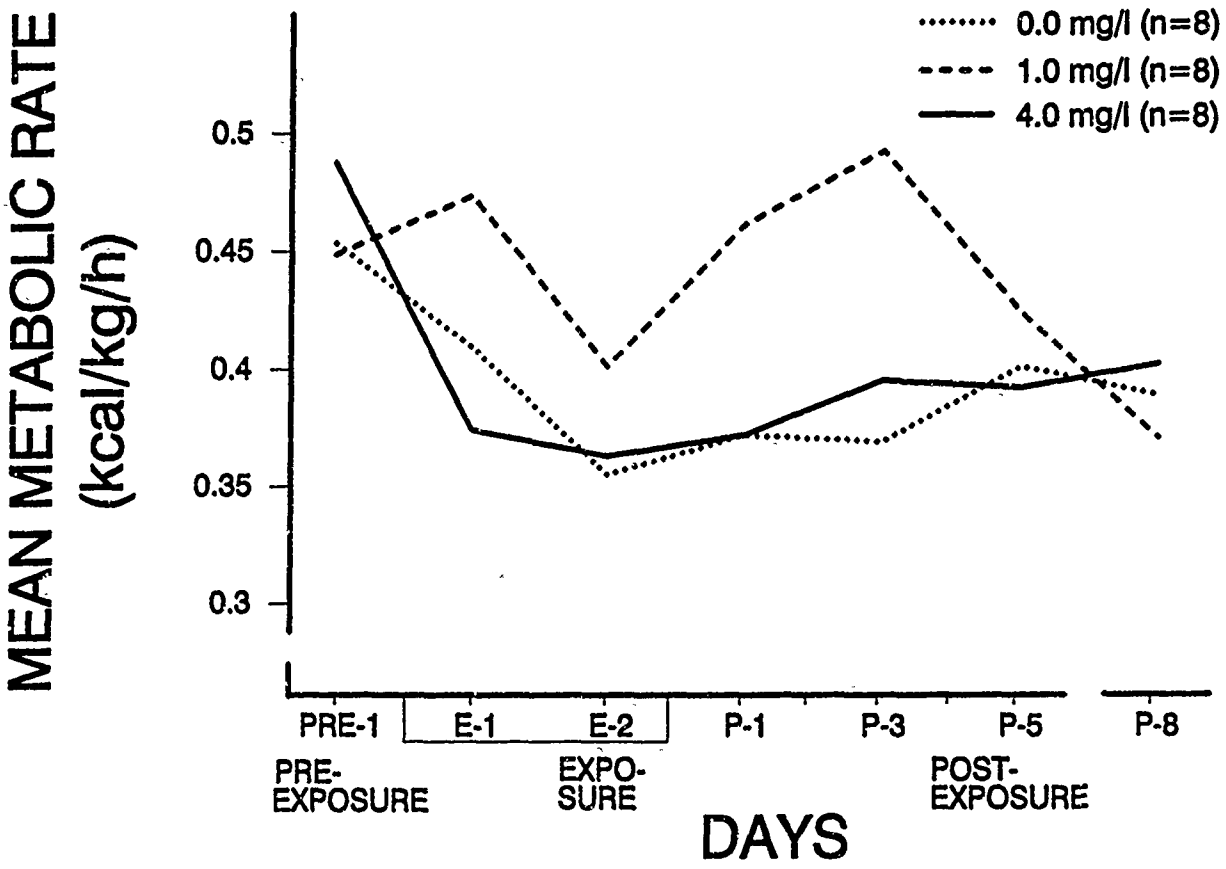

Figure 26. Metabolic rate (MR) Concentration X Day interaction means of rock doves before, during, and after exposure to 3 concentrations of RP/BR smoke.

Day P-3. For example, the RP/BR exposure sequence resulted in birds exposed to 1.0 mg/ℓ RP/BR smoke being exposed and tested soon after consuming food in the morning (within 3-5 h), whereas birds exposed to 4.0 and 0.0 mg/ℓ RP/BR smoke were tested later in the day 4 to 9 h after eating. Since energy intake appears to be the main factor regulating plasma concentration of thyroid hormones (Sturkie, 1986) and because the half-life of thyroid hormones in birds is short (i.e., usually less than 8 h), it is reasonable to assume that the higher pulmonary function values at 1.0 mg/ℓ were caused by diurnal variation in MR. This explanation is not confounded by the fact that the Rf did not significantly vary among the 3 concentrations of RP/BR ($F = 0.94$, $df = 2/18$, $p \geq 0.41$). Change in Vo_2 can occur with little or no change in Rf as long as arterial Po_2 does not decrease below a triggering point where the demand for internal respiration cannot be met (Vander, Sherman, and Luciano, 1985).

Even though Sex was not a statistically significant factor in this Study, it appears that male rock doves are more vulnerable to RP/BR-smoke than female rock doves. Evidence for this can be seen in Figure 27, where mean Rf on Day E-2 for 2 males that subsequently died was 16 percent higher than the corresponding Rf of surviving rock doves. This difference rose to 31 percent by Day P-3; then on Day P-5, the Rf of the 2 dying birds dropped to 28 percent lower than survivors, after which the birds died.

In essence, other than the indication of respiratory distress in the 2 rock doves that died following exposure to 4.0 mg/ℓ RP/BR and the transient significant suppression of Vco_2 on Day E-1 (Figure 24) following exposure to 4.0 mg/ℓ RP/BR, the results provide no evidence that RP/BR smoke caused any significant affects on pulmonary function. It is important to note that birds in this Study were not flown before or after exposure to RP/BR smoke. Therefore, measurements were made under minimum stress conditions. If they had been forced to fly following the exposure schedule, effects may have been more pronounced.

C. Results and Discussion (Blood Chemistry/Hematology)

The automated co-oximeter and blood gas analyzer facilitated rapid and accurate measurement of venous blood chemistry/hematology parameters. However, it should be noted that the co-oximeter was not equipped to analyze blood from prairie dogs and rock doves using a species-specific Programmable Read Only Memory. Therefore, measurements of Hb, O_2Hb, MetHb, and COHb are to be viewed as relative values for comparing the effects of the 3 RP/BR-smoke Concentration Groups, rather than as absolute values.

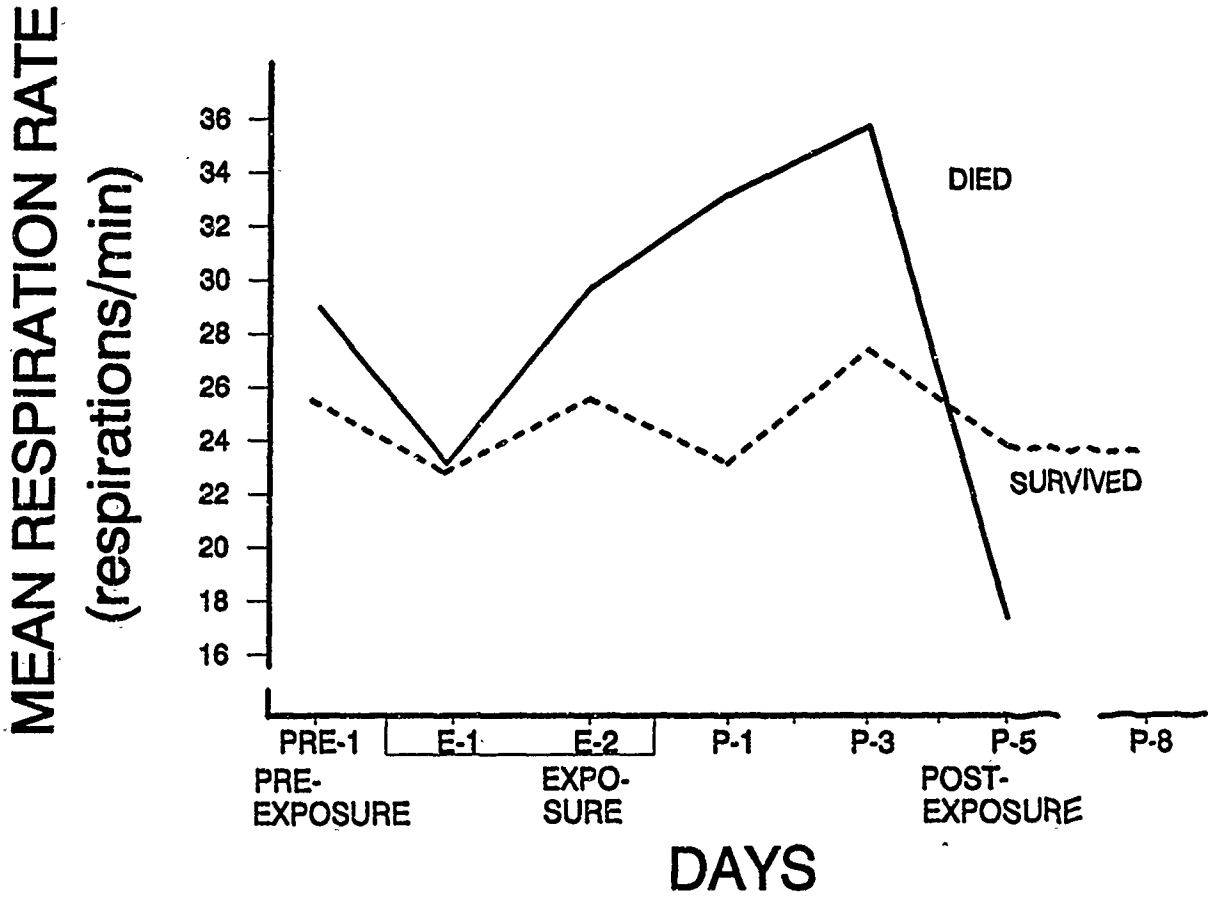

Figure 27. Mean respiration rate (Rf) of 2 male rock doves that died and of 6 rock doves that survived following exposure to a 4.0 mg/ℓ concentration of RP/BR smoke.

1. Black-tailed Prairie Dogs

See Appendix E for the RP/BR-aerosol and Filtered-air characterization data from this Study.

a. Mortality

No prairie dogs died during the course of the Blood Chemistry and Hematology Study.

b. Blood Chemistry and Hematology

Table 8 shows mean (± SD) values for the 7 blood chemistry and 8 hematology variables of prairie dogs exposed to the 3 RP/BR-smoke concentrations. To our knowledge, this is the first report showing blood chemistry and hematology values for this species. As mentioned previously, the Hb, O_2Hb, MetHb, and COHb values cannot be considered as absolute values, however, they fall within the normal mammalian range for venous blood. The pH, Pco_2 and Po_2 values are absolute numbers and fall within the range of expected values for rodent venous blood. The PCV, WBC, and differential WBC values are typical of other rodents (Jain, 1986).

Results of the PROC ANOVAs for blood chemistry showed no significant RP/BR-aerosol effects. However, the ANOVAs showed that the Day effect was significant for 5 of the variables:

Po_2	($F = 4.39$, $df = 3/54$, $p \leq 0.01$),
Hb	($F = 12.82$, $df = 3/54$, $p \leq 0.01$),
O_2Hb	($F = 3.59$, $df = 3/54$, $p \leq 0.02$),
COHb	($F = 4.86$, $df = 3/54$, $p \leq 0.01$), and
MetHb	($F = 2.75$, $df = 3/54$, $p \leq 0.05$).

The PROC ANOVAs for hematology variables showed only 1 significant effect, the Day effect for PCV ($F = 5.97$, $df = 3/54$, $p \leq 0.01$). Table 9 shows the results of Duncan's Tests for the blood chemistry and hematology Day effects. The significant effects among Days may reflect different levels of stress resulting from collecting blood samples and/or differences in depth of anesthesia. During the study, prairie dogs became increasingly sensitive to the anaesthetic and it was necessary to gradually reduce the dosage of ketamine hydrochloride from 50 mg/kg on Day Pre-1 to 38 mg/kg by Day P-6 to insure adequate respiration. Dosage was always the same across treatment groups. Regardless of what caused the Day effect, it was equally distributed across RP/BR treatment groups and therefore not caused by exposure to RP/BR smoke.

These blood chemistry and hematology results clearly show that prairie dogs are very resistant to short term multiple exposures of low and high concentrations of RP/BR smoke.

Table 8. Mean (± SD) blood chemistry and hematology venous values of prairie dogs exposed to 3 concentrations of RP/BR smoke. [a]

Variable	RP/BR Concentrations (mg/ℓ) 0.0	1.0	4.0
Blood Chemistry			
pH	7.279 ± .083	7.265 ± .080	7.302 ± .044
Pco_2 (mm Hg)	56.9 ± 7.2	62.8 ± 7.9	59.6 ± 5.6
Po_2 (mm Hg)	48.8 ± 8.4	45.8 ± 9.9	47.0 ± 7.9
Hb (g/dl)	15.7 ± 1.2	16.2 ± 1.1	15.9 ± .9
% O_2 Hb	80.5 ± 10.7	75.6 ± 14.2	80.0 ± 9.3
% CO Hb	1.32 ± .47	1.23 ± .64	1.44 ± .40
% MetHb	1.84 ± .53	1.74 ± .71	1.98 ± .43
Hematology			
% PCV	45.4 ± 3.2	46.8 ± 3.2	45.7 ± 3.1
Total WBC/μℓ	5,984 ± 1,345	6,888 ± 1,835	6,119 ± 1,602
Differential WBC (%)			
Segmented granulocytes	41.9 ± 8.8	41.8 ± 14.3	41.0 ± 8.2
Band granulocytes	0	0	0
Lymphocytes	53.8 ± 9.9	52.6 ± 13.1	54.5 ± 8.1
Monocytes	0	0	0
Eosinophils	3.2 ± 2.7	4.2 ± 4.0	3.3 ± 2.3
Basophils	0	0	0

[a] Eight prairie dogs per concentration; 4 blood collection days per animal.

Table 9. Means of the significant Day main effect terms for blood chemistry and hematology venous values of prairie dogs as analyzed by Duncan Tests (means with no letter in common are significantly different at an error rate of 0.05.)

Variable	Day	Mean (N=24)	Letter
Po_2 (mm Hg)	Pre-1	43.33	b
	E-4	44.83	b
	P-2	50.38	a
	P-6	50.25	a
Hb (g/dl)	Pre-1	16.53	b
	E-4	16.11	a
	P-2	15.81	a
	P-6	15.35	c
% O_2Hb	Pre-1	74.58	b
	E-4	75.59	b
	P-2	83.38	a
	P-6	81.17	ab
% COHb	Pre-1	1.175	b
	E-4	1.458	a
	P-2	1.571	a
	P-6	1.129	b
% MetHb	Pre-1	1.738	b
	E-4	1.746	b
	P-2	1.821	ab
	P-6	2.117	a
% PCV	Pre-1	47.60	a
	E-4	46.61	ab
	P-2	45.43	bc
	P-6	44.20	c

2. Rock Doves

As noted previously, data characterizing the RP/BR-aerosol chamber conditions during exposures of rock doves in the Blood Chemistry and Hematology Study are presented in Appendix E.

a. Mortality

No rock doves succumbed during the Blood Chemistry and Hematology Study.

b. Blood Chemistry and Hematology

Table 10 shows the means (± SD) for each of the 7 blood chemistry and 7 hematological measurements for the 0.0, 1.0, and 4.0 mg/ℓ Groups of doves. Each value in the 0.0 mg/ℓ RP/BR smoke Group appears to be in the range that is typical of unanesthetized pigeons (Abs, 1983).

Results of the PROC ANOVAs for blood chemistry/hematology values revealed 6 significant effects. These were:

Concentration X Sex interactions

Hb ($F = 3.79$, $df = 2/18$, $p \leq 0.04$)
MetHb ($F = 7.38$, $df = 2/18$, $p \leq 0.005$)

Day Main effects for

pH ($F = 12.00$, $df = 3/54$, $p \leq 0.001$)
Pco_2 ($F = 8.00$, $df = 3/54$, $p \leq 0.001$)
Hb ($F = 14.10$, $df = 3/54$, $p \leq 0.001$)
COHb ($F = 4.31$, $df = 3/54$, $p \leq 0.01$)

The Concentration X Sex effect on Hb was caused by the Hb of females being significantly lower than males at 0.0 mg/ℓ and 1.0 mg/ℓ RP/BR smoke but not significantly different at 4.0 mg/ℓ RP/BR smoke (Figure 28). The interaction indicated that females reacted to the RP/BR smoke at the 4.0 mg/ℓ concentration increasing Hb, whereas the males showed no significant change across Concentrations. The ability of females to significantly increase Hb at 4.0 mg/ℓ RP/BR may explain why they appear to be less vulnerable to theRP/BR smoke than males. It is interesting to note in Figure 28 that Hb of males tended to decrease with increasing concentrations of RP/BR smoke; whereas, with females, it increased across RP/BR concentrations, thereby providing greater blood O_2 carrying capacity to withstand the effects of RP/BR smoke.

Table 10. Mean (± SD) blood chemistry and hematology venous values of rock doves exposed to 3 concentrations of RP/BR smoke. [a]

Variable	RP/BR Concentrations (mg/ℓ) 0.0	1.0	4.0
Blood Chemistry			
pH	7.380 ± .046	7.390 ± .030	7.389 ± .040
Pco_2 (mm Hg)	41.8 ± 5.3	41.5 ± 3.5	40.1 ± 4.0
P_{o2} (mm Hg)	59.9 ± 6.2	58.9 ± 4.9	62.1 ± 3.5
Hb (g/dl)	14.9 ± 1.4	15.6 ± 1.3	15.3 ± .9
% O_2 Hb	90.2 ± 5.0	90.1 ± 4.0	92.4 ± 3.5
% CO Hb	-2.98 ± .35	-2.73 ± .37	-2.99 ± 3.8
% MetHb	.26 ± .33	.13 ± .19	.13 ± .21
Hematology			
% PCV	50.4 ± 3.9	50.9 ± 4.0	50.5 ± 4.3
Total WBC/μℓ	9,425 ± 3,867	6,628 ± 2,873	12,353 ± 5,800
Differential WBC (%)			
Lymphocytes	55.6 ± 14.2	53.8 ± 11.6	49.8 ± 17.3
Monocytes	2.4 ± 2.3	2.1 ± 1.9	1.7 ± 1.5
Eosinophils	1.1 ± 1.7	3.0 ± 3.5	0.6 ± 0.9
Basophils	0	0	0
Heterophils	40.3 ± 14.1	40.2 ± 10.9	47.1 ± 17.6

[a] Eight rock doves per concentration; 4 blood collection days per bird.

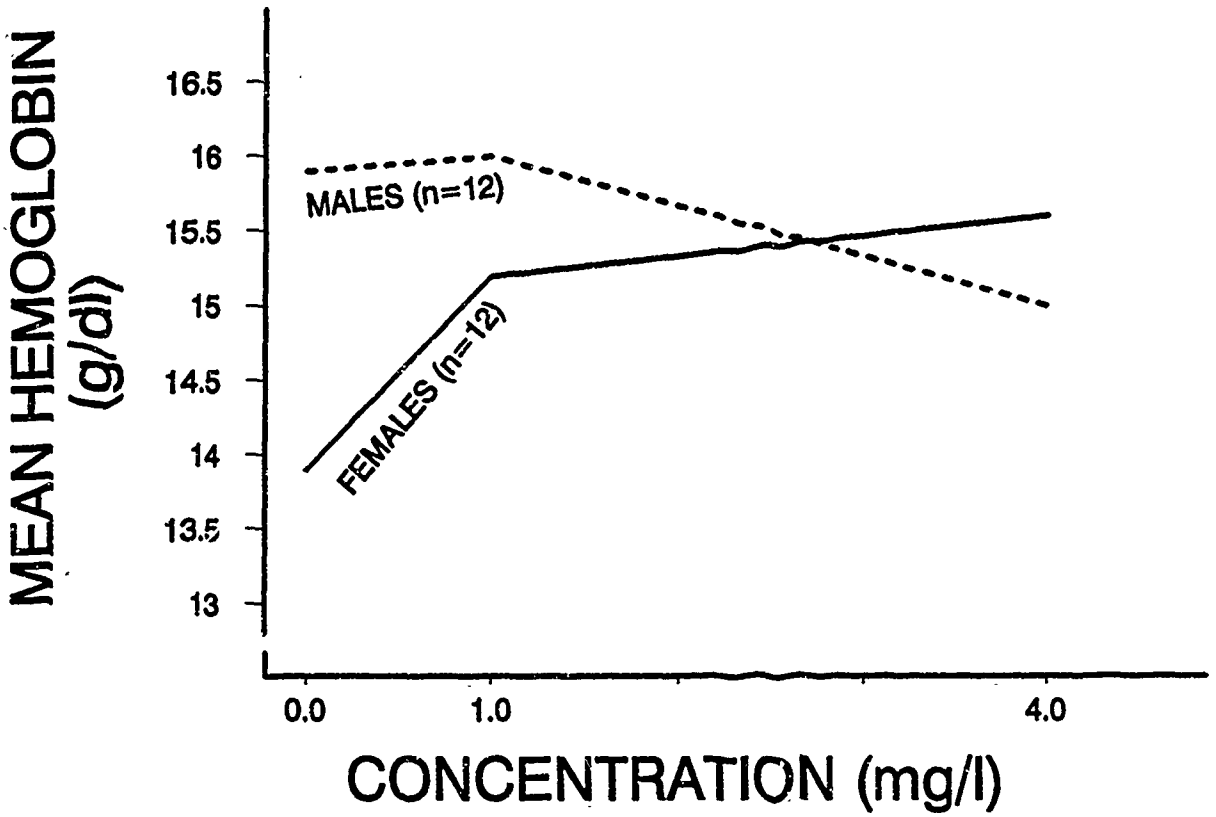

Figure 28. Hemoglobin (Hb) Concentration X Sex interaction means of rock doves exposed to 3 concentrations (0.0, 1.0, and 4.0 mg/ℓ) of RP/BR smoke.

With MetHb, the Concentration X Sex interaction (Figure 29) was caused by MetHb of females being significantly higher than males at 0.0 mg/ℓ and 1.0 mg/ℓ RP/BR smoke but not at 4.0 mg/ℓ RP/BR smoke. The mean MetHb values appear to be too low to interfere with normal oxygen transport. This agrees with normal published values for pigeons (Abs, 1983). However, the MetHb Sex X Concentration interaction may provide an additional indication that male rock doves are more vulnerable to RP/BR smoke than females.

Table 11 shows the results of Duncan's Tests for the blood chemistry and hematology Day effects. The significant Day effect for pH, Pco_2, Hb and COHb appears to have no biological significance relative to RP/BR smoke since there was no Day X Concentration interaction and little variation among means (less than 11% from low to high mean within each variable). The day effect most likely occurs because of handling, bleeding and chamber effects as reported for the Pulmonary Function Study.

The PROC ANOVAs for the hematology variables revealed 4 significant effects in rock doves: Concentration X Day interactions for lymphocytes ($F = 4.03$, $df = 6/66$, $p \leq 0.002$), and heterophiles ($F = 4.38$, $df = 6/66$, $p \leq 0.000$), and Day main effects for PCV ($F = 3.09$, $df = 3/66$, $p \leq 0.033$) and monocytes ($F = 3.98$, $df = 3/66$, $p \leq 0.011$).

The Concentration X Day interaction for lymphocytes resulted from the 4.0 mg/ℓ RP/BR smoke causing lymphocytes to significantly decrease from a mean Pre-exposure level of 61.0 to 25.5 percent on Day E-2 and return to 57.9 percent by Day P-6, whereas the 0.0 and 1.0 mg/ℓ smoke RP/BR concentrations had no significant effect on lymphocytes (see Figure 30).

Conversely, the Concentration X Day interaction with heterophiles resulted from the 4.0 mg/ℓ RP/BR causing heterophiles to significantly increase from a Pre-exposure mean of 35.4 to 71.4 percent on Day E-2 and return to near the Pre-exposure Day by Day P-6, with no corresponding change in heterophiles for the 0.0 and 1.0 mg/ℓ Concentration Groups (see Figure 31).

The marked increase in heterophiles and concomitant decrease in lymphocytes shows that exposure to RP/BR smoke at 4.0 mg/ℓ was a significant environmental stressor. The magnitude of the reaction is suggestive of adrenocortical stimulation, with increased immunological response (see Selye, 1973; Siegel, 1980).

Table 11. Means of the significant Day main effect terms for blood chemistry and hematology venous values of rock doves as analyzed by Duncan Tests (means with no letter in common are significantly different at an error rate of 0.05).

Variable	Day	Mean	Letter
pH	Pre-1	7.365	b
	E-4	7.374	b
	P-2	7.406	a
	P-6	7.399	a
Pco_2 (mm Hg)	Pre-1	44.05	b
	E-4	40.63	a
	P-2	39.75	a
	P-6	40.08	a
Hb (g/dl)	Pre-1	15.83	b
	E-4	15.13	a
	P-2	14.88	a
	P-6	15.14	a
% COHb	Pre-1	-2.979	b
	E-4	-2.800	a
	P-2	-3.046	b
	P-6	-2.783	a

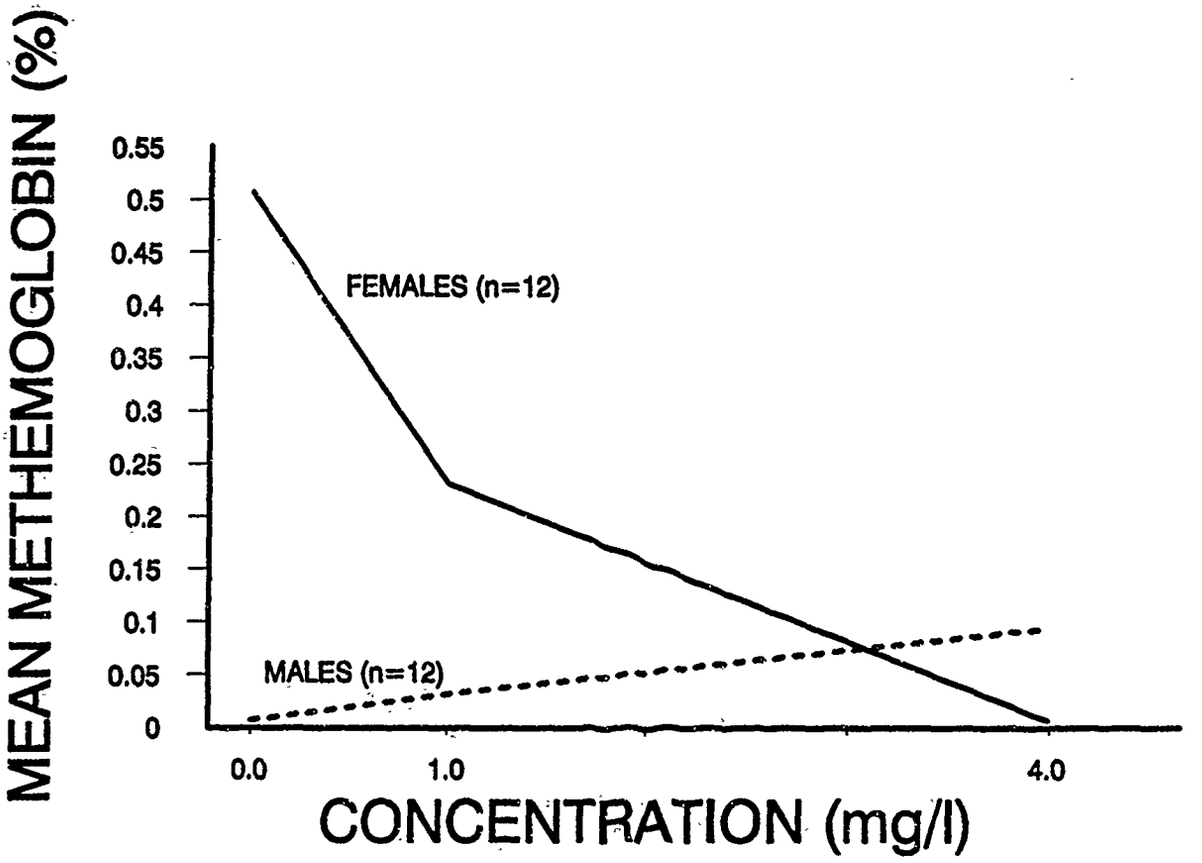

Figure 29. Methemoglobin (MetHb) Sex X Concentration interaction means for rock doves before, during, and after exposure to 3 concentrations (0.0, 1.0, and 4.0 mg/ℓ) of RP/BR smoke.

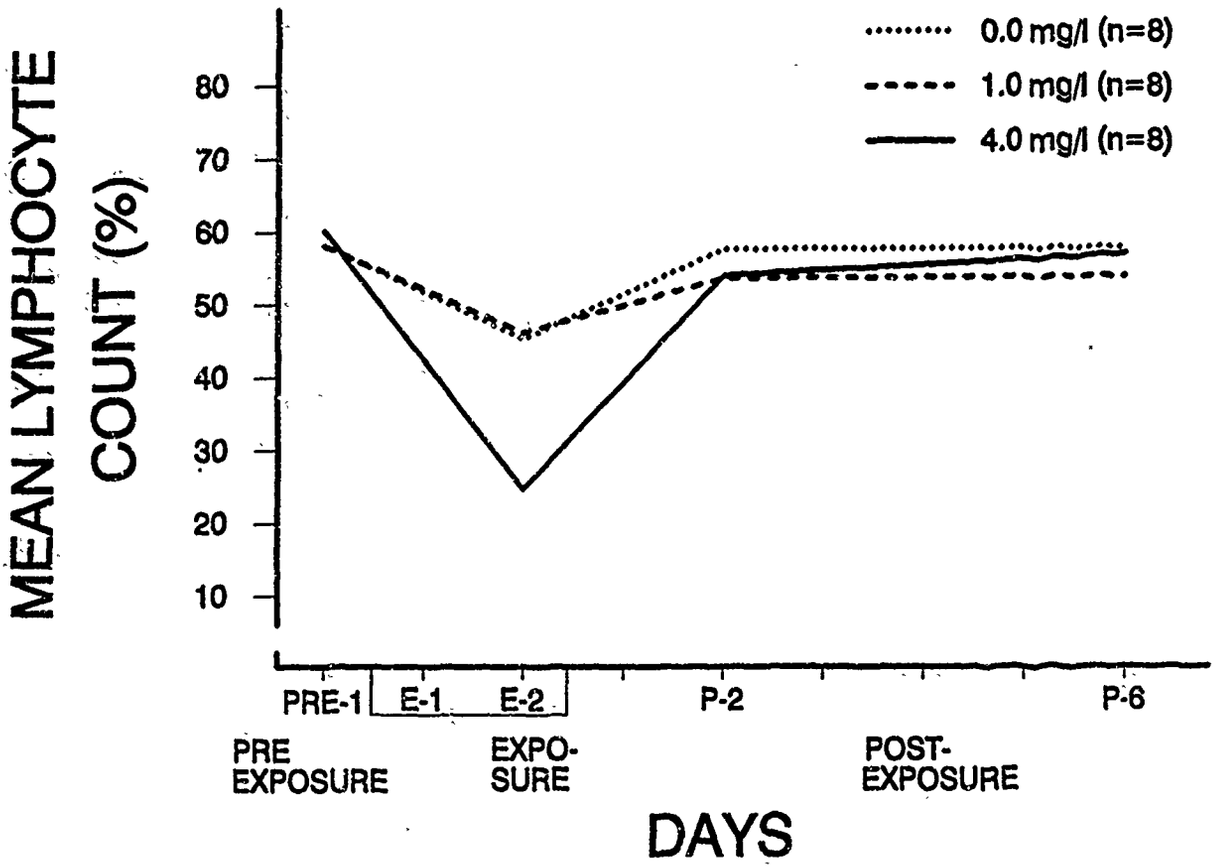

Figure 30. Lymphocyte Concentration X Day interaction means of rock doves before, during, and after exposure to 3 concentrations of RP/BR smoke.

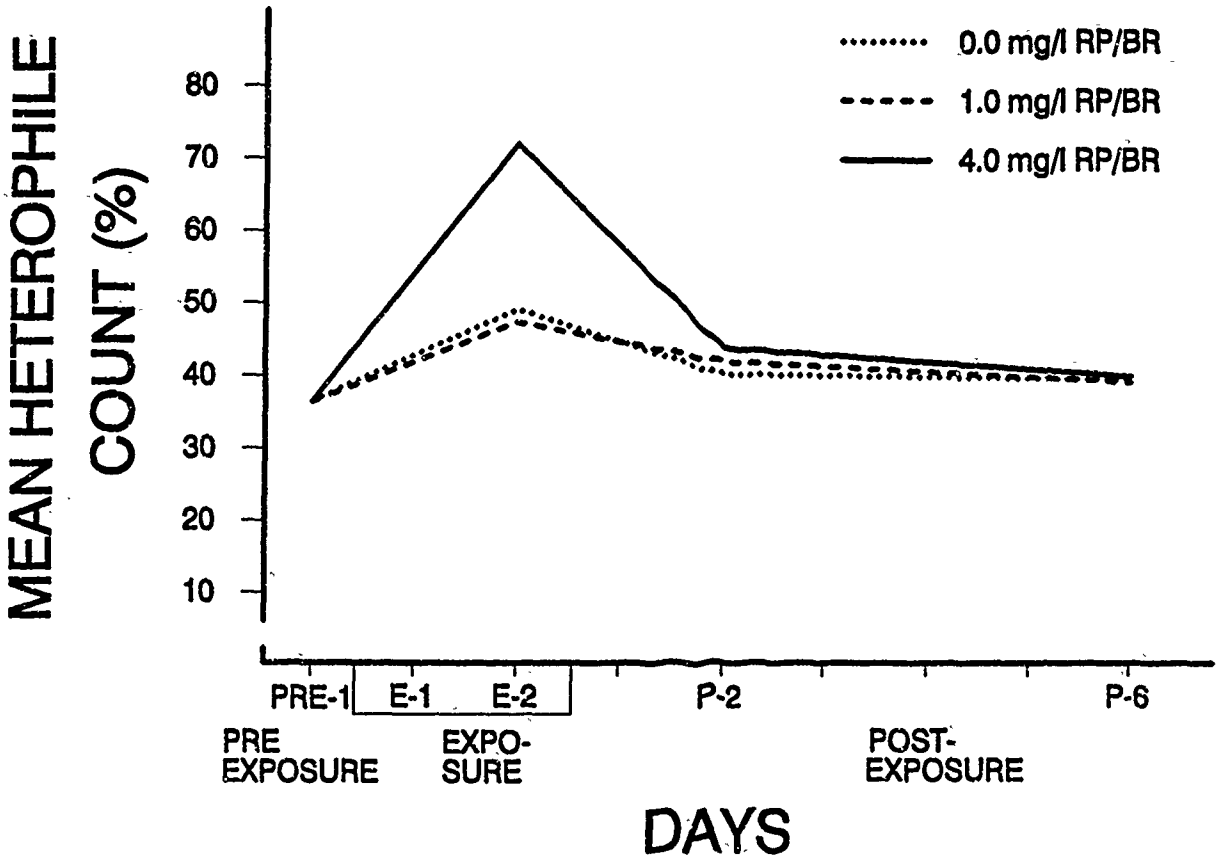

Figure 31. Heterophile Concentration X Day interaction means of rock doves before, during and after exposure to 3 concentrations of RP/BR smoke.

It is interesting to note that lymphocytes and heterophiles for the 4.0 mg/ℓ RP/BR-aerosol Groups returned to near Pre-exposure levels by Day P-6, a response demonstrating return to homeostasis soon after exposures.

With respect to the Day main effects, PCV averaged (mean ± SD) 50.9 (± 3.3) percent on Day Pre-1, 49.2 (± 3.7) percent on Day E-2, and 49.8 (± 4.4) and 52.4 (± 4.1) percent on Days P-2 and P-6, respectively. Similarly, values for monocytes on corresponding Days averaged 2.7 (± 2.3), 2.5 (± 1.5), 1.0 (± 1.3), and 2.1 (± 2.0) percent, respectively. Changes in blood volume are an important consideration in interpreting PCV values, since they are altered with blood volume changes (Swenson, 1984). As such, the Day differences in PCV reflect changes caused by the stress of the handling and the blood-sampling schedules and are unrelated to RP/BR-smoke exposure. The changes in monocytes, although significant, do not necessarily represent changes that are abnormal since considerable variation can occur in normal values. If the values are abnormal, stress induced by handling and bleeding during the test regimen is the most probable cause.

The Heterophile X Day and Lymphocyte X Day interactions clearly show that RP/BR smoke at 4.0 mg/ℓ causes transient physiological stress in rock doves. Also, the significant Hb and MetHb Sex X Concentration interactions, plus the deaths of 2 males in the Pulmonary Function Study, show that perhaps male rock doves are more vulnerable to RP/BR smoke than females.

D. Summary and Conclusions (Pulmonary Function and Blood Chemistry/Hematology Studies)

The physiological effects of exposing prairie dogs and rock doves to 0.0, 1.0, and 4.0 mg/ℓ RP/BR smoke were assessed in terms of a number of pulmonary function (Rf, Vo_2, Vco_2, RER and MR), blood chemistry (pH, Po_2, Pco_2, Hb, O_2Hb, COHb, and MetHb), and hematology variables (PCV, total WBC, and differential WBC). Animals were exposed to each concentration of RP/BR smoke (prairie dogs: 4 daily 80-min exposures; rock doves: 2 daily 80-min exposures) in an inhalation chamber at an air flow rate of 250 ℓ/min. Chamber atmospheres were characterized for RP/BR-aerosol quantity (concentrations and mass), quality (particle sizes, respiratory and contaminant gases) and chamber conditions (duration, temperature and relative humidity) to assure that acceptable test standards were met.

Pulmonary function variables were measured in 2 separate studies, one with 24 prairie dogs and the other with 24 rock doves via a computerized pulmonary function system. Similarly, blood chemistry and hematology variables were measured in 2 separate studies with 24 prairie dogs and 24 rock doves. Blood chemistry variables were

determined using an automated blood gas analyzer and a co-oximeter, whereas hematology variables were determined using standard clinical techniques.

Separate ANOVAS were performed on each variable for each study. A synopsis of the major findings is listed below.

1. Pre-exposure pulmonary function, blood chemistry, and hematologic values were within ranges anticipated for prairie dogs and rock doves.

2. The 3 RP/BR-smoke concentrations (0.0, 1.0 and 4.0 mg/ℓ) had no significant effects on any pulmonary function, blood chemistry or hematology variable of prairie dogs. While there was a significant Sex difference in MR and a significant difference between Days for RER, Po_2, Hb, O_2Hb, COHb, MetHb, and PCV, these differences were not caused by exposure to RP/BR smoke.

3. The 4.0 mg/ℓ concentration of RP/BR smoke caused a significant increase in heterophiles, a significant decrease in lymphocytes, and a significant Sex X Concentration effect on Hb and MetHb of rock doves. Also, male birds appeared to be more vulnerable to RP/BR smoke than females. This was indicated by the death of 2 males approximately 8 days after exposure to 4.0 mg/ℓ RP/BR smoke.

These studies show that RP/BR smoke had no significant effect on pulmonary function, blood chemistry, or hematology of prairie dogs. In rock doves there were only significant transient Concentration X Day interaction effects on Vco_2, RER and MR and significant transient Concentration X Day interaction effects on heterophiles and lymphocytes. Plus there was a significant Sex X Concentration effect on HB and MetHb of rock doves. In essence, other than elevated Rf in 2 male rock doves that died and the marked transient shift in heterophiles and lymphocytes of rock doves exposed to 4.0 mg/ℓ RP/BR smoke, there was no clear evidence that RP/BR smoke caused detrimental effects to either species. The Sex X Concentration Hb and MetHb values are useful information that may be an indicator of RP/BR-smoke exposure. However, they are not in themselves detrimental to the rock doves. The detrimental impacts may have been more pronounced if both species had been subjected to strenuous and/or stressful activity following exposure to RP/BR smoke.

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VIII. APPENDICES

Appendix A. Excerpts from DWRC Work Unit 615.16 Concerning Quarantine and Care of Prairie Dogs and Rock Doves, Plus a Copy of the Report of Animal Care Committee Approving the Task 3 Research Studies.

II. Animals and Animal Care Procedures

Numbers.-- Approximately 110-120 prairie dogs and 110-120 rock doves will be used in Task 2. The sublethal RP/BR exposure phase will involve 48 animals of each species and the basal physiological determinations will involve 70-80 animals of each species.

Capture.-- Black-tailed prairie dogs and rock doves will be caught locally by Project Staff. Every effort will be made to insure the humane care and treatment of animals throughout all phases of the research. All cages and handling procedures will concur with the current regulations concerning the Animal Welfare Act. The attending veterinarians are: Drs. Gary W. Church and Patricia L. White, Veterinary Service, Animal and Plant Health Inspection Service (APHIS), U.S. Department of Agriculture (USDA), Rm. 237, 2490 W. 26th Avenue, Denver, CO 80221. Prairie dogs will be captured at several known colonies in the Denver Area; landowner permission will be obtained prior to these efforts. In general, prairie dogs will be caught using 1 of 2 methods: (1) foaming of burrows or (2) trapping.

The foaming method will involve dispensing a narrow stream of laundry detergent with water into burrow openings known to be occupied by prairie dogs. The suds cause paririe dogs to leave the burrow and the animals are then hand captured with snares and gloves. The repellent action of the detergent has been judged to be non-traumatic and the concentration contacting the animals is less than 0.5 percent as it is mixed with water.

The trapping method will involve positioning #203 Tomahawk traps (61 X 15 X 15 cm) at burrows during daytime. All traps will be covered with white plastic tops to provide shade for animals during trapping in hot weather. Traps will be baited with food, then checked daily at dusk for the presence/absence of a prairie dog. In hot summer weather or cold winter weather, traps will be checked twice daily (i.e., approximately 1300 and 2000 h and 1300 and 1700 h, respectively) to reduce effects of heat and cold stress in captured prairie dogs. The diurnal activity of the animal precludes night trapping. Following capture, animals will be dusted for ectoparasites with Purina Dog Powder (a.i. pyrethrins 0.1%, piperonyl butoxide 1.0% and carbaryl 5.0%) and then transferred to individual holding cages (91 X 61 X 20 cm) for transport to DWRC.

Rock doves will be purchased from a local supplier and housed in 3.0 X 1.5 X 1.8 m 5.1 cm wire mesh cages in a covered outdoor facility. Density of pigeons in each cage will not exceed 30 birds. They will be maintained on Purina pigeon checkers and cracked corn with water and grit available ad libitum. Each wire holding cage shall contain sufficient perch space for

30 birds and an attached 1.0 X .75 X .75 m wood shelter box. After at least 2 weeks of holding in these outdoor cages the birds will be moved to the inside quarantine aviary cage facility.

Quarantine and Holding of Animals Prior to Test.-- Upon arrival at DWRC, prairie dogs will be permanently segregated by sex. Before the quarantine is initiated, these animal will receive a second dusting with the Purina Dog Powder. Each animal will be weighed (nearest g), implanted with a transponder by injection for identification, and checked for outward signs of disease and poor health before the Quarantine Period is started. Unhealthy animals will be euthanized using ether and then incinerated. Throughout the period of research, the prairie dogs will be maintained on water and Purina rabbit checkers (Performance Blend; crude protein of not less than 17.0%; crude fat not less than 2.0%; crude fiber not more than 18.0%; and ash not more than 8.0%).

Quarantine procedures will adhere to the following 10 recommended practices of the DWRC Animal Care Committee:

A. The quarantine area will be isolated from the main research area (Bldg. 16) to provide adequate biosecurity. Animals will be checked daily, and any problems (e.g., health, security) will be reported to the Principal Investigator. Quarantine will begin and end with no animals admitted or removed from the original group in the quarantined area until completion of the Period.

B. The Quarantine Period will be 14 days in length. This will allow for the detection of parasitic and disease problems, as well as, time for the animals to adjust to their captive diet and surroundings.

C. All incoming animals will be examined by one of the Consulting Veterinarians for signs of disease and injury prior to Quarantine. Initial external parasite control will be started either in the field before transport or upon arrival at DWRC depending upon air temperature and length of time elapsed from exposure to the detergent foam treatment. All animals will be re-dusted prior to onset of the Quarantine period.

D. Tests will be performed on pooled fecal samples of all prairie dogs to be quarantined in order to detect potential internal parasites; if positive, the entire group of animals will be treated with an appropriate antiparasitic drug.

   Pooled rock dove fecal samples will be submitted to the National Veterinary Services Laboratory to be inspected for Velogenic Viserotropic Newcastle Disease (VVND) and Psittacosis. If positive for VVND the entire group of birds will be euthanized using ether and incinerated; all cages, bedding, food, and any other materials in contact with the birds will be disinfected or incinerated. If positive for Psittacosis, each bird will be assessed individually with cloacal swabs; those birds showing positive confirmation will be euthanized.

E. All unusual symptoms and deaths of quarantined animals will be promptly reported and recorded by the Principal Investigator. Post mortems and diagnostic workups will be performed, if indicated by the consulting veterinarians.

F. Food storage will be located adjacent to the Quarantine Area and not in contact with food to be given to nonquarantine animals. Storage of other supplies and water containers used will also be located in the Quarantine Area.

G. Vermin control will be strictly monitored and enforced using glue boards and traps in the Quarantine Areas. Insect control will be accomplished using appropriate insecticides prior to the Quarantine Period.

H. Respirator masks must be worn by personnel at all times when working around avian subject in the Quarantine Area.

I. All personnel will change their outer wear clothing including boots, before entering and leaving the Quarantine Area.

J. After the Quarantine Period is over, the entire Area will be cleaned and disinfected. This includes cages, holding pens, water and food dishes.

K. Animals showing signs of debilitation or poor health following these quarantine procedures will be euthanized, necropsied, and incinerated. All animals displaying good health will then be available for transport to appropriate laboratory animal colony holding rooms in Building 16.

Prairie dogs will be held in a separate heated and air-conditioned brick building on the DFC. This building is temperature ($23^{o} \pm 5^{o}$ C) and light controlled. The animals will be reweighed every 14 days; animals losing more than 15 percent body weight will not be used for research until determined "healthy" by the consulting veterinarian. Lactating or pregnant females will not be used in research studies. The animals will be euthanized using ether.

Regarding rock doves, each will be checked for outward signs of disease and poor health, dusted with Purina Dog Powder, weighed, and banded for individual identification before the quarantine Period is initiated. Throughout the period of research, doves will be maintained on water and Purina Pigeon Checkers with grit available ad libitum.

Quarantine of rock doves will involve placing the males and females in either of three wire mesh aviary cages (1.6 X 3.3 X 2.6 m; 2.0 X 6.6 X 2.6 m; 3.9 X 3.9 X 2.6 m) located within a 11.5-m-diameter Butler building on the DFC. At the end of the Quarantine Period and each 14-day interval of captivity, all birds will be re-weighed. Those animals losing more than 20 percent body weight (i.e., relative to initial weight) will be

held until weight and status are acceptable, then made available for research studies. All birds showing severe body weight loss or other unhealthy signs will be treated as appropriate to restore health or euthanized. Animals not responding to veterinary treatment will be euthanized and incinerated. All doves not surviving Quarantine will be refrigerated for later necropsy examination by the Veterinarians. Birds appearing healthy and maintaining body weight after the 14-day Quarantine Period will be available for RP/BR smoke exposure tests in Building 16.

Holding of Animals During Test.-- During specific studies, animals will be separately housed in rabbit-type cages (Hazleton Systems H-1432, Aberdeen, MD -- 61 X 62.5 X 41 cm; Wahman Mfg. Co., Baltimore, MD -- 51 X 54 X 38 cm) and allowed 14 days adaptation prior to use in experiments. The research will be conducted in isolated rooms of Building 16. Throughout the studies, all prairie dogs and doves will be maintained on a 12:12-h light/dark schedule, with temperature controlled at $23^{o} \pm 2^{o}$ C. A restricted feeding schedule will be maintained with food available between the hours of 0630 and 1430 MST (8 hrs) to counteract overeating by these individually caged animals.

Use of Anesthetics and Euthanasia Drugs.-- If possible, pain alleviating drugs will not be used during behavioral or physiological measurement procedures because of potential distortion of measured effects. If and when drugs are administered to animals, specific drug and dose procedures will be provided as Standard Operating Procedures to the contractor, and appropriate control groups will be included in theses designs. In the case of severe debilitation in the animals produced by RP/BR smoke exposures, the animals will be observed daily for recovery or mortality. Euthanasia would not be used as this would obviate mortality-effects data.

For collection of blood specimens during certain physiological procedures, animals will be restrained. If restraint proves overly stressful to the animals, Methoxyflurane will be used to anesthetize both species.

For necropsy examinations the drug to be used for euthanasia of prairie dogs will be sodium pentobarbital administered according to the procedures recommended by the American Veterinary Medical Association (Smith, Houpt, Kitchell, Kohn, McDonald, Passaglia, Thurmon, Ames, 1986). Cervical dislocations will be used as the means of euthanasia with pigeons for necropsy purposes. (Note: Cervical dislocation proved unacceptable, all Task 3 doves were euthanized with sodium pentobarbital -- RTS 7/22/89.)

REFERENCE

Smith, A.W., K.A. Houpt, R.L. Kitchell, D.F. Kohn, L.E. McDonald, M. Passaglia, J.C. Thurmon, and E.R. Ames. 1986. 1986 Report of the AVMA Panel on Euthanasia. Journal of the American Veterinary Medical Association. 188(3):252-268.

REPORT OF ANIMAL CARE COMMITTEE

United States Department of Agriculture | Animal and Plant Health Inspection Service | Animal Damage Control

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

Principal Investigator:	Research Study Title:
R. T. Sterner, S. A. Shumake, B. E. Johns, R. D. Thompson	RP/BR Smoke Inhalation: Effects Upon Spontaneous Activity, Startle Response, Pulmonary Function, & Blood Chemistry, Task 3.

This committee has reviewed the above described project with respect to the rights and safety of the animal subjects. The following are our findings:

Risks (Check one)

___ The planned research involves little foreseeable risk and adequate precautions have been taken for the safety and comfort of the subjects unless the plan is modified.

✓ The foreseeable risk is justified by the anticipated benefit to society and the plans include adequate and appropriate measures to ensure the comfort and safety of the subjects insofar as feasible.

___ The risk is justified but further measures seem advisable to protect the subjects. Comments are attached.

___ The risk seems greater than can be justified by the research as planned and the research study is not approved as presented.

Further Comments:

Recommendation (Check one)

✓ The research study be approved as submitted. ___ The research study be revised in keeping with our comments and resubmitted. ___ The research study as described be rejected.

Signature of Committee Chairperson	Title	Date Signed
I have not signed since I am out of town. Brad A. Johns	Research Physiologist	5-16-88
Signature of Committee Veterinarian Gary W. Church	Title Veterinary Medical Officer, VS, APHIS, USDA	Date Signed 5/26/88
Signature of Committee Member Peter J. Savarie Peter J. Savarie	Title Pharmacologist	Date Signed 5/26/88
Signature of Committee Member James E. Davis	Title Biological Laboratory Technician (Wildlife)	Date Signed 5/18/88
Signature of Committee Member Jennifer L. Keller Jennifer L. Keller	Title Biological Aid (Animal)	Date Signed 6/2/88
Signature of Committee Member Louis Ray Burke	Title Representative of Community	Date Signed 5/26/88

Appendix B. Detailed Descriptions of RP/BR-aerosol and Filtered-air Inhalation Chamber Systems Plus the In-chamber Atmospheric Monitoring Techniques.

Two separate inhalation systems were used to expose animals to either RP/BR aerosol or equivalent durations of filtered air. The Modified RP/BR Extruder and Inhalation Chamber System and the Filtered-air Inhalation Chamber system were described in the Task 1 and 2 Reports (Sterner et al., 1988; Shumake et al., 1989).

Essentially, each system was constructed of identical materials and components. Each had independent closed-air supplies with separate air-filtration, air-humidification and air-movement equipment. Negative air pressure produced by individual ceiling vents (approx. 15-room air exchanges/h) within each system-housing room prevented any inadvertent smoke contamination of the animal-holding areas.

A. Modified RP/BR Extruder and Inhalation Chamber System

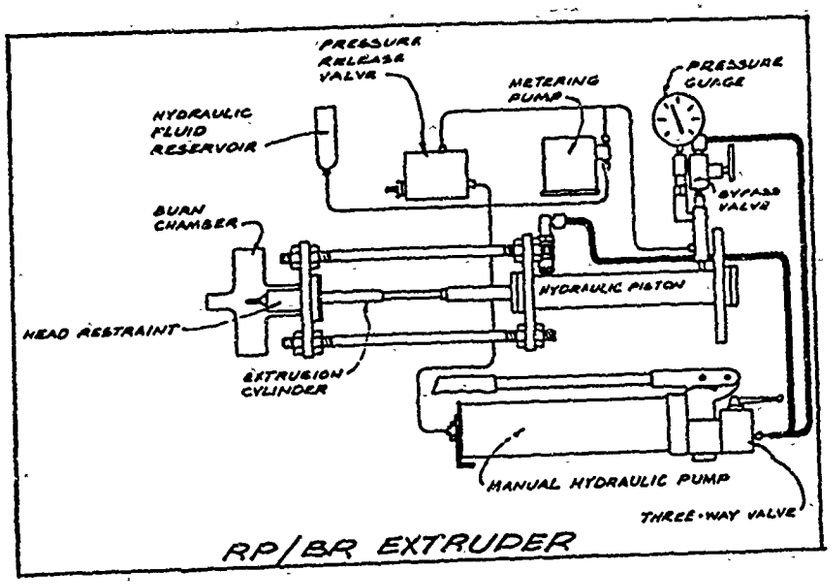
Figure B1 illustrates the Modified RP/BR Extruder and Inhalation Chamber System. The insert of Figure B1 is a detailed drawing of the RP/BR extruder equipment. This System is similar to that described by Holmberg, Moneyhun, and Gayle (1985) and Aranyi (1983a, 1983b, 1984, and 1986). Operation of the RP/BR-aerosol System involved 4 elements: (1) Formulation of RP/BR Product, (2) RP/BR Extruder/Generator Subsystem, (3) Inhalation Chamber Subsystem, and (4) Air-movement/-condition/-filtration Subsystem.

1. Formulation of RP/BR Product

The RP/BR product was formulated by the staff of the Bio/Organic Analysis Section, Analytical Chemistry Division, Oak Ridge National Laboratory (ORNL) in accordance with APHIS Interagency Agreements (IAGs) 87-74-01 and 34-WT-88 12-34-74-006 (IA). The mixture was formulated from 2.5 kg lots of a 95% RP (2.375 kg) and 5% BR (0.125 kg) product. Following mixing of the dry RP and BR substances, the product was placed in a vacuum desiccator and hexane (C_6H_{14})was introduced until 7-8 percent (wt/wt) was absorbed. This "softened product" was then loaded into 11.45-cm sections (1.91 cm i.d.) of stainless steel pipe (billets). Each of these "billets" contained approximately 40 g of pliable RP/BR material that was sealed with Teflon-lined steel caps to prevent drying. Billets were shipped to DWRC as needed, and only billets $\leq$ 3 months old were used to produce RP/BR aerosol for Task 3 Studies.

2. RP/BR Extruder/Generator Subsystem

Operation of the RP/BR extruder/generator required loading the extrusion cylinder with the formulated RP/BR product (see Figure B1). This material was 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



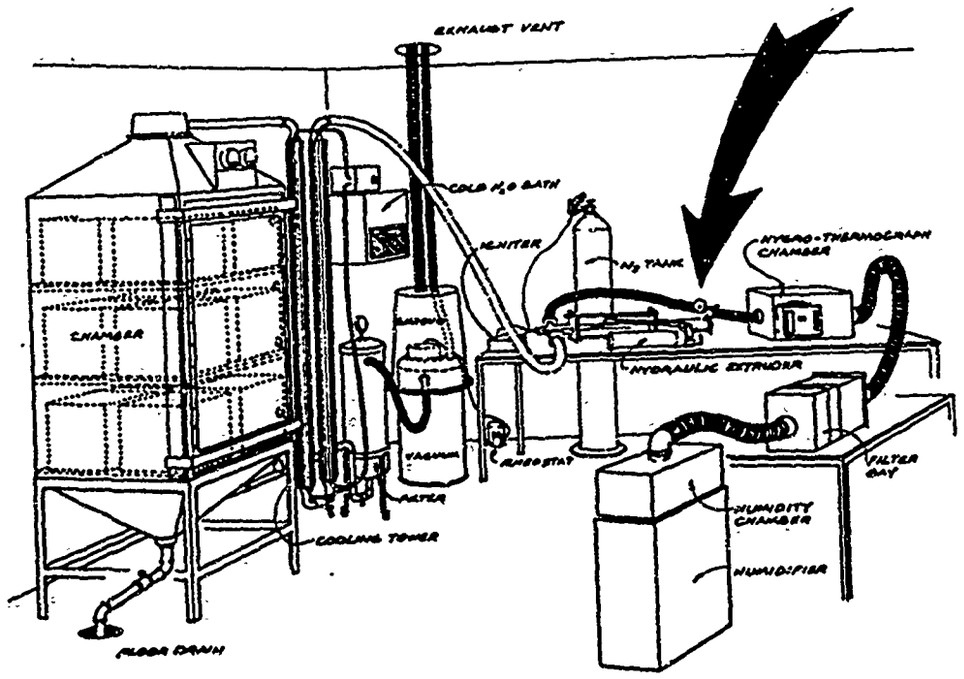


Figure B1. Technical illustration of the Modified RP/BR Extruder and Inhalation Chamber System, with schematic diagram of the RP/BR extruder/generator shown in the insert. (Note.-- Components of the System are scaled relative to the perspective, i.e., 1 cm equals 0.236 m, but the location of some components has been drawn to improve the visual display.)

(approx. 2 mm-dia.) was extruded into the custom-blown glass burn chamber where it was ignited to produce the RP/BR aerosol. A small envelope of nitrogen (N_2) gas was bled continuously into the RP/BR extrusion tip to prevent a backburn of RP/BR.

3. Inhalation Chamber Subsystem

The inhalation chamber was a standard stainless steel unit (91.5 X 91.5 X 91.5 cm) with autoclave door (Bertke and Young, Cincinnati, OH). The door consisted of 1.3 cm thick opaque Plexiglas. The internal chamber had 3 shelves each containing 4 stainless steel wire mesh animal cages. A PVC drain valve was plumbed to the bottom of the chamber for flushing residues from the interior out a floor drain.

4. Air-movement/-condition/-filtration Subsystem

Air flow was caused by an in-line industrial vacuum (Dayton Electrical Mfg. Co., Chicago, IL). This vacuum source was located at the end of the closed, air-flow line. Calibrated air flow was determined using a Pneumotachograph Air Pressure Gauge (OEM Medical, Inc., Richmond, VA). The operator regulated a variable voltage auto transformer (Staco Energy Products, Dayton, OH) which controlled the vacuum source so that a stable 250 ℓ/min flow of air was maintained through the System.

Humidification of the intake air was accomplished using a commercial console humidifier (Emerson Electric Co., St. Louis, MO) with a custom-fabricated Plexiglas humidity-collection chamber located over the humidifier's exhaust (Figure B1). From the humidity-collection chamber, air was routed through flexible PVC tubing to a custom-made Plexiglas filter bay containing an Absolute Filter Unit (Young and Bertke Co., Cincinnati, OH). This was a pleated, coarse filter (American Air Filter, Louisville, KY) with an inserted charcoal bed and HEPA filter (Mine Safety Appliances Co., Pittsburgh, PA).

Humidified, filtered air then flowed through PVC tubing to the Hygrothermograph Chamber. This Chamber was custom-fabricated of Plexiglas. A Hygrothermograph (Belfort Instrument Co., Baltimore, MD) was placed inside the chamber and the lid of the chamber was sealed. The Hygrothermograph was calibrated prior to each day's RP/BR burns using a fixed-position battery-powered psychrometer (Belfort Instrument Co., Baltimore, MD). Humidity in this air-flow line was maintained at between 40 and 60 percent.

From the Hygrothermograph Chamber, air moved through flexible PVC tubing to the glass burn chamber. The aerosol-laden, heated air then moved from the burn chamber through flexible and rigid sections of stainless steel pipe to the apex of the inhalation chamber. A large portion of this pipe was surrounded by a water jacket; and, cold water was circulated between the jacket and a cold water bath (Massagerata-Werk Lauda, West Germany) to cool the intake pipe and the aerosol. Activation of this temperature-control subsystem was a decision of the operator. Generally, circulation of cold water was required only if

the room temperature exceeded 21° C or if 4.0 mg/ℓ RP/BR burns were planned.

After the aerosol-laden air reached the apex of the inhalation chamber, it dispersed throughout the chamber. A uniform flow was assumed to occur from apex to base. The aerosol was exhausted from the base of the chamber via PVC pipe. From the RH-recording port, the aerosol moved to a 7-bank, DX-grade coalescent filter unit (Balston Filter Products, Lexington, MA) which removed aerosol and associated contaminants from the chamber exhaust (Holmberg et al., 1985). Finally, the "scrubbed air" flowed to the vacuum source (Dayton Electrical Mfg. Co., Chicago, IL) via flexible PE tubing, and exited the System (building) through a ceiling vent. A 30-gallon PVC shroud, with exhaust hose connected to the ceiling vent, covered the vacuum source to prevent any residual smoke products from entering the room.

B. Filtered-air Inhalation Chamber System

Figure B2 is a technical illustration of the Filtered-air Inhalation Chamber System. The Filtered-air Inhalation Chamber System was used to expose "control groups" of animals to roughly equivalent durations of filtered air.

Briefly, air flow was produced by an in-line vacuum (Dayton Electrical Mfg. Co., Chicago, IL). Air-flow rates were calibrated using a Fleisch Pneumotachograph Air Pressure Gauge (OEM Medical, Inc., Richmond, VA); and, calibration procedures were identical to those used with the RP/BR System (see Sterner et al., 1988).

Humidification of the intake air was accomplished using a commercial console humidifier (Emerson Electric Co., St. Louis, MO). The humidified air was routed through a flexible PVC tube to a Plexiglas filter bay and an Absolute Filter Unit (Young and Bertke Co., Cincinnati, OH). This filter unit was identical to that used with the RP/BR System.

Next, the filtered air flowed through a 1.22 m section of 10.16 cm (i.d.) flexible PVC pipe to the Hygrothermograph Chamber. The same Hygrothermograph (Belfort Instrument Co., Baltimore, MD) used for the RP/BR-aerosol exposures was placed into this chamber during measurement sessions. Humidity was maintained between 40 and 60 percent RH. Air then moved through flexible PVC tubing to the glass burn chamber of a RP/BR extruder (i.e., an extruder was placed in the air intake line, but was never loaded with RP/BR).

From the "blank" burn chamber, air flowed through a length of 5.6-cm diameter flexible stainless steel pipe. A U-shaped 5-m length of 6.35 cm diameter stainless steel pipe was run from the end of the flexible pipe to the apex of the inhalation chamber (i.e., the intake pipe was a duplicate of that used in the RP/BR System). The base of the U-shaped column also was joined by 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 form the condensate drain

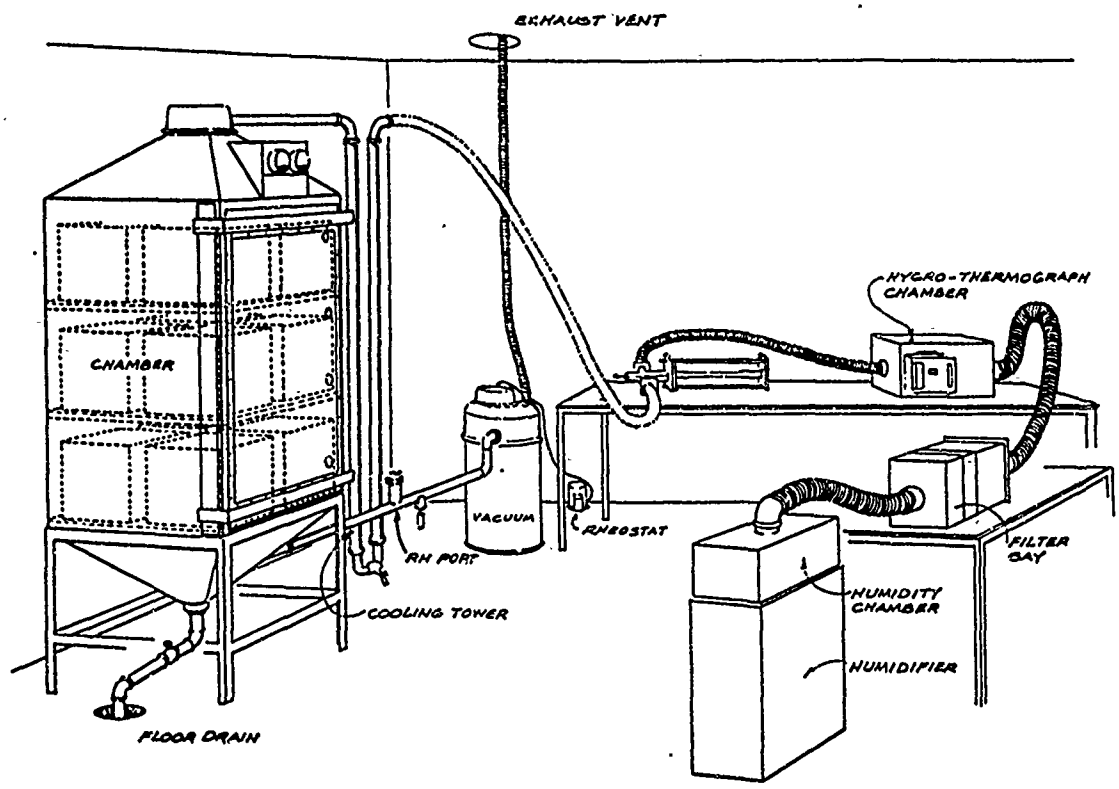


Figure B2. Technical illustration on the Filtered-air Inhalation Chamber System, with the floorplan for the research areas of the DWRC Laboratory shown as an insert. (Note.-- Components of the System are scaled relative to the perspective, i.e., 1 cm equals 0.305 m, but the locations of some components have been drawn to improve the display.)

on the RP/BR System. No water jacket cooling column surrounded the stainless steel intake line of the Filtered-air System.

Air then flowed from the apex to base of the chamber where air was exhausted via a standard PVC pipe. Similar to the RP/BR System, a special RH-recording port was located 24 cm from the chamber outlet (see Figure B2). At the end of each exposure session, a measurement of in-chamber RH was obtained by inserting a standard wet-/dry-bulb thermometer in the chamber exhaust line for approximately 5 min.

From the RH-recording port, air moved to the vacuum source (Dayton Electrical Mfg. Co., Chicago, IL). No 7-bank, DX-grade filter unit was present in the Filtered-air Inhalation Chamber System. The air was then vented from the System and from the building through wire-ribbed PE tubing (5.02 cm dia.) via the room's ceiling vent. No 30-gal PVC shroud covered the vacuum source of this System.

C. RP/BR Aerosol and Filtered-air Monitoring

Sampling of in-chamber conditions differed for the 2 chamber Systems. Checks of the Modified RP/BR Extruder and Inhalation Chamber System atmosphere involved 7 sets of variables: (1) aerosol mass, (2) phosphoric acid (H_3PO_4) titration, (3) aerosol opacity, (4) aerosol particle size, (5) respiratory gases, (6) contaminate gases and (7) temperature/humidity. Checks of the Filtered-air Inhalation System atmosphere involved 5 sets of variables: (1) aerosol mass, (2) phosphoric acid, (3) respiratory gases, (4) contaminant gases, and (5) temperature/humidity. No opacity or particle size readings were taken for the Filtered-air System; and checks of the 5 aforementioned variables were limited due to the high consistency of the previous data and lack of contaminants observed for the System.

1. Aerosol Mass

Aerosol mass 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). The filter holders were each fitted with a Millipore Limiting Flow Orifice (Millipore Corp., Bedford, MA); these orifices provided a uniform flow rate of 1 ℓ/min ± 5% for sampled aerosol. During collection, a filter holder was mounted onto the downstream leg of interconnected plastic and PE tubing running from the center of each chamber to a vacuum pump. In-line sampling connections were made air-tight with Teflon tape.

The Aerosol Sampling System is shown in Figure B3. Filtered-air mass collections were made using essentially this same type of equipment.

Aerosol mass of both RP/BR-aerosol and filtered air was measured gravimetrically. This 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 each RP/BR aerosol or filtered-air collection. The difference between the

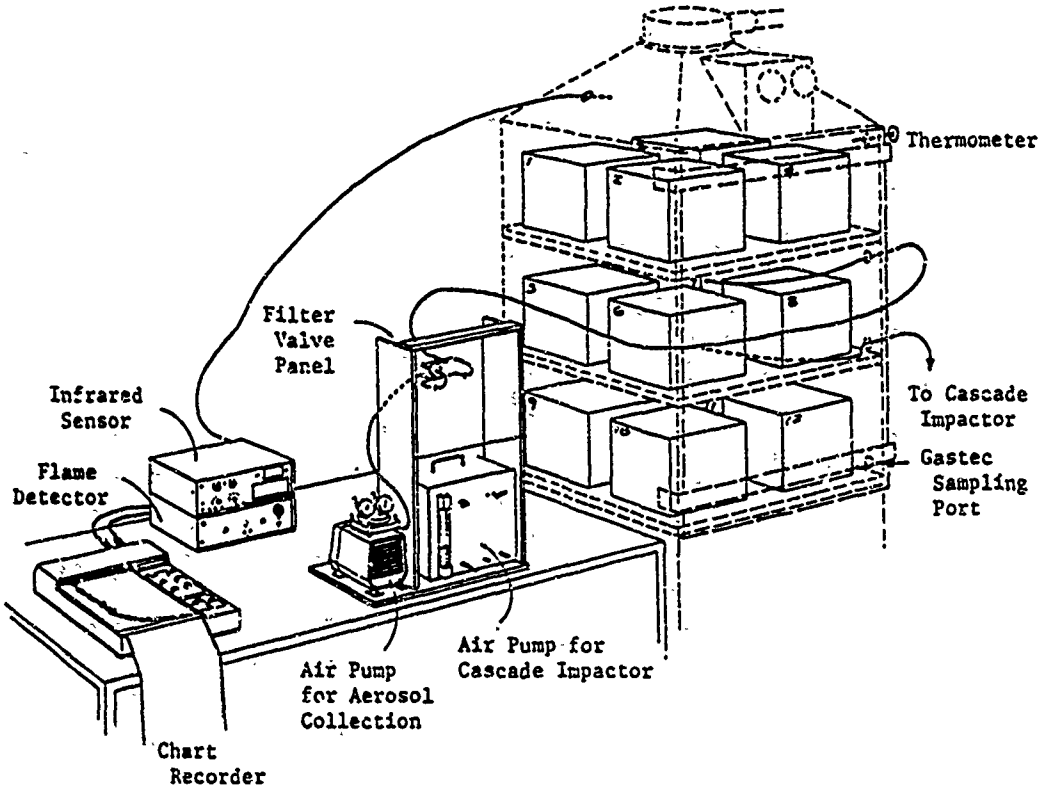


Figure B3. Technical illustration of the Aerosol Sampling System used to collect RP/BR aerosol mass and H_3PO_4 titration filters, opacity measurements, and particle size measurements during behavioral-physiological studies. (Note.-- Cage 12 was unused throughout Task 3; a localized dilution of RP/BR aerosol was believed to occur in the vicinity of this cage during Gastec Tube sampling.)

pre- and post-weight of filters was the mass of aerosol or filtered air accumulated during the sampling period (i.e., approx. 80-min exposure).

2. Phosphoric Acid (H_3PO_4) Titration

Titration analysis was used to determine the amount of H_3PO_4 contained on each filter-disc. This measure is an indicator of the total phosphorus content of the aerosol (Burton et al., 1982).

Following gravimetric analysis, individual filter discs were deposited into covered plastic Petri dishes (Miles Laboratories Inc., Naperville, IL). The petri dishes were then stored in a ventilated cabinet for between 48 and 168 h to allow for complete hydrolysis of the acids. This aging process was used to allow hydrolysis of aerosol acids to mainly H_3PO_4 (Burton et al., 1982). A number of unused, "blank" discs, exposed to ambient room air during RP/BR-aerosol and filtered-air sessions, were also stored in this manner for purposes of quality assurance analyses.

Titration analysis involved the use of a Radiometer DTS-800 Multi-titration System (Radiometer America Inc., Cleveland, OH). Upon removal of the filter from storage, acid residue from each disc was extracted using 60 mℓ of boiled deionized water in a 400 mℓ glass beaker and agitated with a magnetic stir bar for 10 min. When 2 pads were involved, the solutions were combined after extraction. Subsequently, a 20 mℓ sample of the extracted solution was used for titration analysis with 0.1N or 0.01N NaOH. The titrator was programmed to calculate mg of H_3PO_4 in total extracted sample by inflection-point titration. The formula used to make the calculation for single filter pads was:

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

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). Assuming that hydrolization to H_3PO_4 is complete, the first inflection point is a direct measure of the total number of phosphorus atoms (i.e., mg of H_3PO_4) present in the extracted sample. If only H_3PO_4 is present, the amount of NaOH required to titrate to the first inflection point is equal to the NaOH needed to titrate from the first to the second inflection point. Comparisons of H_3PO_4 standard solutions indicated that all aerosol filters had sufficiently hydrolized prior to titration.

3. Aerosol Opacity

The density of RP/BR aerosol within the inhalation chamber was monitored continuously during the burns using an ORNL Aerosol Sensor (Higgins, Gayle and Stokely, 1978; Holmberg et al., 1985). This sensor consisted of an infrared light-emitting diode mounted beside, but optically separated from, a photo-transistor. Aerosol particles

scatter the infrared light, raising the mV output of the transistor and providing an analog record on a chart recorder (Cole-Palmer Instrument Co., Chicago, IL). The sensor consisted of a 16 cm-long probe which was inserted into the top of the inhalation chamber so as to minimize interference due to animal cage reflectance back to the sensor probe (see Figure B3).

Opacity data were used to calculate "average asymptotic (steady-state) concentrations" of the burns comprising each behavioral-physiological study (see Appendix C of this report). Charts of the sensor measurements and the total integrated sensor counts for each RP/BR burn have been archived to provide visual records of flame outs and stability of aerosol levels during each burn.

4. Aerosol Particle Sizes

Particle sizes were derived from aerosol sample measurements using a Piezo-electric Quartz-Crystal-Micro-Balance (QCM) Cascade Impactor (California Measurements Inc., Sierra Madre, CA). All sampling and calibration procedures were conducted according to the manufacturer's instructions.

Measurements were collected on alternate RP/BR-smoke-exposure days (i.e., half of the burns). Samples of aerosol particle sizes were obtained between 20 to 60 min after ignition of the RP/BR material. No particle size measurements were made of the filtered-air exposures.

The particle size sampling procedure involved 2 successive RP/BR-aerosol samplings from near the center of the chamber, along with 2 samples of the room air taken immediately after the RP/BR-aerosol readings. Aerosol sampling involved connecting a plastic sampling tube to the ORNL vacuum flow pump and to the high concentration slide valve on the QCM Cascade Impactor. Interconnected lengths of PE and rigid plastic tubing ran from the slide valve of the Cascade Impactor to the center of the inhalation chamber. The aerosol sampling flow rate was 4.3 ℓ/min. During room air measurements, samples of ambient air were collected from outside of the inhalation chamber.

A 10-sec (300 μl) sample of aerosol or air for each respective measurement was circulated a minimum of 90 sec within the impactor column. Injections of aerosol or air were drawn into the stack of matched frequency quartz crystal oscillator pairs using the high concentration slide valve. Actual determinations of MMAD for each sample were completed using a graphical procedure; the cumulative normalized percentage of total mass detected was plotted for the particle size range limit of each impactor stage as outlined by Chuan (1986).

5. Respiratory Gases

Oxygen (O_2) and carbon dioxide (CO_2) levels within the inhalation chambers were measured using the Gastec Gas Detection System (Gastec Inc., Newark, CA) -- a standard industrial-hygiene-type analyzer tube and pump system (see Section D of this Appendix). Oxygen and CO_2 were measured using Gastec Analyzer Tubes +31 (% O_2) and 2LL (ppm CO_2), respectively. Sampling was conducted between 20 and 60 min following ignition of RP/BR or start of a filtered-air exposure. Respective tubes were inserted into the chamber and the Gastec Pump extended using a port on the right-front base of the chamber. Respiratory gas readings were determined by noting the farthest migration of dye along the graduated marking on the side of each analyzer tube. Actual percent O_2 and ppm CO_2 were corrected for atmospheric pressure at 1646 m (5400 ft) elevation based upon the following formula:

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

6. Contaminant Gases

Determination of the amounts of CO, phosphine (PH_3), and hexane (C_6H_{14})were performed using identical procedures as those described for respiratory gases. Gastec Analyzer Tubes 1LL (CO), 7L (PH_3), and 102L (C_6H_{14})were used (see Section D of this Appendix). Few contaminant readings were taken for filtered-air exposures. Data were again corrected for atmospheric pressure at 1646 m (5400 ft) elevation using the formula cited for respiratory gases.

7. Temperature/Humidity

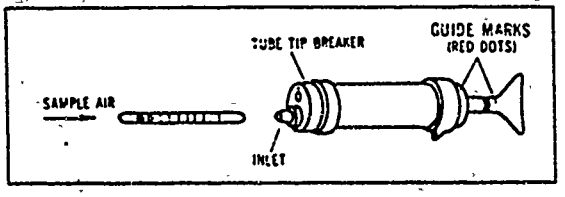
In-chamber temperatures were recorded at successive 20-min intervals throughout each exposure from a VWR Digital Thermometer (Van Waters and Rogers, Denver, CO). The thermometer was permanently mounted into a port at the top right-front side of each chamber.

A special RH-recording port in the main air exhaust line exiting the inhalation chambers permitted assessment of in-chamber RH (see Figures B1 and B2). A standard wet-/dry-bulb thermometer was inserted into the port for approximately 5 min near the end of each RP/BR burn or filtered-air exposure, and the RH was then determined using standard charts corrected for altitude (Department of Commerce, 1965).

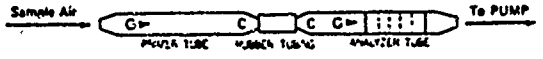
D. Operating Guide for the Gastec Analyzer Pump and Specifications of O_2, CO_2, CO, PH_3 and C_6H_{14} Analyzer Tubes. (We thank Sensidyne, Inc., the U.S. distributor for Gastec Corp., for permission to print these instructions/specifications.)

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 atomospheric pressure.

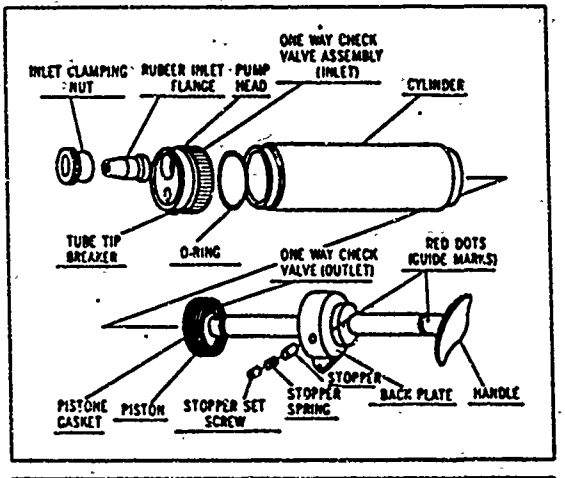
1. No correction is normally required for tube temperatures of 0°–40°C (32°–104°F) and for relative humidity range of 20–90%.
2. Where detecting reagent is abnormally sensitive to temperature or humidity, correction table or chart is provided in each box of tubes. In this case, tube reading must be corrected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

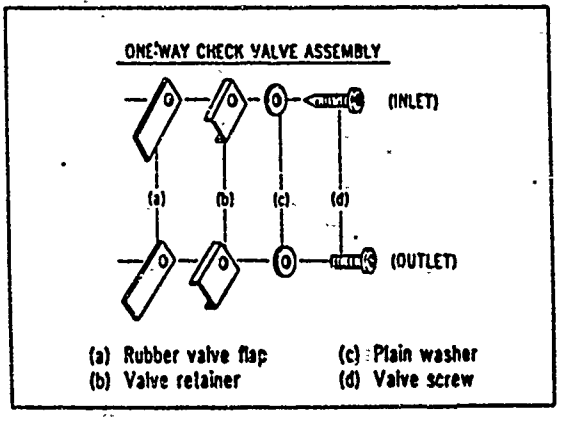
$$\frac{760}{\text{Atmospheric Pressure (mmHg)}}$$

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.





CHECKING PUMP PERFORMANCE

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

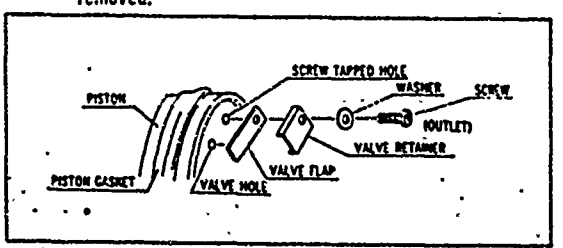
B. Valve Leak Check

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.

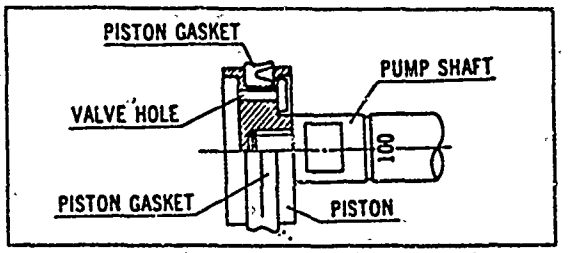
C. Field Volume Check
1. Insert a fresh sealed detector tube into pump.
2. Align red dots on pump body and handle.
3. Pull handle firmly and at a moderate speed until handle locks into position. Wait 1 minute.
4. Unlock handle by turning it and guide it back.
TO PROTECT PUMP STOPPER from breakage, do 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 tolerance 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—11,500ppm
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 3,000 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
86E-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 Change	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 1pump 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 OR PRESSURE:

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 non-dispersive infared adsorption (NDIR) or gas chromatographic technique.

DETECTION PRINCIPLE:

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

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

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
86K-1LL-S

Printed in Japan

GASTEC

PHOSPHINE LOW RANGE DETECTOR TUBE NO. 7L

The Gastec Detector Tube No. 7L provides a rapid, fully quantitative analysis of the concentration of PHOSPHINE in air with a minimum accuracy of ±25% at 1, 2 and 5 times TLV or ±35% at 1/2 TLV utilizing the Gastec Multi-Stroke Gas Sampling Pump.

PERFORMANCE:

Calibration Scale	0.3—5 ppm (based on 5 pump strokes)	
Color Change	Dull Yellow—Purple	
Shelf Life	3 years	
Measuring Range	0.15—2.5 ppm	0.3—5 ppm
Detecting Limit *	0.04 ppm	0.08 ppm
Pump Strokes	10	5
Sampling Time	1 minute per pump stroke	

* The minimum detectable concentration

MEASURING 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 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.
5. Repeat this sampling procedure four (4) more times without removing the tube. For repeated pump stroke sampling, the handle must be 1/4 turn in either direction to unlock the pump so the handle can be returned to the starting position.
6. Read concentration at the interface of the stained-to-unstained reagent when staining stops after completion of 5 pump stroke (500 ml) sampling.
7. If the discoloration is before the first calibration mark (0.3 ppm), repeat the above sampling procedure five (5) more times without removing the tube. Obtain true concentration by dividing the tube reading by 2.

CORRECTION FOR TEMPERATURE HUUIDITY OR PRESSURE:

Calibration of the Gastec detector tube No. 7L is based on a tube temperature of 20℃ (68℉) and not the temperature of the gas being sampled, approximately 50% relative humidity, and normal atmospheric pressure. No correction is required for tube temperature of 0°—40℃ (32°—104℉) 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. 7L is carefully calibrated as an integral part of the munufacturing process. Calibration and accuracy test are performed using combination of dynamic dulution board system and gas chromatographic technique.

DETECTION PRINCIPLE:

PH_3 + Gold compound ⟶ Colloidal gold

DANGEROUS AND HAZARDOUS PROPERTIES:

Threshold Limit Value-Time Weighted Average by ACGIH (1984): 0.3 ppm (7—8 hours)

Threshold Limit Value-Short Term Exposure Limit by ACGIH (1984): 1 ppm (15 minutes)

INTERFERENCES:

Interferent	Concentration	Result	Comment
Arsine		Plus error	Produce similar stain by it self
Hydrogen Chloride		〃	〃
Hydrogen Selenide		〃	〃
Hydrogen Sulfide		〃	〃
Mercaptans		〃	〃

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

Manufacturer: Gastec Corporation, Yokohama, Japan
85A-7L-2

Printed in Japan

• GASTEC

N-HEXANE LOW RANGE DETECTOR TUBE NO. 102L

The Gastec Detector Tube No. 102L provices a rapid, fully quantitative analysis of the concentration of n-HEXANE in air with a minimum accuracy of ±25% at 1, 2, and 5 times TLV or ±35% at 1/2 TLV utilizing the Gastec Multi-Stroke Gas Sampling Pump.

PERFORMANCE:

Calibration Scale	50—1200 ppm (based on 1 pump stroke)		
Measuring Range	10—50 ppm	50—1200 ppm	1200—2640 ppm
Number of Pump Stroke	5	1	1/2
Correction Factor	Tube reading ÷ 5	Tube reading × 1	Tube Reading × 2.2
Detecting Limit*	1 ppm	—	—
Sampling Time	2 minutes per pump stroke		
Color Change	Orange—Brownish Green		
Shelf Life	3 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 towards the pump.
3. Make certain the pump handle is all the way in. Align the 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.
5. Read concentration at the interface of the stained-to-unstained reagent. If the stain produces channeling, take the stain length of farthest extended and least extended along the tube's longitudinal axis and read the concentration at the mean value.
6. When the measuring concentration is over the 1200 ppm, or if the stain length extends over the highest calibration mark by 1 pump stroke sampling, use 1/2 stroke sampling (50 ml), in which case the true concentration is obtained by multiplying the tube reading by 2.2.
7. For more accurate measurement of such a lower concentration as less than 50 ppm use 5 pump stroke sampling, in this case the true concentration is obtained by dividing the tube reading by 5.
8. To unlock the pump, turn the handle by making 1/4 turn in either direction.

CORRECTION FOR TEMPERATURE, HUMIDITY AND PRESSURE:

Calibration of the Gastec Detector Tube No. 102L 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 temperature correction is required for tube temperatures of 0°—40°C (32°—104°F). Moisture in the sample is controlled in the prelayer, therefore, does not affect accurate tube readings. Tube reading is proportional to absolute pressure. To correct the tube reading for pressure, multiply by

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

CALIBRATION AND ACCURACY:

The Gastec detector tube No. 102L is carefully calibrated as an integral part of the manufacturing process. Calibration and accuracy test are performed using combinations of dynamic diffusion tube method and gas chromatographic technique.

DETECTION PRINCIPLE:

n-Hexane reduces potassium dichromate to form chromic sulfate, which is green in color.

$$C_6H_{14} + K_2Cr_2O_7 + H_2O \longrightarrow Cr_2(SO_4)_3$$

INTERFERENCES:

Interferent	Concentration	Result	Comment
Other organic vapors except halogenated hydrocarbons			Produce similar stain by themselves.

DANGEROUS AND HAZARDOUS PROPERTIES:

Threshold Limit Value-Time Weighted Average (TLV-TWA) by ACGIH (1986): 50 ppm (7—8 hours)
Flammable Limit: 1.2—7.5%

APPLICATION FOR OTHER GASES:

The detector tube No. 102L can also be used for the measurement of tert-Butyl Alcohol in air. Concentration of the substance can be obtained from the table below:

tert-Butyl Alcohol $(CH_3)_3COH$

Tube Reading 102L	50	100	200	400	600	800	1000	1200
$(CH_3)_3COH$ ppm 2 pump strokes	1000	1700	2700	4100	5400	6600	7700	8900

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

Manufacturer: Gastec Corporation, Yokohama, Japan
85G-102L-5
Printed in Japan

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Appendix C. Procedural Description and Validation Data for Estimates of the RP/BR-aerosol Concentration Characterizing the Steady-state Concentration Period (i.e., Min 21-60 inclusive) of the 80-min Exposures in Tasks 2 and 3.

This appendix explains the approach used to obtain maximum, steady-state estimates of RP/BR-aerosol concentration during the Asymptotic-concentration Period (i.e., Min 21-60 inclusive) of the 80-min exposures comprising Tasks 2 and 3. The estimation procedure was necessitated by: (1) the inadvertent omission of a separate RP/BR-aerosol-collection filter during the Period and (2) the intent to provide readers with an approximation of the "actual target concentration" attained during Task 2 and 3 exposures of animals.

Remember that animals in Task 2 and 3 were typically exposed to RP/BR aerosol for approximately 80 consecutive min. Each exposure also involved a 3-phase cycle of RP/BR-aerosol concentration: (1) a 20-min Chamber-fill Period, (2) a 40-min Steady-state Concentration Period and (3) a 20-min Chamber-vent Period.

Procedures.-- Precise tracings of the chart recording obtained with the ORNL Infrared Detector were made for a representative number of the RP/BR burns comprising each study in Tasks 2 and 3 using a Lasico Planimeter (Model 120; Los Angeles Scientific Instrument Co., Los Angeles, CA). Each chart was divided into 3 segments corresponding to the 20-min Chamber-fill, 40-min Steady-state Concentration and 20-min Chamber-vent Periods. Vertical lines were drawn on each chart to demarcate the portions of each chart corresponding to these Periods -- at points corresponding to 20 and 60 min after ignition of RP/BR. The lines were constructed perpendicular to the "zero baseline" and extended to the intersection with the ORNL Infrared Detector Chart Record. Next, 2 separate planimeter tracings of (1) the total area beneath the ORNL infrared recording and (2) the area corresponding to the Steady-state Concentration Period were obtained.

To compute the "average asymptotic concentration," the proportion of the 2 planimeter measurements was calculated (i.e., planimeter area for a variable 36 to 40 min portion of the 21-60 min chart record ÷ planimeter area traced for Min 0-80). This proportion was then multiplied times the "total aerosol mass" collected for the respective RP/BR burn; the resultant number represented the amount of aerosol mass associated with the Steady-state Concentration Period. Finally, this aerosol mass value was divided by 36-40 ℓ (i.e., aerosol mass collected at a rate of 1 ℓ/min throughout Tasks 2 and 3) to determine the Steady-state Concentration Estimate (mg/ℓ).

Consider the following example:

> A RP/BR burn was conducted to yield a target concentration of 4.0 mg/ℓ. The aerosol mass collected for 80 min was .2729 mg. Planimeter measurements for the total 80 min ORNL Infrared Detector Chart Record and the Steady-state Concentration Period were 4079 and 2424 units, respectively. The appropriate calculation of the Steady-state Concentration Estimate is:
>
> $$((2424 \text{ units} + 4079 \text{ units}) \times 2729 \text{ mg}) \div 36 \ \ell = 4.5 \text{ mg}/\ell.$$

Validation.-- To validate the Steady-state Concentration Estimate, data from the temporal-homogeneity portion of Task 1 (Sterner, Shumake, Johns, and Thompson, 1988) were used. Remember that RP/BR burns in Task 1 involved 60 min aerosol mass collections. Also, 3, 10-min aerosol mass filter collections were obtained from near the center of chamber during the 20-30, 35-45 and 50-60 min intervals after RP/BR ignition; and, 8 burns each were conducted at target concentrations of 0.4, 1.5 and 3.0 mg/ℓ and 3.0, 4.5 and 6.0 mg/ℓ RP/BR aerosol mass at a 500 and 250 ℓ/min air flow rate. For current purposes, 5 representative burns at each of the 3.0, 4.5 and 6.0 mg/ℓ target concentrations with a 250 ℓ/min air flow rate were selected for planimeter measurements.

Tracings were made of each ORNL Infrared Detector Chart Record from the 15 selected RP/BR burns using the Lasico Planimeter. Perpendicular lines were constructed on each chart between the "zero baseline" and the intersection with the ORNL infrared chart function at a point corresponding to 20 and 60 min after RP/BR ignition. A planimeter measurement of the area subsumed beneath the ORNL infrared recording for this 40 min time period (i.e., Steady-state Concentration Period) and the total 60 min chart record was determined. The proportion of these 2 areas was obtained, and multiplied times the cumulative aerosol mass of the 3 consecutive 10-min temporal-homogeneity filter collections. This value, in turn, was divided by 40 ℓ (i.e., sampling volume). (Note.-- Although a more accurate measure would have been obtained by making separate planimeter measurements of the areas beneath the 3, 10-min segments corresponding to the actual times of filter pad collections, the stability of most infrared charts essentially equated with the addition of 2, 5-min constant areas to each estimate.)

Final validation involved computation of a linear regression between (1) the aerosol mass concentration estimates (mg/ℓ) for the Steady-state Concentration Periods of the 15 selected Task 1 burns and (2) the mean of the 3, 10-min aerosol mass concentration filter collections (mg/ℓ) obtained for these burns. Table C1 is a presentation of the actual data and variance table obtained from the statistical analysis of the 2 aforementioned variables. Figure C1 is a graphical plot of the linear regression. As shown, the linear regression accounted for a significant amount of variance in the score space ($F = 592.942$, $df = 1/13$, $p < 0.0001$). The adjusted coefficient of determination (Adj. R-sq) was 0.9769 -- indicating that practically all of the variation in the dependent variable (aerosol mass, planimeter-based estimate) is explained by the actual mean aerosol mass values for the 3, 10-min filter collections.

Table C1. Actual validation data for the Steady-state Concentration Estimation Procedure selected from Sterner et al. (1988), plus results of the linear regression analysis for these data.

Burn	Planimeter-based aerosol mass concentration (mg/ℓ)	Actual mean aerosol mass of 3, 10-min filter collections (mg/ℓ)
1	2.92	2.88
2	3.15	2.98
3	3.33	3.33
4	3.22	3.39
5	2.96	3.20
6	4.32	4.42
7	4.43	4.23
8	4.79	4.61
9	4.41	4.05
10	4.42	4.26
11	6.00	5.94
12	6.15	6.32
13	6.00	5.73
14	5.69	5.68
15	6.36	6.03

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	19.76271	19.76271	592.942	0.0001
Error	13	0.43329	0.03333		
C Total	14	20.19600			

Root MSE	0.18256	R-square	0.9785
Dep Mean	4.47000	Adj R-sq	0.9769
C.V.	4.08422		

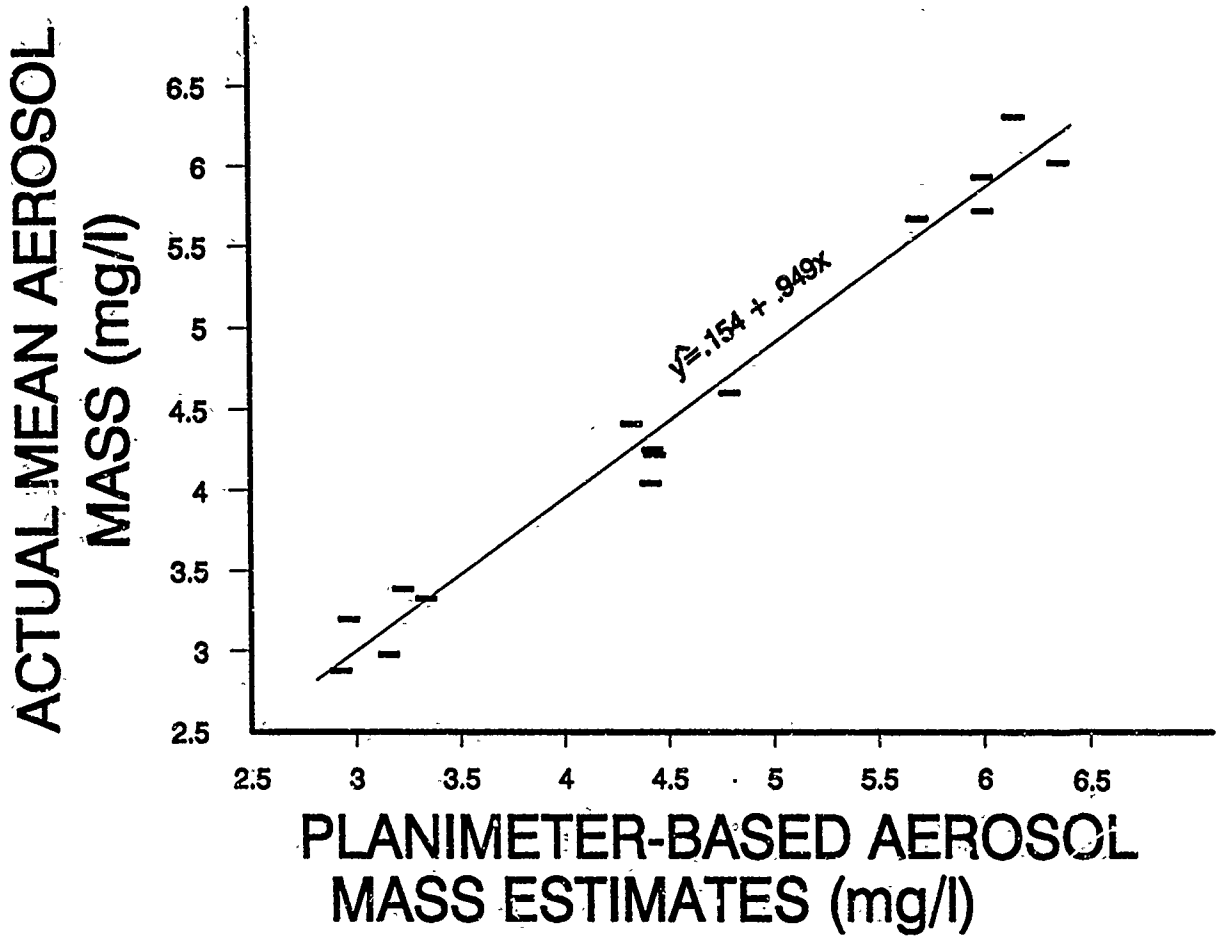

Figure C1. Scatterplot and linear regression of the steady-state estimates of RP/BR-aerosol concentrations derived for 15 representative burns reported by Sterner et al. (1988). The regression reflects the relationship between mean aerosol mass concentrations (mg/ℓ) and planimeter-based aerosol mass estimates of asymptotic concentration for 20-60 min portions of burns conducted to assess temporal homogeneity of chamber conditions.

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Sterner, R.T., S.A. Shumake, B.E. Johns, and R.D. Thompson. 1988. Behavioral-physiological Effects of Red Phosphorus Smoke Inhalation on Two Wildlife Species. Task 1 Report. (Inhalation Equipment Development/Ambient CO Evaluation/Aerosol Distribution and Air Quality Study). Project Order No. 85PP5847. (AD-A196753). USDA/APHIS/S&T, Denver Wildlife Research Center: Denver, CO. 75 pp.

Appendix D. Subsidiary Report of Body Weight, Food Consumption, and Water Consumption Collected during the Spontaneous Activity Studies.

Body weight, food intake, and water intake are used as clinical indices of toxicosis (e.g., Annau, 1972; Benitz, 1970; Shumake, Sterner, Johns, and Thompson, 1989). As part of the Spontaneous Activity Studies, auxiliary measurements of these consummatory and body weight variables were collected to confirm and extend results of Shumake et al. (1989).

Two prior reports involving clinical signs (i.e., body weight and water consumption) of RP/BR-aerosol exposure merit note. Aranyi (1983b) exposed numerous groups of male and female laboratory rats daily to 0.5 mg/ℓ RP/BR aerosol for 1 and 3.5 h during 4 weekly bouts of 4 consecutive days. Weight loss typically ranged between 1 and 3 percent, with depressed weight evident until 39 days of post-exposure recovery. Shumake et al. (1989) also noted depressed body weights in both black-tailed prairie dogs and rock doves using diverse exposure schedules. Prairie dogs showed 1-2 days of suppressed weight after 1-4 successive 80-min daily exposures to 6.0 mg/ℓ RP/BR aerosol; whereas, rock doves showed depressed weight relative to controls for 22 days post exposure when administered 2 successive 1 h daily exposures to 6.0 mg/ℓ aerosol. Water consumption in these animals typically increased during the latter part of a 28-day, post-exposure period -- Days 10-28 (prairie dogs) and Days 7-25 (rock doves) following the aforementioned RP/BR-aerosol shedule. No food consumption data were reported by either Aranyi (1983b) or Shumake et al. (1989). Clinical indices of body weight, food consumption and water consumption were collected as part of the Spontaneous Activity Studies with black-tailed prairie dogs and rock doves to verify and extend data on clinical signs (i.e., Shumake et al., 1989) associated with RP/BR-aerosol inhalation in these species.

Relatively greater intake of water and food, plus increased body weight, were hypothesized for the 4.0 mg/ℓ RP/BR-aerosol Group immediately following chamber confinement (≤3 h out-of-chamber) in prairie dogs. These immediate data were not collected for rock doves. It was posited that the relatively greater stress associated with the 4.0 mg/ℓ aerosol concentration would produce greater urination and defecation during handling/confinement of chamber exposures with a subsequently greater drive for nutrient and fluid replenishment during the 2-h immediately out-of-chamber period.

It was also hypothesized that 4- and 2-successive, 80-min exposures to approximately 4.0 mg/ℓ concentrations of RP/BR aerosol (relative to equivalent exposures to 0.0 and 1.0 mg/ℓ aerosol) should cause <u>acute (≤ 6 days post exposure)</u> decrements in the body weight, and food intake of both species. Based on Shumake et al. (1989), no acute shifts in water intake of these species were expected during 6-days of post-exposure measurements; Shumake et al. reported increased water intake only 10-28 days after these species were exposed to 6.0 mg/ℓ of RP/BR-aerosol. All effects were predicted to occur and recover rapidly (≤ 6 days after exposure) due to the sub-lethal exposures involved.

A. Effects in Black-tailed Prairie Dogs

1. Methods

All prairie dogs, group assignments, housing conditions, and procedures were the same as those described for the Spontaneous Activity Study with prairie dogs (See IV. Studies of RP/BR-aerosol Effects Upon Spontaneous Activity in Black-tailed Prairie Dogs and Rock Doves).

2. Experimental Designs and Data Analyses

Evaluations of the potential immediate and acute effects of RP/BR-aerosol exposure upon body weight, food consumption, and water consumption involved a total of 6 separate ANOVAs. Where missing data occurred, these ANOVAs, were computed using the General Linear Model (i.e., PROC GLM Program and Type III sums of squares; SAS Institute, Inc. 1985); otherwise balanced data sets were analyzed using the PROC ANOVA Program (SAS Institute, Inc., 1985). Throughout all ANOVAS, significant terms were further assessed using post-hoc Duncan Multiple Range Tests (Waller and Duncan, 1969); and, the 0.05 level of significance was used to test all ANOVA terms and Duncan Comparisons.*

a. Immediate Effects

Three ANOVAs were computed to assess immediate consequences of RP/BR exposure and chamber confinement upon clinical indices in prairie dogs. Change in body weight (g), amount of food consumed (g), and amount of water consumed (mℓ) for the 2-h period immediately following respective chamber confinements were analyzed separately to assess relative differences among the 0.0, 1.0, and 4.0 mg/ℓ RP/BR-aerosol Concentration Groups during the Exposure Phase. Each of these variables were analyzed as balanced 3 (RP/BR-aerosol Concentration) X 2 (Sex) X 4 (Day) ANOVAs, where Day was a repeated measures factor (Winer, 1971).

b. Acute Effects

Three ANOVAs were computed to assess acute effects of RP/BR-aerosol exposure upon clinical indices in prairie dogs. Body weight (g), food intake (g), and water intake (mℓ) were analyzed as 3 (RP/BR-aerosol Concentration) X 2 (Sex) X 12 (Day) factorial ANOVAs, with Day treated as a repeated measures factor (Winer, 1979). The total daily body weight, food intake and water intake measurements of animals were considered to have ample time to compensate for the approximately 2.5 h deprivation associated with inhalation-chamber confinement. Thus, these analyses were believed to reflect RP/BR-caused differences over the course of the Study.

*See footnote on Page 27.

3. Results and Discussion

a. Immediate Effects

Results of the ANOVAs to assess the "immediate, 2-h post-exposure effects" associated with the 4 successive chamber confinements yielded a rather consistent pattern of results for the 3 clinical variables (i.e., change in body weight, food intake, and water intake). Significant Concentration and Sex main effects characterized these data.

Figure D1 presents bar graphs of the Concentration main effects for weight change ($F = 8.53$, $df = 2/18$, $p < 0.0025$), food consumption ($F = 5.33$, $df = 2/18$, $p < 0.015$), and water consumption ($F = 12.01$, $df = 2/18$, $p < 0.0027$) during the 2-h, post-exposure periods of the Exposure Phase for prairie dogs. As shown, the 3 variables reflect a highly similar pattern of means for these Concentration effects. Post-hoc Duncan Range Tests confirmed that the Filtered-air Group mean weight change, food, and water intake was significantly greater than these respective variables for the 1.0 and 4.0 mg/ℓ RP/BR-aerosol Groups, but that the means of the respective variables for the RP/BR-aerosol groups were not different from each other.

The Sex main effects for these 3 clinical signs were all significant: change in body weight ($F = 9.67$, $df = 1/18$, $p \leq 0.003$), food intake ($F = 12.01$, $df = 1/18$, $p \leq 0.001$), and water intake ($F = 9.73$, $df = 1/18$, $p \leq 0.0029$). Results indicated that female prairie dogs gained significantly more weight, as well as displayed greater food and water consumption during these 2-h post-exposure periods than male prairie dogs. Mean weight change, food intake, and water intake was 4.8 versus -3.6 g (loss), 5.2 versus 3.4 g, and 20.8 versus 11.8 mℓ, respectively, for females compared to males.

Together, these findings demonstrate that exposure to RP/BR-aerosol causes dramatic short-term suppression of ingestive behaviors plus concomitant weight loss in prairie dogs, with males more affected than females. Confinement of the animals within the inhalation chambers was obviously stressful -- prairie dogs urinated, defecated, barked, and bit repeatedly during the chamber loading and unloading procedures. This excitement, coupled with the unavailability of food and water during the approximately 2 h loading/confinement/unloading schedules may have depleted nutrient and fluid reserves of the animals. If unaffected by the exposure(s), the prairie dogs would have been expected to quickly replenish nutrients and fluids once food and water became available. This is exactly how the control animals (0.0 mg/ℓ Group) behaved. The RP/BR-aerosol Groups, on the other hand, refrained from eating and drinking during the 2-h post-exposure periods. This caused added weight loss even after RP/BR-aerosol inhalation for these Groups.

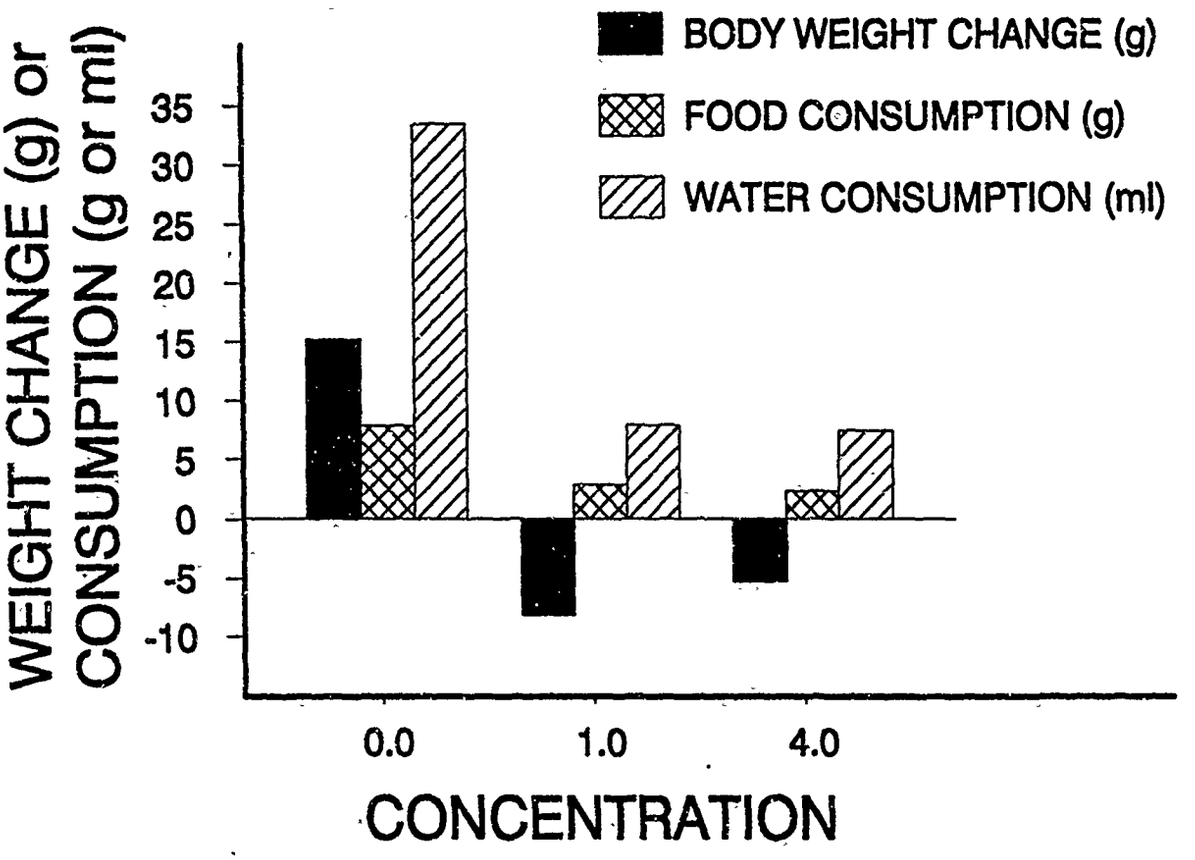

Figure D1. Bar graphs of the mean weight changes, food consumption, and water consumptions for prairie dogs in the 0.0 (filtered-air), 1.0, and 4.0 mg/ℓ RP/BR-aerosol Groups during the 2 h after chamber confinements across the 4 Exposure Days (E-1 to E-4).

The somewhat enhanced recovery of ingestive behaviors and weight by female prairie dogs immediately following chamber confinement is difficult to explain. Females showed roughly twofold larger food and water consumptions, plus threefold larger weight gain, immediately after confinements relative to males. This implies inherent differences in either stress tolerance or recovery potential by females. Of course, a myriad of uncontrolled factors (e.g., hormonal, genetic, morphological) could have produced these results, and no unequivocal interpretation is possible. Still, these effects are intriguing. Sex differences have been a recurrent theme in prior studies of RP/BR-aerosol exposure with animals. Aranyi (1983b) reported less body weight loss among groups of female laboratory rats (relative to males) given 5 daily, 1-h exposures of between 1.6 and 3.0 mg/ℓ RP/BR-aerosol concentrations. Similarly, Shumake et al. (1989) found significantly greater weight loss (0-7 days post-exposure) and less water consumption for male than female rock doves several weeks (i.e., 7 to 25 days) after multiple exposures to between 1 and 3 80-min sessions of 3.0 or 6.0 mg/ℓ RP/BR aerosol, with enhanced survivability by female doves. Overall mortality ratios of 1:21 versus 10:27 for female versus male doves, respectively, were reported in Task 2. What does this pattern of sex-related differences mean? I contend that the consistency of the greater recover by females reflect important physiological/biological tolerance differences to either RP/BR aerosol or, possibly, acid aerosols in general. Future studies should address potential hormonal or genetic factors related to these sex differences.

b. Acute Effects

The ANOVAs for the 23-h body weight (g), food consumption (g), and water consumption (mℓ) variables across the 3 Phases of the design yielded a highly consistent pattern of Sex and Day main effects for prairie dogs.

Analyses of the 23-h body weight data yielded 2 significant main effects: Sex ($F = 14.46$, $df = 1/18$, $p < 0.0013$) and Day ($F = 5.28$, $df = 11/198$, $p \leq 0.0001$). The Day main effect was the only significant effect obtained for the 23-h food intake ($F = 35.54$, $df = 11/198$, $p \leq 0.0001$) and water intake ($F = 12.89$, $df = 11/198$, $p \leq 0.001$) variables.

Regarding the Sex main effect, the male prairie dogs weighed 12.4 percent more than the females (i.e., means of 1252 vs 1098 g, respectively) across the 12 days of the Spontaneous Activity Study. The result simply confirmed that the males weighted more than the female prairie dogs and this mean weight difference was maintained across the 3 Phases of the Study.

Figure D2 is a graph of the Day main effects for mean body weight, food consumption, and water consumption of the 24 prairie dogs across the 12 days of the Study. Post-hoc Duncan Range

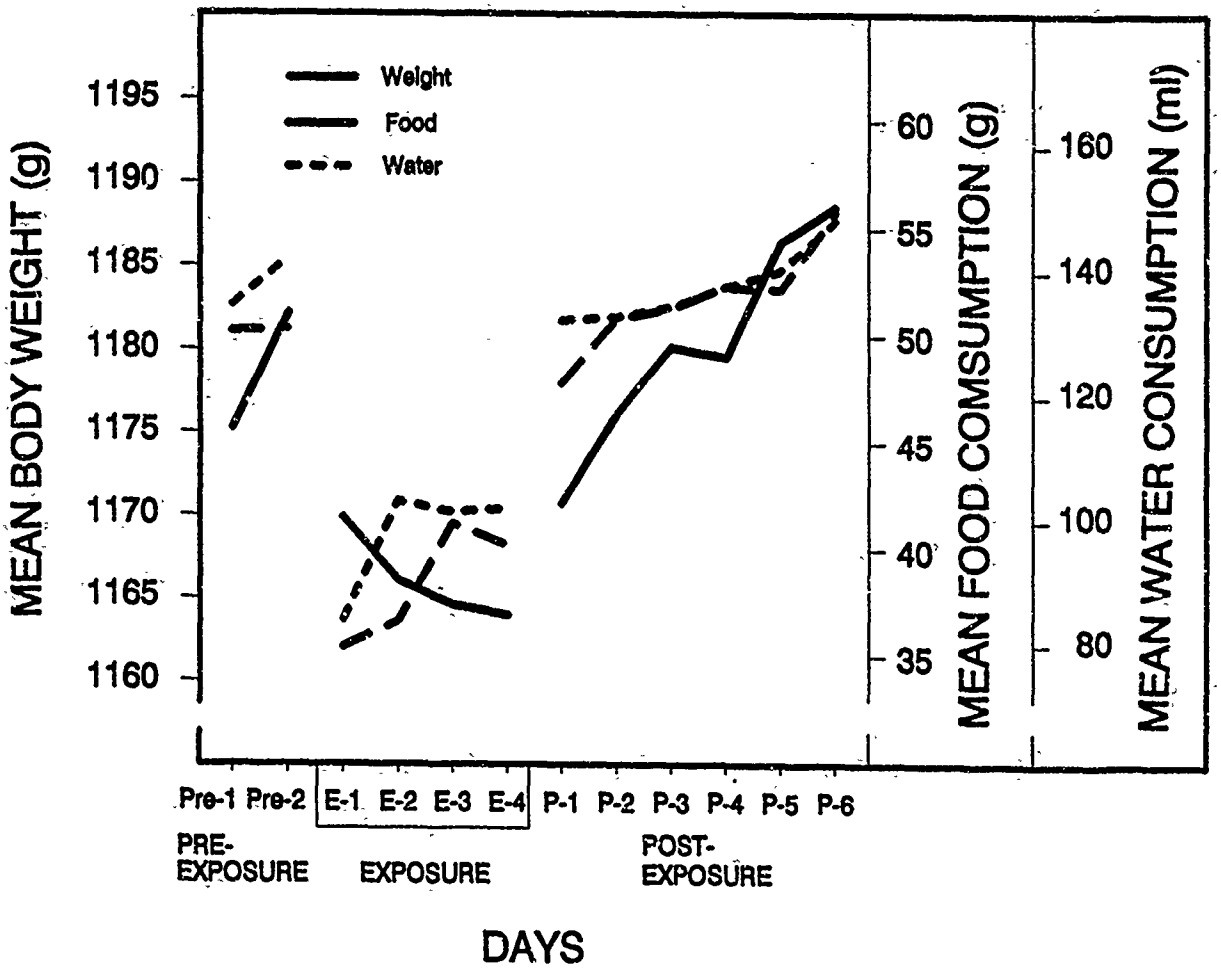

Figure D2. Graph of the mean daily body weight, food consumption, and water consumption for all prairie dogs (n = 24) across the 12 days of the Spontaneous Activity Study -- the Day main effects for these variables.

Tests for each variable showed that respective measurements for either all Exposure Days (E-1, E-2, E-3, and E-4), or some combination of these Days, were significantly less than most Pre- and Post-exposure Days. Specifically, comparisons among body weight means revealed that the animals: (a) gained weight between Pre-1 and Pre-2 of the Pre-exposure Phase, (b) had significantly reduced weight relative to Pre-2 during all of the Exposure Days (E-1, E-2, E-3, and E-4) and the first half of the Post-exposure Days (P-1, P-2, and P-3), and (c) started to gain weight relative to Pre-2 during the latter half of the Post-exposure Phase. Essentially, means for the animals' food and water intake reflected this same pattern; however, recovery of Pre-exposure consumption levels for food and water was accomplished by Day P-1 (i.e., only Exposure Day intakes were suppressed).

These data confirm the acute nature of the Day effects for these clinical variables, with recovery of Pre-exposure intakes and weights complete within 0 and 4 days after the Exposure Phase, respectively. The occurrence of significant Day main effects for the 23-h consummatory and weight variables is indicative of "handling/chamber-confinement stress," not "RP/BR-aerosol stress." In the absence of RP/BR-aerosol Group differences (i.e., Concentration X Day interaction), these Day terms must be due to factors experienced by all animals.

Regarding prior evidence, Shumake et al. (1989) did not analyze Exposure Phase data; these authors compared Pre- versus Post-exposure water intake and weight data of prairie dogs. A number of significant interaction effects involving concentration group, exposure treatment, and day factors were found in the Toxicity Range-finding Studies that are not present in this Study. Still, there are no discrepancies. The interactions involving RP/BR-aerosol Concentration upon water consumption in Shumake et al. (1989) occurred during the latter part of the Post-exposure Phase, with prairie dogs exposed to 6.0 mg/ℓ aerosol showing increased water intake between Days 10 and 28. Similar to current findings, no shorter-term effects of RP/BR-aerosol exposure were noted. This implies that inhalation of high aerosol concentrations of multiple sessions causes longer-term acute differences in water intake (≥ 10 days), but has no overt short-term acute consequence. These results are still compatible with a model that postulates delayed increases in water consumption as a result of acid-caused edema (i.e., fluid loss).

B. Effects in Rock Doves

1. Methods

All doves, group assignments, housing conditions, and procedures were the same as those described for the Spontaneous Activity Study with prairie dogs (see IV. Studies of RP/BR-aerosol Effects upon Spontaneous Activity in Black-tailed Prairie Dogs and Rock Doves).

2. Experimental Designs and Data Analyses

a. Immediate Effects

No immediate food, water, or weight measurements were collected in this Study.

b. Acute Effects

Three PROC GLM ANOVA's and Type III sums of squares (SAS Institute, Inc., 1985) were computed to assess acute effects of RP/BR-aerosol exposure upon clinical indices in rock doves.* Daily body weight (g), food intake (g), and water intake (mℓ) variables were analyzed as 3 (Concentration) X 2 (Sex) X 10 (Day) factorial ANOVAs, where Day was a repeated measures factor (Winer, 1971). As for prairie dogs, the birds were viewed to have ample time to compensate daily body weight, food intake, and water intake throughout all phases of the Study (i.e., including Exposure Days).

2. Results and Discussion

a. Acute Effects

As stated, PROC GLM ANOVAs for body weight, food intake, and water intake variables yielded significant Day main effects (i.e., $F = 2.53$, $df = 9/159$, $p \leq 0.0097$; $F = 9.99$, $df = 9/159$, $p \leq 0.0001$; and $F = 6.46$, $df = 9/159$, $p \leq 0.0001$, respectively). Sex main effects for body weight ($F = 8.72$, $df = 1/18$, $p = \leq 0.0085$), food intake ($F = 9.56$, $df = 1/18$, $p < 0.0063$) and water intake ($F = 7.44$, $df = 1/18$, $p < 0.0138$) were also significant.

Figure D3 is a composite graph of the mean daily food and water intake (plus concomitant weight) for all doves across days of the Study. As was reported for prairie dogs, the "generalized stress effect" of the exposure schedules upon the "body maintenance variables" is evident. Post-hoc Duncan Tests confirmed that food, water and weight of the birds were depressed during both days of the Exposure Phase. Depressed food intake was quickly alleviated on the first day of the Post-exposure Phase (P-1). The acute effect of the chamber-confinement stress on water consumption was not alleviated during the 6 days of Post-exposure (i.e., all Post-exposure Day means less than at least Day Pre-1). Body weight depression was alleviated on Day P-6. Thus, the effects of the chamber-confinement stress had a major clinical impact upon the consummatory and weight loss/recovery of the doves. This, as will become clear, substantiates the toxicity range-finding results of Shumake et al. (1989) for this species; as well as further documents the greater vulnerability of wild doves to inhalation chamber schedules.

*See footnote on Page 27.

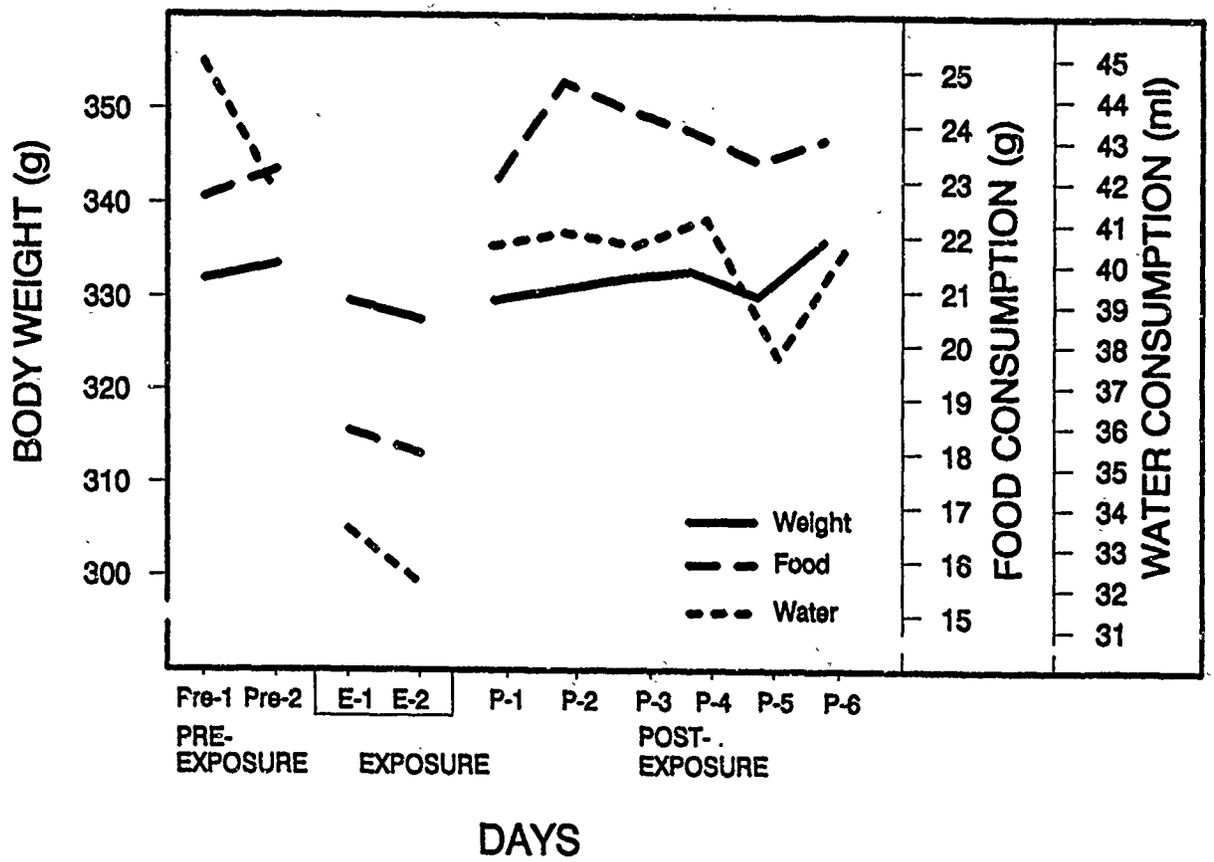

Figure D3. Graph of the mean daily body weight, food consumption, and water consumption for all rock doves (n = 24) across the 10 days of the Spontaneous Activity Study -- the Day main effects for these variables.

The Sex effects were expected, with female doves weighing 8 percent less, eating 20 percent less, and drinking 20 percent less than male doves throughout the 10 day study period.

With these Day and Sex effects presented, now consider the 2 RP/BR-aerosol Group interactions evident in these results. First, Figure D4 is a graph of the significant Concentration X Day interaction found for the food intake variable. Despite the exceptionally high food intakes shown for the 0.0 mg/ℓ (Filtered-air) Group during Pre-exposure Phase (Pre-1 and Pre-2), Duncan Range Tests confirmed that 4.0 mg/ℓ and 0.0 mg/ℓ-exposed doves significantly decreased food intake on the Exposure Days (E-1 and E-2), with the 4.0 mg/ℓ Group cutting food intake approximately 35 percent. Results for the 1.0 mg/ℓ Group were decreased only relative to Day Pre-2 of the Pre-exposure Phase. Recovery of food intake behavior during Post-exposure was rapid and involved some hyperphagia for the 4.0 mg/ℓ RP/BR-aerosol Group. Means comparisons indicated that all Post-exposure Day (P-1 to P-6) means for the 4.0 mg/ℓ Group exceeded Day Pre-1 and Pre-2 values. The 1.0 mg/ℓ Group showed 3 days (Days P-1 to P-3) of hyperphagia, with no differences from Pre-2 throughout the remaining 3 days; whereas, the 0.0 mg/ℓ Group matched its Day Pre-2 food intake on Day P-1, but never exceeded Pre-exposure consumptions.

Obviously, the variability of the Pre-exposure food intakes (particularly the 0.0 mg/ℓ Group consumptions) imposes certain restrictions on possible inferences; no satisfactory explanation can be derived for the high Pre-exposure intakes for the Filtered-air Condition. Still, the Concentration X Day interaction does indicate an inverse type relationship exists for food intake during the Exposure Phase. The 4.0 mg/ℓ-exposed doves showed increased feeding after exposure, possibly in an attempt to replenish lost food stores.

The remaining interaction is the complex Concentration X Day X Sex term noted for water consumption. The 3-way interaction is plotted in Figures D5a and D5b, but very little interpretation is afforded by this graph due to obvious significant variation among groups prior to exposure. Only 3 points are noted. First, high variances for day-to-day water ingestions are the rule, rather than the exception, for this species of birds (see Shumake et at., 1989). Second, the fact that male water intakes were generally greater than those of the females during the Pre-exposure Phase led to numerous crossovers in daily consumption as the sub-groups showed differing magnitudes of hypodipsia during the Exposure Days. Note, in particular, that male doves exposed to 4.0 mg/ℓ aerosol showed a delayed hypodipsic response as compared to other Groups. Third, the male and female sub-groups of doves in the 4.0 mg/ℓ RP/BR-aerosol Group essentially reversed their respective water intake patterns during Days P-4 to P-6 of Post-exposure. That is, these male birds showed decreased and the female doves

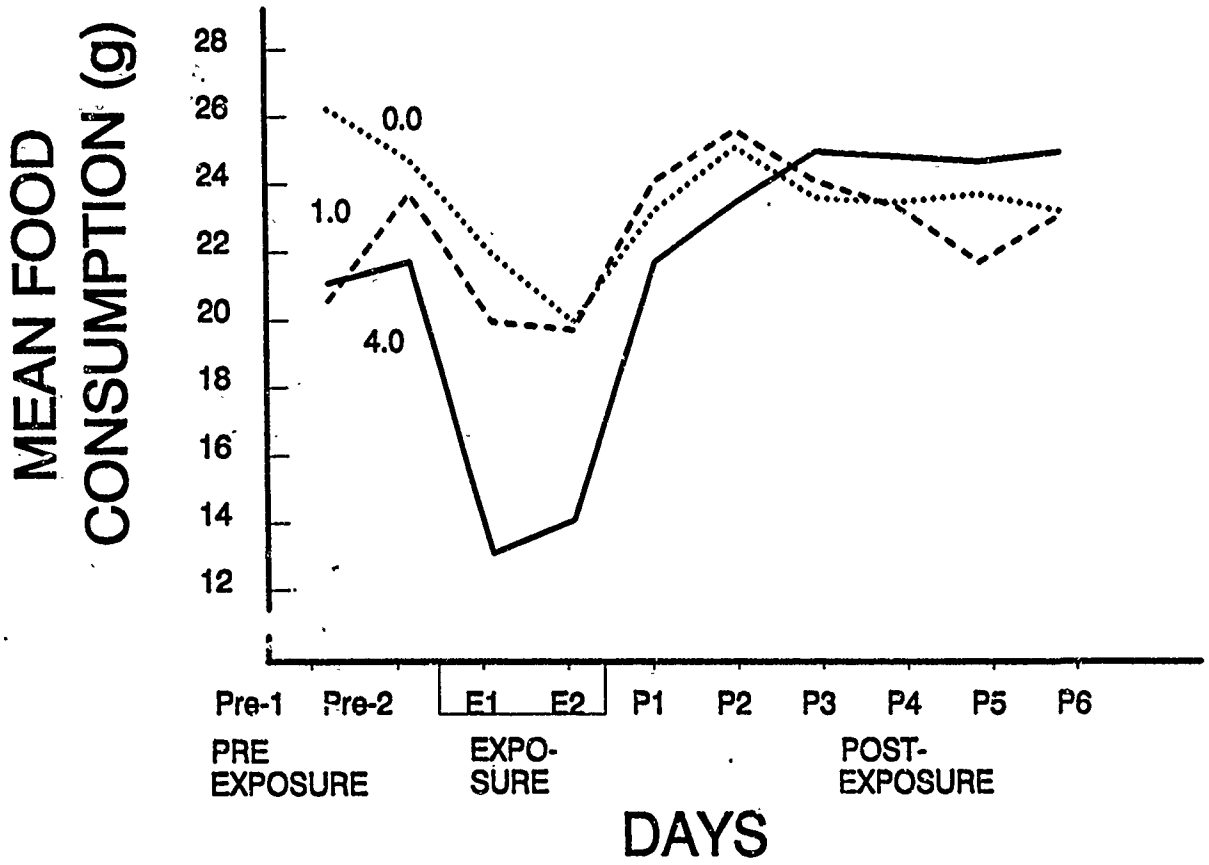

Figure D4. Graph of the mean daily food consumptions for the 3 RP/BR-aerosol Groups of rock doves (0.0, 1.0, and 4.0 mg/ℓ) across the 10 days of the Spontaneous Activity Study -- the Concentration X Day interaction.

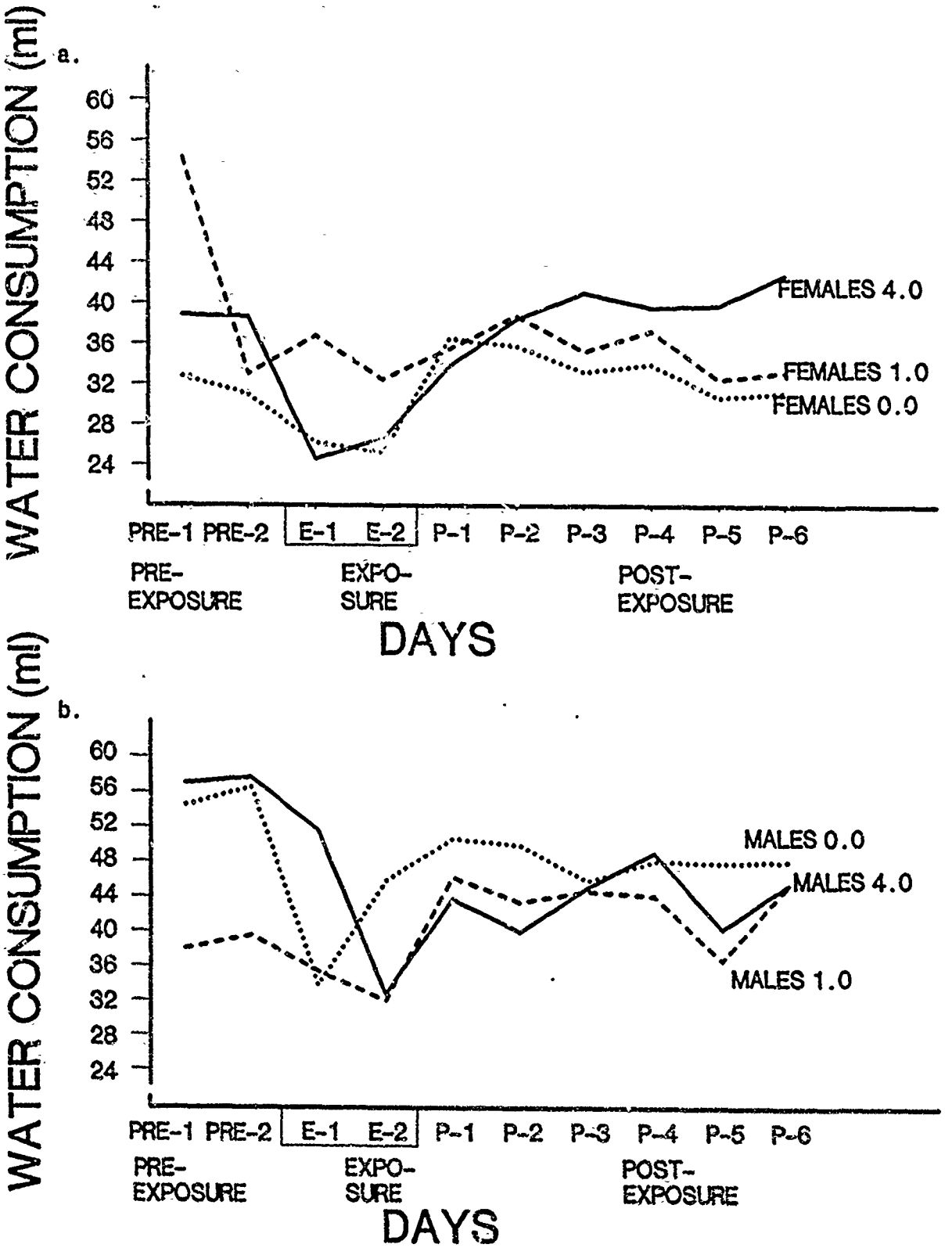

Figure D5a and b. Graph of the mean water consumptions for male (top) and female (bottom) rock doves in each of the 3 RP/BR-aerosol Groups (0.0, 1.0, and 4.0 mg/ℓ) across the 10 days of the Spontaneous Activity Study -- the Concentration X Sex X Day interaction.

showed increased water intakes several days after RP/BR-aerosol inhalation. This again points out the persistent recurrence of enhanced water replenishment among female doves several days after exposure (see Shumake et al., 1989). Although an adequate explanation for this recurrent effect is difficult to provide, this fluid-replenishment difference between male and female birds is a key outcome of the current work. The direct correlation of these male and female hypo-and hyper-dipsic responses to greater and lesser lethality warrant manipulation in other research, if undertaken.

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Appendix E. Tables and Descriptions of RP/BR-aerosol and Filtered-air Measurements Characterizing the Chamber Atmospheres during each of the 8 Behavioral-physiological Studies with Prairie Dogs and Rock Doves (i.e., Exposure Conditions).

Spontaneous Activity Study (Prairie Dogs)

Table E1 presents median and range statistics for the RP/BR-aerosol and air quality measurements used to characterize chamber atmospheres during the Spontaneous Activity Study with prairie dogs. Statistics represent the 12 exposures conducted at each of the 0.0, 1.0, and 4.0 mg/ℓ target concentrations (i.e., 3 replications of 4 successive daily 80-min sessions). Due to the high consistency among particle size measurements in the Filtered-air Chamber during Task 2 (Shumake et al., 1989), and the absence of any anomalous respiratory or detectable contaminant gas readings, limited measurements of the Filtered-air Chamber were collected.

Total aerosol mass (mg) across the 12 RP/BR burns displayed minimum to maximum percent variation (i.e., ((maximum - minimum) ÷ minimum) X 100) of 20 and 36 percent for burns at the 1.0 and 4.0 mg/ℓ target concentrations, respectively. Inspection of these data revealed that the dispersion associated with the 4.0 mg/ℓ burns was due to a single outlier (i.e., Replication 3, Day 4). Without this 248.9 mg value, maximal variation was 24 percent. Considering the large differences in inhaled aerosol for the current exposure concentrations (1.0 and 4.0 mg/ℓ target concentrations) during 4 repeated 80-min exposures, this dispersion was essentially insignificant.

Median aerosol mass concentrations for the 12, 80-min exposures at the 1.0 and 4.0 mg/ℓ targets were 0.76 and 3.46 mg/ℓ, respectively; whereas, estimated median steady-state concentrations for 36-min portions of 8 RP/BR burns at these target concentrations were 1.03 and 4.96 mg/ℓ, respectively.

The H_3PO_4 titration data further confirmed the acceptability of the aerosol-exposure data, with the acid accounting for a median 73 percent of the aerosol mass at each target concentration. Again, similar to Task 2 (Shumake et al., 1989), actual aerosol measurements generally exceeded those reported in Task 1 (Sterner et al., 1988) at equivalent target concentrations; however, this was expected due to the added 20-min filter collection associated with the 80-min exposures used in the animal studies.

Regarding particle size, MMAD values were also in line with earlier results for the RP/BR-aerosol System. Median MMADs were 0.40 μm for burns at both 1.0 and 4.0 mg/ℓ target concentrations. The point here is that MMADs were always found to be < 1 μm and within the respirable range.

Respiratory and contaminant gases within the chambers were acceptable. Oxygen readings consistently yielded 22 percent. Median CO_2 values were between 696 and 847 ppm for these conditions. Although the 847 ppm value is elevated, no CO_2 reading exceeded the clinically troublesome value of 1,000 ppm (i.e., 1%). Interestingly, CO was effectively controlled by the selection of the 1.0 and 4.0 mg/ℓ target concentrations, with median values of 13 and 18 ppm, respectively -- levels well below the 35 ppm EPA standard for a 1-h Short-term Limit Threshold

Table E1. Median (minimum-maximum) statistics of the RP/BR-aerosol and air quality variables characterizing the aerosol and filtered-air exposures conducted at the 0.0 (filtered air), 1.0 , and 4.0 mg/ℓ target concentrations for the Spontaneous Activity Study with prairie dogs in Task 3 [a].

Variable	Target Concentration (extrusion setting) 0.0 mg/ℓ (control)	1.0 mg/ℓ (50 μm)	4.0 mg/ℓ (180 μm)
Aerosol			
Aerosol Mass(mg)	1.9 (-20.0 to 19.2)	62.3 (57.1-68.5)	290.2 (248.9-338.7)
Aerosol Mass Concentration (mg/ℓ)	0.2 (0.00-0.19)	0.76 (0.4-0.86)	3.46 (1.50-4.03)
Steady-State Concentration (mg/ℓ)	-- ND	1.03 (0.95-1.12)	4.96 (4.5-5.5)
H_3PO_4 Titration (mg)		45.06 (40.06-49.82)	211.2 (83.5-238.9)
H_3PO_4 Concentration (mg/ℓ)	ND	0.55 (0.37-0.62)	2.57 (1.04-2.78)
Percent H_3PO_4 of Aerosol Mass	--	73 (60-84)	73 (65-80)
Particle Size			
MMAD (μm) [b]	--	.40 (.40-.40)	.40 (.20-.80)
Respiratory Gases [c]			
O_2 (%)	22 [d]	22 (21-23)	22 (21-22)
CO_2 (ppm)	847 [d]	696 (484-968)	847 (726-968)
CO_2 From Burn and Animal Respirations (ppm) [e]	290 [d]	139 (-72-411)	290 (169-411)
Contaminant Gases [c]			
CO (ppm)	ND [d]	13 (10-22)	18 (15-21)
PH_3 (ppm)	ND [d]	ND	ND
C_6H_{14}(ppm)	ND [d]	ND	ND
Exposure Duration/Chamber Conditions			
Length Of Exposure (min)	86 (80-106)	80.5 (80-86)	81.5 (80-86)
Temperature (C^o)	(21-25)	(20-24)	(21-24)
Relative Humidity (%)	54 (50-63)	59.5 (52-67)	59 (48-82)

[a] Median (minimum-maximum) statistics shown as measures of central tendency and dispersion because of the decreased influence of outliers on the indices ("--" = Not Measured; ND = Not Detected).

[b] Determinations of MMAD were completed using a graphical analysis procedure (Log particle size diameter versus cumulative probability of sampling detections) as outlined by Chuan (1986). No particle size measurements were obtained for the filtered-air (0.0 mg/l) exposures; small volume samples were insufficient to determine MMAD values for filtered-air exposures.

[c] All Gastec analyzer tube values were corrected for 1646 m elevation (i.e., 628 mm Hg) in accordance with the manufacturer's recommendations. The formula for this correction was: $\text{Actual Tube Value} \times \frac{760 \text{ mm Hg}}{628 \text{ mm Hg}}$.

[d] Only a single measurement taken during 1 exposure; datum is only value, not median and range.

[e] A corrected mean of 557 ppm CO_2 was obtained for 10 CO_2 readings made under ambient conditions in Room 158 of DWRC; this was subtracted from the respective within-chamber medians to estimate CO_2 production associated with each extrusion setting/animal confinement.

[f] The EPA Short-term Limit Threshold for CO is 35 ppm maximum for a 1 h average exposure (National Research Council, 1977). Note that all of the CO tube readings (Tube No. 1LL) were ≤ 22 ppm.

(National Research Council, 1977). No PH_3 nor C_6H_{14} was detected during any sampled burn.

Regarding durations, temperatures, and humidities of exposures, these data were highly consistent and acceptable. Median in-chamber confinements for prairie dogs were 86, 80.5, and 81.5 min for the 0.0, 1.0 and 4.0 mg/ℓ, respectively. Granted, certain filtered-air confinements exceeded 100 min; however, this should produce greater stress for control animals. Within-chamber temperatures during all exposures were held between 20 and 25° C -- temperatures congruent with those for Task 2. Although 1 RH value (4.0 mg/ℓ Group) was 82%, the RH for all other burns met previously established criteria of between 40 and 60 percent (Sterner et al., 1988).

Spontaneous Activity Study (Rock Doves)

Table E2 presents median and range statistics for the RP/BR-aerosol and air quality measurements used to characterize chamber atmospheres during the Spontaneous Activity Study with rock doves. Statistics represent the 6 exposures conducted at each of the 0.0, 1.0, and 4.0 mg/ℓ target concentrations (i.e., 3 replications of 2 successive daily 80-min exposures).

Similar to the aerosol characterization data for the Spontaneous Activity Study with prairie dogs (see Table E1), the RP/BR-aerosol and air quality data for rock dove exposures are acceptable. The only result requiring some elaboration is the sizable dispersion in aerosol mass noted for the 1.0 mg/ℓ target concentration burns.

Total aerosol mass (mg) across the 6 RP/BR burns displayed minimum to maximum percent variation (i.e., ((maximum - minimum) ÷ minimum) X 100) of 48 and 15 percent for burns at the 1.0 and 4.0 mg/ℓ target concentrations, respectively. The 48 percent variation at the 1.0 mg/ℓ concentrations is sizable; however, the large separation in mass exposure, coupled with the extremely low dose of RP/BR that would be associated with this concentration, ensures significantly different inhalation treatments between the 1.0 and 4.0 mg/ℓ Groups.

All other aerosol and air quality data were indicative of highly uniform and acceptable inhalation exposures. The H_3PO_4 measurements accounted for roughly two-thirds to three-fourths of the RP/BR-aerosol mass. Mean aerodynamic diameters of aerosol particles were less than 1 μm. Median O_2 readings were 22 percent for all exposures; whereas, median CO_2 was less than 0.8 percent. Carbon monoxide never exceeded 25 ppm, and no PH_3 nor C_6H_{14} were detected during any exposure.

Finally, exposure durations were well controlled at approximately 80 min, median in-chamber temperatures hovered at about 23° C, and in-chamber RH values were kept between 52 and 66 percent.

Startle Response Study (Prairie Dogs)

Table E3 lists the medians and ranges of the aerosol and air quality variables that were used to characterize the chamber conditions during the Exposure Phase of the Startle Response Study with prairie dogs. The 3 columns contain

Table E2. Median (minimum-maximum) statistics of the RP/BR-aerosol and air quality variables characterizing the aerosol and filtered-air exposures conducted at the 0.0 (filtered air) 1.0, and 4.0 mg/ℓ target concentrations for the Spontaneous Activity Study with rock doves in Task 3 [a].

Variable	Target Concentration (extrusion setting) 0.0 mg/ℓ (control)	1.0 mg/ℓ (50 μm)	4.0 mg/ℓ (180 μm)
Aerosol			
Aerosol Mass (mg)	3.85 (0.5-7.2)	71 (61.4-91.4)	299.5 (281.7-322.9)
Aerosol Mass Concentration (mg/ℓ)	.056 (0.008-0.12)	.887 (0.768-1.142)	3.74 (3.52-4.04)
Steady-State Concentration (mg/ℓ)	--	1.17 (0.96-1.62)	5.12 (4.79-5.33)
H_3PO_4 Titration (mg)	ND	46.91 (45.26-50.04)	217.6 (210.7-235.1)
H_3PO_4 Concentration (mg/ℓ)	ND	.586 (0.566-0.626)	2.72 (2.63-2.94)
Percent H_3PO_4 of Aerosol Mass	--	67.2 (51.6-73.7)	74.3 (69.7-73.7)
Particle Size			
MMAD (μm) [b]	--	0.4 (0.2-0.4)	0.6 (0.4-0.8)
Respiratory Gases [c]			
O_2 (%)	22 [d] (21-22) [d]	22 (18-22)	22 (20-22)
CO_2 (ppm)	605 [d] (484-605)	605 (605-726)	787 (605-847)
CO_2 From Burn and Animal Respirations (ppm) [e]	48 [d] (-73-48)	48 (48-169)	230 (46-290)
Contaminant Gases [c]			
CO (ppm) [f]	ND [d]	12 (11-18)	20 (15-25)
PH_3 (ppm)	ND [d]	ND	ND
C_6H_{14} (ppm)	ND [d]	ND	ND
Exposure Duration/Chamber Conditions			
Length Of Exposure (min)	85.5 (80-92)	80 (80-80)	80 (80-80)
Temperature (C°)	23 (21-26)	22.5 (21.5-24)	24 (23.5-26)
Relative Humidity (%)	57.5 (52-62)	59 (52-66)	59 (52-63)

[a] Median (minimum-maximum) statistics shown as measures of central tendency and dispersion because of the decreased influence of outliers on the indices ("--" = Not Measured; ND = Not Detected).

[b] Determinations of MMAD were completed using a graphical analysis procedure (Log particle size diameter versus cumulative probability of sampling detections) as outlined by Chuan (1986). No particle size measurements were obtained for the filtered-air (0.0 mg/l) exposures; small volume samples were insufficient to determine MMAD values for filtered-air exposures.

[c] All Gastec analyzer tube values were corrected for 1646 m elevation (i.e., 628 mm Hg) in accordance with the manufacturer's recommendations. The formula for this correction was: $\text{Actual Tube Value} \times \frac{760 \text{ mm Hg}}{628 \text{ mm Hg}}$.

[d] Only a single measurement taken during 1 exposure; datum is only value, not median and range.

[e] A corrected mean of 557 ppm CO_2 was obtained for 10 CO_2 readings made under ambient conditions in Room 150 of DWRC; this was subtracted from the respective within-chamber medians to estimate CO_2 production associated with each extrusion setting/animal confinement.

[f] The EPA Short-term Limit Threshold for CO is 35 ppm maximum for a 1 h average exposure (National Research Council, 1977). Note that all of the CO tube readings (Tube No. 1LL) were ≤ 25 ppm.

Table E3. Median (minimum-maximum) statistics of the RP/BR-aerosol and air quality variables characterizing the 4 aerosol and filtered-air exposures conducted at the 0.0 (filtered air), 1.0, and 4.0 mg/ℓ target concentrations for the Startle Response Study with prairie dogs in Task 3. [a]

Variable	Target Concentration (extrusion setting) 0.0 mg/ℓ (control)	1.0 mg/ℓ (50 μm)	4.0 mg/ℓ (180 μm)
Aerosol			
Aerosol Mass (mg)	-9.7 [b] (-126.9 to 0.0)	51.1 (43.4-55.7)	283.2 (277.9-303.2)
Aerosol Mass Concentration (mg/ℓ)	-0.12 (-.27 to 0.0)	.64 (.54-.70)	3.54 (3.51-3.79)
Steady-State Concentration (mg/ℓ)	--	.86 (.71-.94)	4.71 (4.49-5.00)
H_3PO_4 Titration (mg)	ND	38.93 (38.81-39.52)	214.4 (209.1-217.2)
H_3PO_4 Concentration (mg/ℓ)	ND	.49 (.49-.49)	2.68 (2.61-2.72)
Percent H_3PO_4 of Aerosol Mass	--	76.2 (70.9-89.4)	75.7 (71.6-75.2)
Particle Size			
MMAD (μm)	--	.40 (.40-.40)	.79 (.76-.82)
Respiratory Gases			
O_2 (%)	--	20.9 (20.0-21.8)	20.0 (19.4-20.6)
CO_2 (ppm)	--	605.1 (605.1-605.1)	925.8 (641.4-1210.2)
CO_2 From Burn and Animal Respirations (ppm) [c]	--	48.1 (48.1-48.1)	368.8 (84.4-653.2)
Contaminant Gases			
CO (ppm)	--	17.0 (9.7-24.2)	16.4 (14.5-18.2)
PH_3 (ppm)	--	ND (ND-ND)	<0.1 (ND-<0.1)
C_6H_{14} (ppm)	--	ND (ND-ND)	30.3 (ND-60.5)
Exposure Duration/Chamber Conditions			
Length Of Exposure (min)	81.5 (80-87)	80 (80-80)	80 (80-80)
Temperature (C^o)	(19.4-22.4)	(20.0-22.6)	(20.3-21.2)
Relative Humidity (%)	57.5 (51-65)	57 (51-58)	57.5 (57-58)

[a] ND = not detected; -- = not appropriate or not collected.

[b] Operation of the filtered-air chamber (0.0 mg/ℓ) produced several odd (i.e., high negative) aerosol mass readings; these were attributed to evaporation of moisture from filter discs during sampling.

[c] A corrected mean of 557 ppm CO_2 was obtained for 10 readings made under ambient conditions in Room 158 of DWRC; this was subtracted from the respective within-chamber median to estimate CO_2 production associated with each extrusion setting.

statistics related to the 0.0, 1.0, and 4.0 mg/ℓ RP/BR-aerosol target concentrations, respectively, with each set of statistics referencing a total of 4, 80-min exposures since all animals were exposed during 1 replication.

The total aerosol mass values for the 1.0 mg/ℓ burns varied up to 28 percent across burns; whereas, mass values for the 4.0 mg/ℓ burns varied up to 9 percent across burns when the difference in range values was compared to the lowest measured value. Although the percentage variation in aerosol mass for the 1.0 mg/ℓ condition exceeded 20 percent heterogeneity among burns, this was of minor concern because of the low exposure level involved. The H_3PO_4 titration data indicated that deposition of acid made up approximately 76 percent of the total aerosol mass -- data in close agreement with previous research (Sterner et al., 1988; Moneyhun et al., 1988; Shumake et al., 1989).

Planimeter-based estimates of maximal steady-state concentrations indicated that median values of 0.86 and 4.71 mg/ℓ were associated with the 1.0 and 4.0 mg/ℓ RP/BR-aerosol target concentrations, respectively (see Appendix B). These concentrations were within ± 20 percent of target levels (i.e., -14 and +18% for 1.0 and 4.0 mg/ℓ burns, respectively).

Median particle sizes based on graphical MMAD determinations for the 1.0 and 4.0 mg/ℓ target concentration levels were .40 and .79 μm, respectively. These values agree very closely with those previously determined for Task 1 (Sterner et al., 1988) and Task 2 (Shumake et al., 1989) under this Project Order. All MMAD values were < 1.0 μm and well within the respirable range.

Respiratory gases and contaminant gases were found to be within acceptable limits. However, one exposure at the 4.0 mg/ℓ target concentration did yield a 60.5 ppm reading for C_6H_{14}, but as indicated in the Moneyhun et al. (1988) Report, this may have been an anomalous reading as determined by Gastec Analyzer Tubes.

Length of exposure, chamber temperature, and RH were uniform and well-controlled throughout the exposure sessions of this study.

Startle Response Study (Rock Doves)

Table E4 lists the median and range values of aerosol and air quality variables used to characterize chamber conditions during the Exposure Phase of the Startle Response Study with rock doves. Each column shows statistics pertaining to the 0.0, 1.0, and 4.0 RP/BR-aerosol exposures. Each target concentration group received a total of 2, approximately 80-min exposures on successive days.

Aerosol mass concentrations in mg varied 7.0 percent and 14.1 percent between exposures for the 1.0 and 4.0 mg/ℓ target concentrations, respectively, when the difference in range values were each compared to the lowest measured values. Thus, uniformity of concentration between the 2 exposure sessions was very acceptable. The H_3PO_4 titration weight data indicated that deposition of this acid made up approximately two-thirds of the total aerosol mass collected, again agreeing closely with prior reports (Sterner et al., 1988; Moneyhun et al., 1988; Shumake et al., 1989).

Table E4. Median (minimum-maximum) statistics of the RP/BR-aerosol and air quality variables characterizing the aerosol and filtered-air exposures -- conducted at the 0.0 (filtered air), 1.0, and 4.0 mg/ℓ target concentrations for the Startle Response Study with rock doves in Task 3. [a]

Variable	Target Concentration (extrusion setting) 0.0 mg/ℓ (control)	1.0 mg/ℓ (50 μm)	4.0 mg/ℓ (180 μm)
Aerosol			
Aerosol Mass (mg)	+0.1 (-0.5 to 1.0)	66.3 (64.0-68.5)	317.8 (296.8-338.7)
Aerosol Mass Concentration (mg/ℓ)	.0003 (-.0006 to .0012)	.83 (.80-.86)	3.97 (3.71-4.23)
Steady-State Concentration (mg/ℓ)	--	1.12	4.98
H_3PO_4 Titration (mg)	ND	45.63 (43.78-47.48)	214.8 (211.0-218.5)
H_3PO_4 Concentration (mg/ℓ)	ND		
Percent H_3PO_4 of Aerosol Mass	--	68.8 (68.4-69.3)	67.6 (64.5-71.9)
Particle Size			
MMAD (μm)	--	.53 (.51-.54)	.63 (.62-.64)
Respiratory Gases			
O_2 (%)	--	23.0	21.8
CO_2 (ppm)	--	968.2	847.1
CO_2 From Burn and Animal Respirations (ppm) [b]	--	411.2	290.1
Contaminant Gases			
CO (ppm)	--	14.5	18.2
PH_3 (ppm)	--	ND	ND
C_6H_{14} (ppm)	--	ND	ND
Exposure Duration/Chamber Conditions			
Length Of Exposure (min)	85.5 (85-86)	80 (80-80)	80 (80-80)
Temperature (C^o)	(22.3-23.1)	(20.9-22.4)	(21.4-23.9)
Relative Humidity (%)	62.5 (59-66)	63.5 (62-65)	78.5 (66-91)

[a] Where only 1 value is listed in the table, only 1 was obtained during the 2 exposure sessions. (ND = not detected; -- = not appropriate or not collected.)

[b] A corrected mean of 557 ppm CO_2 was obtained for 10 readings made under ambient conditions in Room 158 of the DWRC; this value was subtracted from the respective within-chamber CO_2 readings to estimate CO_2 production associated with each extrusion setting.

Planimeter-estimated steady-state concentrations of RP/BR aerosol, using ORNL opacity sensor records, indicated median values of 1.12 and 4.98 mg/ℓ with the extruder set to deliver 1.0 and 4.0 mg/ℓ target concentrations, respectively (see Appendix B). These steady-state levels indicated that the lower concentration was only 12 percent above the target, but the higher concentration was 24.5 percent higher than the target. Variations in the consistency of the RP/BR material could have generated these higher than expected steady state concentrations; however, our asymptotic concentration value was only based on one usable graphic tracing from the ORNL sensor for the 4.0 mg/ℓ condition.

Particle sizes for both RP/BR-aerosol concentration levels were similar to one another (medians of 0.53 and 0.63 μm), and less differentiated than those obtained during the prairie dog exposures. The consistency of measured MMAD values was acceptable and all MMAD readings were again < 1 μm -- well within the respirable range.

Respiratory gases were also all within expected, acceptable ranges. Exposure durations, temperatures, and RH levels were also all well-controlled throughout testing; however, RH did reach an extreme value of 91 percent during one of the 4.0 mg/ℓ exposures.

Pulmonary Function Study (Prairie Dogs)

Table E5 presents median and range statistics for the RP/BR-aerosol and air quality measurements used to characterize chamber atmospheres during the Pulmonary Function Study with prairie dogs. Statistics represent the 20 exposures conducted at each of the 0.0, 1.0, and 4.0 mg/ℓ target concentrations (i.e., 5 replications at 4 successive daily 80-min sessions). Due to the high consistency previously noted (Shumake et al., 1989) among particle size measurements, and the absence of any anomalous respiratory or contaminant gas readings in the Filtered-air Chamber, limited measurements of these conditions were obtained.

Total aerosol mass (mg) across the 20 RP/BR burns displayed acceptable dispersion. Minimum to maximum percent variation (i.e., ((maximum - minimum) ÷ maximum) X 100) was 30 and 22 percent for burns at the 1.0 and 4.0 mg/ℓ target concentrations, respectively. Median aerosol mass concentrations for the 20, 80-min exposures at the 1.0 and 4.0 mg/ℓ targets were 0.78 and 3.41 mg/ℓ, respectively; whereas, estimated median steady-state concentrations for 36-min portions of 10 RP/BR burns at these target concentrations were 1.00 and 4.45 mg/ℓ, respectively (see Appendix B). The H_3PO_4 titration data supported the gravimetric data, with the acid accounting for a median 72 and 77 percent of the aerosol mass at the 1.0 and 4.0 mg/ℓ target concentration respectively.

Regarding particle size, MMAD values were also in line with earlier results for the RP/BR-aerosol System. Median MMADs were 0.47 and 0.81 μm with ranges of 0.45-0.50 and 0.75-1.00 for the 1.0 and 4.0 mg/ℓ target concentrations, respectively. The point here is that all particles were found to be respirable, with approximately 25 percent of the particles being deposited in the respiratory tract (Phalen, 1984).

Table E5. Median (minimum-maximum) statistics of the RP/BR-aerosol and air quality variables characterizing the aerosol and filtered-air exposures conducted at the 0.0 (filtered air) 1.0, and 4.0 mg/ℓ target concentrations for the Pulmonary Function Study with prairie dogs in Task 3. [a]

Variable	Target Concentration (extrusion setting) 0.0 mg/ℓ (control)	1.0 mg/ℓ (50 μm)	4.0 mg/ℓ (180 μm)
Aerosol			
Aerosol Mass(mg)	0.0 (-8.0 - 16.0)	62.5 (48.4 - 69.2)	272.4 (247.0 - 318.1)
Aerosol Mass Concentration (mg/ℓ)	0.0 (-0.10 - 0.21)	0.78 (0.61 - 0.87)	3.41 (3.09 - 3.98)
Steady-State Concentration (mg/ℓ)	--	1.00 (0.80 - 1.10)	4.45 (4.10 - 5.00)
H_3PO_4 Titration (mg)	ND	44.16 (28.04-49.14)	207.0 (141.9-223.3)
H_3PO_4 Concentration (mg/ℓ)	ND	0.55 (0.35 - 0.61)	2.58 (1.77 -2.79)
Percent H_3PO_4 of Aerosol Mass		72 (45 - 89)	77 (52 - 82)
Particle Size			
MMAD (μm) [b]	--	0.47 (0.45 - 0.50)	0.81 (0.75 - 1.00)
Respiratory Gases [c]			
O_2 (%)	21 (17 - 22)	22 (18 - 23)	21 (17 - 22)
CO_2 (ppm) [d]	484 (363 - 605)	605 (363 - 726)	726 (605 - 968)
CO_2 From burn and animal respiration (ppm)	-93 (-194 -48)	48 (-194 -169)	169 (48 - 412)
Contaminant Gases [c]			
CO (ppm) [e]	ND	10 (2 - 15)	16 (8 - 21)
PH_3 (ppm)	ND	ND	ND
C_6H_{14} (ppm)	ND	ND	ND
Exposure Duration/Chamber Conditions			
Length Of Exposure (min)	80 (80 - 80)	80 (80 - 81)	80 (80 - 83)
Temperature (C°)	24 (21 - 28)	22 (19 - 24)	24 (20 - 26)
Relative Humidity (%)	48 (39 - 54)	52 (46 - 64)	47 (42 - 57)

[a] Median (minimum-maximum) statistics shown as measures of central tendency and dispersion because of the decreased influence of outliers on the indices ("--" = Not Measured; ND = Not Detected).

[b] Determinations of MMAD were completed using a graphical analysis procedure (Log particle size diameter versus cumulative probability of sampling detections) as outlined by Chuan (1986). No particle size measurements were obtained for the filtered-air (0.0 mg/l) exposures; small volume samples were insufficient to determine MMAD values for filtered-air exposures.

[c] All Gastec analyzer tube values were corrected for 1646 m elevation (i.e., 628 mm Hg) in accordance with the manufacturer's recommendations. The formula for this correction was:

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

[d] A corrected mean of 557 ppm CO_2 was obtained for 10 CO_2 readings made under ambient conditions in Room 158 of DWRC; this was subtracted from the respective within-chamber medians to estimate CO_2 production associated with each extrusion setting and the animals in the chamber.

[e] The EPA standard for CO os 35 ppm maximum for a 1 h average exposure (National Research Council, 1977). Note that all of the CO tube readings (Tube No. 1LL) were < 21 ppm.

Respiratory and contaminant gases within the chambers were also acceptable. Oxygen readings consistently yielded 21 or 22 percent medians across all RP/BR-aerosol and filtered-air exposures. Median CO_2 values were between 484 and 726 ppm for these conditions. Carbon monoxide was effectively controlled by the selection of the 1.0 and 4.0 mg/ℓ target concentration, with median values of 10 and 16 ppm, respectively -- levels well below the 35 ppm 1 h Threshold Limit (National Research Council, 1977). No PH_3 nor $C_6H_{14}$was detected during any burn.

Regarding durations, temperatures, and humidities of exposures, the data showed acceptable consistency. The median in-chamber confinements for prairie dogs all equalled 80.0 min for all 3 Groups. Within-chamber temperatures during all exposures with the exception of 3, 28° C 0.0 exposures, were held within 19 - 26 C° -- data congruent with those for Task 2. Although 1 RH value (0.0 mg/ℓ Group) was 39% and another 64% (1.0 mg/ℓ Group), the RH for all other burns met previously stated criteria of between 40 to 60 percent (Sterner et al., 1988).

Pulmonary Function Study (Rock Doves)

Table E6 presents median and range statistics for the RP/BR-aerosol and air quality measurements used to characterize chamber atmospheres during the Pulmonary Function Study with rock doves. Statistics represent the 8 exposures conducted at each of the 0.0, 0.1, and 4.0 mg/ℓ target concentrations (i.e., 4 replications having 2 successive daily 80-min exposures each). Due to the high consistency previously noted among particle size measurements (Shumake et al, 1989), and the absence of any anomalous respiratory or contaminant gas readings in the Filtered-air chamber, limited measurements of these conditions were obtained.

Median aerosol mass concentrations for the 8, 80-min exposures at the 1.0 and 4.0 mg/ℓ targets were 0.86 and 3.49 mg/ℓ, respectively; whereas, estimated median steady-state concentrations were 1.17 and 5.00 mg/ℓ, respectively (see Appendix B). The H_3PO_4 titration data supported the aerosol-exposure data, with the acid accounting for a median 70 and 76 percent of the aerosol mass at the 1.0 and 4.0 mg/ℓ each target concentration respectively.

Median MMADs were 0.46 and 0.80 μm with ranges of 0.39-0.50 and 0.57-0.97 at 1.0 and 4.0 mg/ℓ target concentrations respectively. The point here is that all particles were found to be respirable with approximately 25 percent of the particles being deposited in the respiratory tract (Phalen, 1984).

Respiratory and contaminant gases within the chambers were also acceptable. Oxygen readings consistently yielded 22 percent medians across all RP/BR-aerosol and filtered-air exposures. Median CO_2 values were between 546 and 726 ppm for these conditions. Carbon monoxide was effectively controlled by the selection of the 1.0 and 4.0 mg/ℓ target concentrations, with median values of 15 and 16 ppm, respectively. No PH_3 nor $C_6H_{14}$was detected during any burn.

The median in-chamber confinements for rock doves equaled 80 min for the 0.0, 1.0 and 4.0 mg/ℓ, respectively. Within-chamber temperatures during all

Table E6. Median (minimum-maximum) statistics of the RP/BR-aerosol and air quality variables characterizing the aerosol and filtered-air exposures conducted at the 0.0 (filtered air) 1.0, and 4.0 mg/ℓ target concentrations for the Pulmonary Function Study with rock doves in Task 3.[a]

Variable	Target Concentration (extrusion setting) 0.0 mg/ℓ (control)	1.0 mg/ℓ (50 μm)	4.0 mg/ℓ (180μm)
Aerosol			
Aerosol Mass (mg)	1.0 (-14.6-13.2)	68.6 (59.6-73.2)	279.5 (248.0-304.3)
Aerosol Mass Concentration (mg/ℓ)	0.01 (-0.18-0.16)	0.86 (0.75-0.92)	3.49 (3.06-3.80)
Steady-State Concentration (mg/ℓ)	--	1.17 (0.99-1.20)	5.00 (4.30-5.10)
H_3PO_4 Titration (mg)	ND	46.97 (42.22-50.61)	214.2 (203.7-223.7)
H_3PO_4 Concentration (mg/ℓ)	ND	0.59 (0.53-0.63)	2.68 (2.52-2.80)
Percent H_3PO_4 of Aerosol Mass		70 (67-73)	76 (72-83)
Particle Size			
MMAD (μm)[b]	--	0.46 (0.39-0.50)	0.80 (0.57-0.97)
Respiratory Gases[c]			
O_2 (%)	22 (21 - 22)	22 (21 - 23)	22 (19 - 22)
CO_2 (ppm)[d]	546 (363 - 605)	546 (484 - 726)	726 (605 - 968)
CO_2 From Burn and Animal Respirations (ppm)	-11 (-194 - 48)	-11 (-73 - 169)	169 (48 - 411)
Contaminant Gases[c]			
CO (ppm)[e]	ND	15 (7 - 22)	16 (12 - 24)
PH_3 (ppm)	ND	ND	ND
C_6H_{14} (ppm)	ND	ND	ND
Exposure Duration/Chamber Conditions			
Length Of Exposure (min)	80 (80 - 90)	80 (80 - 81)	80 (80 - 81)
Temperature (C^o)	24 (22 - 26)	23 (21 - 25)	24 (22 - 25)
Relative Humidity (%)	48 (39 - 66)	52 (37 - 63)	51 (43 - 59)

[a] Median (minimum-maximum) statistics shown as measures of central tendency and dispersion because of the decreased influence of outliers on the indices ("--" = Not Measured; ND = Not Detected).

[b] Determinations of MMAD were completed using a graphical analysis procedure (Log particle size diameter versus cumulative probability of sampling detections) as outlined by Chuan (1986). No particle size measurements were obtained for the filtered-air (0.0 mg/l) exposures; small volume samples were insufficient to determine MMAD values for filtered-air exposures.

[c] All Gastec analyzer tube values were corrected for 1646 m elevation (i.e., 628 mm Hg) in accordance with the manufacturer's recommendations. The formula for this correction was:

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

[d] A corrected mean of 557 ppm CO_2 was obtained for 10 CO_2 readings made under ambient conditions in Room 158 of DWRC; this was subtracted from the respective within-chamber medians to estimate CO_2 production associated with each extrusion setting and the animals in the chamber.

[e] The EPA standard for CO os 35 ppm maximum for a 1 h average exposure (National Research Council, 1977). Note that all of the CO tube readings (Tube No. 1LL) were < 25 ppm.

exposures were held within 21-26 C^{o} -- data congruent with those for Task 2. Although 3 RH values during 0.0 mg/ℓ burns (i.e., 39, 63, and 66%) and 2 during 1.0 mg/ℓ burns (i.e., 37 and 63%) exceeded the 40 to 60 percent limits established prior to the research (Sterner et al., 1988), the RHs for all other burns met previous criteria.

Blood Chemistry and Hematology Study (Prairie Dogs)

Table E7 presents median and range statistics for the RP/BR-aerosol and air quality measurements used to characterize chamber atmospheres during the Blood Chemistry/Hematology Study with prairie dogs. Statistics represent the 8 exposures conducted at each of the 0.0, 1.0, and 4.0 mg/ℓ target concentrations (i.e., 2 replications at 4 successive daily 80-min sessions). Due to the high consistency previously noted among particle size measurements (Shumake et al., 1989), and the absence of any anomalous respiratory or contaminant gas readings in the filtered-air Chamber, limited measurements of these conditions were obtained.

Total aerosol mass (mg) across the 8 RP/BR burns at each concentration displayed acceptable dispersion. Minimum to maximum percent variations (i.e., ((maximum - minimum) maximum) X 100) were 19 and 11 percent for burns at the 1.0 and 4.0 mg/ℓ target concentrations, respectively. Median aerosol mass concentrations for the 8, 80 min exposures at the 1.0 and 4.0 mg/ℓ targets were 0.78 and 3.60 mg/ℓ, respectively; whereas, estimated median steady-state concentration for 36-min portions of 2 RP/BR burns at each of these target concentrations were 0.94 and 4.70 mg/ℓ respectively (see Appendix B). The H_3PO_4 titration data supported the aerosol-exposure data, with the acid accounting for a median 73 and 75 percent of the aerosol mass at the 1.0 and 4.0 mg/ℓ concentrations respectively.

Regarding particle size, MMAD values were also in line with earlier results for the RP/BR-aerosol System. Median MMADs were 0.46 and 0.85 μm at 1.0 and 4.0 mg/ℓ target concentrations respectively. The point here is that all particles were found to be respirable with approximately 25 percent of the particles being deposited in the respiratory tract (Phalen, 1984).

Respiratory and contaminant gases within the chambers were also acceptable. Oxygen readings consistently yielded 21 or 22 percent medians across all RP/BR-aerosol and filtered-air exposures. Median CO_2 values were between 666 and 1029 ppm for these conditions. Carbon monoxide was effectively controlled by the selection of the 1.0 and 4.0 mg/ℓ target concentrations, with median values of 13 and 19 ppm, respectively -- levels well below the 35 ppm EPA standard for 1 h Threshold Limit (National Research council, 1977) No PH_3 nor C_6H_{14} was detected during any burn.

Regarding durations, temperatures, and humidities of exposures, the data showed acceptable consistency. The in-chamber confinements for prairie yielded medians of 89, 80, and 80 min for the 0.0, 1.0, and 4.0 mg/ℓ, respectively. Within-chamber temperatures during all exposures were held within 19-26° C. Although 1 outlier RH value was 61, the RH for all other burns (49-61) met previously stated criteria of between 40 to 60 percent (Sterner et al., 1988).

Table E7. Median (minimum-maximum) statistics of the RP/BR-aerosol and air quality variables characterizing the aerosol and filtered-air exposures conducted at the 0.0 (filtered air), 1.0, and 4.0 mg/ℓ target concentrations for the Blood Chemistry/Hematology Study with prairie dogs in Task 3 [a].

Variable	Target Concentration (extrusion setting) 0.0 mg/ℓ (control)	1.0 mg/ℓ (50 μm)	4.0 mg/ℓ (180 μm)
Aerosol			
Aerosol Mass(mg)	0.8 (-3.9 - 19.2)	62.6 (52.5 - 64.8)	288.4 (278.3 - 312.1)
Aerosol Mass Concentration (mg/ℓ)	0.11 (-0.05 - 0.24)	0.78 (0.66 - 0.81)	3.60 (3.48 - 3.90)
Steady-State Concentration (mg/ℓ)	--	0.94 (0.92 - 1.03)	4.70 (4.60 - 5.10)
H_3PO_4 Titration (mg)	ND	44.32 (40.13 - 49.73)	217.2 (207.7 - 238.9)
H_3PO_4 Concentration (mg/ℓ)	ND	0.55 (0.50 - 0.62)	2.72 (2.60 - 2.99)
Percent H_3PO_4 of Aerosol Mass	--	73 (70 - 77)	75 (73 - 77)
Particle Size			
MMAD (μm) [b]	--	0.46 (0.45 - 0.48)	0.85 (0.82 - 0.89)
Respiratory Gases [c]			
O_2 (%)	22 (22 - 22)	21 (21 - 22)	21 (21 - 21)
CO_2 (ppm) [d]	847 (847 - 847)	666 (726 - 847)	1029 (605 - 1210)
CO_2 From Burn and Animal Respirations (ppm)	290 (290 - 290)	109 (48 - 290)	472 (48 - 653)
Contaminant Gases [c]			
CO (ppm) [e]	ND	13 (10 - 18)	19 (18 - 24)
PH_3 (ppm)	ND	ND	ND
C_6H_{14} (ppm)	ND	ND	ND
Exposure Duration/Chamber Conditions			
Length Of Exposure (min)	89 (80 - 100)	80 (80 - 80)	80 (80 - 80)
Temperature (C°)	23 (20 - 24)	22 (19 - 26)	23 (21 - 25)
Relative Humidity (%)	52 (49 - 58)	58 (51 - 61)	56 (49 - 60)

[a] Median (minimum-maximum) statistics shown as measures of central tendency and dispersion because of the decreased influence of outliers on the indices ("--" = Not Measured; ND = Not Detected).

[b] Determinations of MMAD were completed using a graphical analysis procedure (Log particle size diameter versus cumulative probability of sampling detections) as outlined by Chuan (1986). No particle size measurements were obtained for the filtered-air (0.0 mg/l) exposures; small volume samples were insufficient to determine MMAD values for filtered-air exposures.

[c] All Gastec analyzer tube values were corrected for 1646 m elevation (i.e., 628 mm Hg) in accordance with the manufacturer's recommendations. The formula for this correction was:

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

[d] A corrected mean of 557 ppm CO_2 was obtained for 10 CO_2 readings made under ambient conditions in Room 158 of DWRC; this was subtracted from the respective within-chamber medians to estimate CO_2 production associated with each extrusion setting and the animals in the chamber.

[e] The EPA standard for CO os 35 ppm maximum for a 1 h average exposure (National Research Council, 1977). Note that all of the CO tube readings (Tube No. 1LL) were < 24 ppm.

Blood Chemistry and Hematology Study (Rock Doves)

Table E8 presents median and range statistics for the RP/BR-aerosol and air quality measurements used to characterize chamber atmospheres during the Blood Chemistry and Hematology Study with rock doves. Statistics represent the 4 exposures conducted at each of the 0.0, 1.0, and 4.0 mg/ℓ target concentrations (i.e., 2 replications having 2 successive daily 80-min exposures each).

Total aerosol mass (mg) across the 4 RP/BR burns displayed acceptable dispersions. Minimum to maximum percent variation (i.e., ((maximum - minimum) ÷ maximum) X 100) was 20 and 10 percent for burns at the 1.0 and 4.0 mg/ℓ target concentrations, respectively. Median aerosol mass concentrations for the 4, 80-min exposures at the 1.0 and 4.0 mg/ℓ targets were 0.72 and 3.49 mg/ℓ, respectively; whereas, estimated median steady-state concentrations for 36-min portions of 2 RP/BR burns at each of these target concentrations were 0.95 and 4.45 mg/ℓ, respectively. The H_3PO_4 titration data supported the aerosol-exposure data, with the acid accounting for a median 70 and 74 percent of the aerosol mass at the 1.0 and 4.0 mg/ℓ concentrations respectively.

Median MMADs were 0.47 and 0.75 μm at 1.0 and 4.0 mg/ℓ target concentrations, respectively. The point here is that all particles were found to be respirable, with approximately 25 percent of the particles being deposited in the respiratory tract (Phalen,1984).

Respiratory and contaminant gases within the chambers were also acceptable. Oxygen readings consistently yielded 21 or 22 percent medians across all RP/BR-aerosol exposures. Median CO_2 values were between 545 and 666 ppm for these conditions. Carbon monoxide was effectively controlled by the selection of the 1.0 and 4.0 mg/ℓ target concentrations, with median values of 11 and 21 ppm, respectively. Except for a single 8 ppm C_6H_{14} value in the 4.0 mg/ℓ Group, no PH_3 nor C_6H_{14} was detected during any other burns.

Regarding durations, temperatures, and humidities of exposure, the data showed acceptable consistency. The in-chamber confinements for rock doves yielded medians of 98, 80, and 80 min for the 0.0, 1.0 and 4.0 mg/ℓ, respectively. Within-chamber temperatures during all exposures were held within 22-24° C -- data congruent with those for Task 2. Although 2 outlier RH values for the 1.0 and 0.0 mg/ℓ Groups were 62 and 66%, respectively, the RH for all other burns met previously stated criteria of between 40 to 60 percent (Sterner et al., 1988).

Table E8. Median and range (minimum-maximum) statistics of the RP/BR-aerosol and air quality variables characterizing the aerosol and filtered-air exposures conducted at the 0.0 (filtered air) 1.0, and 4.0 mg/ℓ target concentrations for the Blood Chemistry and Hematology Study with rock doves in Task 3 [a].

Variable	Target Concentration (extrusion setting) 0.0 mg/ℓ (control)	1.0 mg/ℓ (50 μm)	4.0 mg/ℓ (180 μm)
Aerosol			
Aerosol Mass (mg)	-1.4 (-6.6 - 3.0)	57.3 (53.5 - 66.5)	279.0 (260.9 - 288.9)
Aerosol Mass Concentration (mg/ℓ)	0.07 (-0.08 - 0.04)	0.72 (0.67 - 0.83)	3.49 (3.26 - 3.61)
Steady-State Concentration (mg/ℓ)	--	0.95 (0.90 - 1.00)	4.45 (4.40 - 4.50)
H_3PO_4 Titration (mg)	ND	40.83 (36.26 - 42.33)	207.5 (205.6 - 209.8)
H_3PO_4 Concentration (mg/ℓ)	ND	0.51 (0.45 - 0.53)	2.60 (2.57 - 2.62)
Percent H_3PO_4 of Aerosol Mass		70 (60 - 73)	74 (72 - 79)
Particle Size			
MMAD (μm) [b]	--	0.47 (0.46 - 0.49)	0.75 (0.71 - 0.79)
Respiratory Gases [c]			
O_2 (%)	--	21 (21 - 22)	21 (21 - 22)
CO_2 (ppm) [d]	--	545 (484 - 605)	666 (605 - 726)
CO_2 From Burn and Animal Respirations (ppm)	--	-12 (-73 - 48)	109 (48 - 169)
Contaminant Gases [c]			
CO (ppm) [e]	--	11 (10 - 12)	21 (14 - 27)
PH_3 (ppm)	--	ND	ND
C_6H_{14} (ppm)	--	ND	8
Exposure Duration/Chamber Conditions			
Length of Exposure (min)	98 (88 - 108)	80 (80 - 80)	80 (80 - 80)
Temperature (C°)	23 (22 - 24)	21 (20 - 22)	23 (22 - 24)
Relative Humidity (%)	52 (52 - 66)	56 (51 - 62)	56 (52 - 59)

[a] Median (minimum-maximum) statistics shown as measures of central tendency and dispersion because of the decreased influence of outliers on the indices ("--" = Not Measured; ND = Not Detected).

[b] Determinations of MMAD were completed using a graphical analysis procedure (Log particle size diameter versus cumulative probability of sampling detections) as outlined by Chuan (1986). No particle size measurements were obtained for the filtered-air (0.0 mg/l) exposures; small volume samples were insufficient to determine MMAD values for filtered-air exposures.

[c] All Gastec analyzer tube values were corrected for 1646 m elevation (i.e., 628 mm Hg) in accordance with the manufacturer's recommendations. The formula for this correction was:

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

[d] A corrected mean of 557 ppm CO_2 was obtained for 10 CO_2 readings made under ambient conditions in Room 158 of DWRC; this was subtracted from the respective within-chamber medians to estimate CO_2 production associated with each extrusion setting and the animals in the chamber.

[e] The EPA standard for CO is 35 ppm maximum for a 1 h average exposure (National Research Council, 1977). Note that all of the CO tube readings (Tube No. 211) were < 27 ppm.

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