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Denver Wildlife Research Center
Building 16, P.O. Box 25266
Denver Federal Center
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BEHAVIORAL-PHYSIOLOGICAL EFFECTS OF RED PHOSPHORUS SMOKE INHALATION ON TWO WILDLIFE SPECIES

FINAL REPORT

(Project Summary)

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 report summarizes a 3-year Project Order (85PP5847) to determine lethal and sub-lethal symptomatological, behavioral and physiological effects of red phosphorous/butyl rubber (RP/BR) smoke exposure(s) upon black-tailed prairie dogs (Cynomys ludovicianus) and rock doves (Columba livia). Use of this mammalian and avian model extended the Army's comparative database of RP/BR-smoke effects. All research involved whole-body, inhalation chamber studies designed to assess immediate, acute or sub-acute effects. Research comprising the project was divided into 3 tasks.

Task I involved: (1) setup and modification of a RP/BR-aerosol and inhalation system developed by the Analytical Chemistry Division, Oak Ridge National Laboratory, (2) checks of ambient carbon monoxide (CO) at the test site and (3) studies to assess spatial and temporal uniformity of the RP/BR smoke within the inhalation chamber. Outcomes were:

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(1) the addition of air filtration, humidification, and cooling equipment afforded sufficient control of the chamber atmosphere for the conduct of animal studies, (2) no ambient CO was ever detected at the research site, (3) RP/BR-smoke concentrations were generally homogeneous throughout the chamber (i.e., except for Cage Site 12 which was excluded from use in later studies) and (4) air quality was acceptable for the conduct of animal studies.

Task 2 involved a series of toxicity range-finding studies with the 2 species. Prairie dogs were given between 1 and 4 successive 80-min per day exposures to 0.0, 2.0, 4.0 and 6.0 mg/ℓ of RP/BR smoke; rock doves received similar exposures to 0.0, 3.0 and 6.0 mg/ℓ of RP/BR smoke. All studies involved a 3-phase paradigm (Pre-exposure, Exposure, and Post-exposure). Mortality, plus qualitative and quantitative symptomatology, was assessed for up to 30 days after these exposures. Results showed that: (1) no prairie dogs died, but 11 of 42 (26%) rock doves succumbed within 8 days after exposure, (2) of the birds that died, roughly one-third were males, (3) both species displayed dramatic loss, raspy, softer or less frequent vocalization and (4) both species displayed acute, quickly-recovered decreases in food intake, water intake and body weight following exposure(s), with significant increases in water intake noted between 10 to 28 days after exposure.

Task 3 involved 4 pairs of separate behavioral-physiological studies to assess sub-lethal consequences of RP/BR smoke in the 2 species. Each study evaluated the effects of 4 or 2 successive 80-min per day exposures upon selected measures of spontaneous activity, startle response, pulmonary function, and blood chemistry/hematology in prairie dogs and rock doves, respectively. Each study also involved a 3-phase paradigm (Pre-exposure, Exposure, and Post-exposure) and 3 RP/BR-smoke concentrations (0.0, 1.0, and 4.0 mg/ℓ). Twenty-four prairie dogs and rock doves were tested in each study (8 per concentration), with sex included as a factor in each design. Analyses of these data yielded several immediate and acute behavioral-physiological effects to RP/BR smoke for these species, with rock doves showing the greatest sensitivity. Examples of key findings were: (1) the ambulatory activity of prairie dogs, and the preening and ambulatory activity of rock doves, was decreased during the 2-h period following exposure(s) to 4.0 mg/ℓ smoke, (2) female rock doves showed brief, acute hypersensitivity (lowered latencies) to photoflash startle stimuli during the Post-exposure Phase, (3) respiration rates of 2 male rock doves that died following 4 exposures to 4.0 mg/ℓ smoke decreased temporarily, but then increased sharply prior to death and (4) heterophiles and lymphocytes of rock doves were raised and lowered, respectively, within 3 h of exposure to 4.0 mg/ℓ smoke.

Basically, the research indicated that prairie dogs and rock doves are extremely tolerant of RP/BR-smoke exposure(s). Multiple, high-concentration exposures to RP/BR smoke were needed to produce any symptomatological, behavioral and physiological effects in these species. Nevertheless, rock doves were more vulnerable to this obscurant than prairie dogs, with male rock doves relatively more sensitive than females. Future studies need to address: (1) the basis of the observed vocalization impairments, (2) the consequences of RP/BR smoke exposure to animals experiencing stressors (e.g., cold, food deprivation, exertion) and (3) detailed observations of these (and other sentinel) species to RP/BR-smoke effects under field conditions.

EXECUTIVE SUMMARY

This report summarizes the test procedures, major findings, conclusions and recommendations resulting from a 3 year study to determine the effects of RP/BR smoke on 2 species of wildlife -- black tailed prairie dogs (Cynomys ludovicianus) and rock doves (Columba livia). The research was conducted in 3 sequential tasks and the research procedures and technical findings have been reported in 3 separate Task Reports.

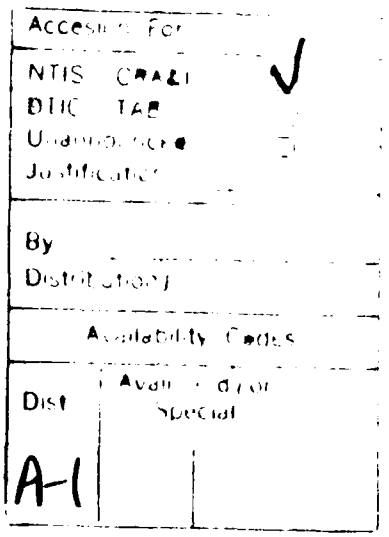
Task 1 consisted of the development, installation and modification of an inhalation chamber system and the conduct of studies to characterize the chamber atmosphere for Task 2 and Task 3 Studies. The smoke was quantified for aerosol mass, phosphoric acid, particle sizes and for respiratory and contaminant gases (oxygen, carbon dioxide, carbon monoxide, phosphine and hexane). Test results showed that RP/BR concentration was homogenous throughout the chamber (below a 20% maximum allowable deviation between the center of chamber and cage sites) and that all particle sizes were respirable ($\leq$ 1.0 μm). Respiratory and contaminant gas components were within limits considered unlikely to cause test animal impairment.

Task 2 consisted of a series of toxicity range-finding studies with both species to establish the RP/BR-smoke concentrations for Task 3 Studies. To conduct the tests, animals were subjected to 1 to 4 successive 80-min exposures of RP/BR aerosol (prairie dogs: 0.0, 2.0, 4.0, and 6.0 mg/ℓ; rock doves: 0.0, 3.0, and 6.0 mg/ℓ) and monitored for mortality (number of deaths and days to death) and sub-lethal symptomatology (body posture, respiratory congestion, coat or plumage condition, vocalization effects, water consumption and body weight) for up to 28 days after exposure. No prairie dogs died following exposure to the smoke, but there was 26 percent mortality in rock doves (11 of 42 birds; 6% of females and 42% of males). All except one death occurred at a concentration of 6.0 mg/ℓ. The smoke exposures affected or caused the loss of vocalization in both species. At 6.0 mg/ℓ, RP/BR smoke caused increased respiratory congestion in both species and abnormal body posture and weight loss in rock doves. The smoke also caused a significant increase in water consumption in both species. No significant RP/BR-smoke effects were detected in the gross pathology or histopathology of either species. The findings led to the selection of 1.0 and 4.0 mg/ℓ as the target concentrations for exposing animals to RP/BR smoke in Task 3 Studies. It was recognized that 4.0 mg/ℓ is a high smoke concentration and unlikely to occur under field exposure conditions; however, it provided a stringent challenge to each wildlife species.

In Task 3, 8 separate behavioral and physiological studies were conducted. Each study evaluated the effects of 4 or 2 successive 80-min RP/BR-smoke exposures with prairie dogs and rock doves, respectively. Measurements consisted of spontaneous activity, startle response, pulmonary function and blood chemistry/hematology variables. All studies involved a 3-phase paradigm (Pre-exposure, Exposure and Post-exposure) and 3 RP/BR-smoke concentrations (0.0, 1.0 and 4.0 mg/ℓ). Twenty-four prairie dogs or 24 rock doves were tested in each study (8/concentration), with sex included as a factor. Immediate ($\leq$3 h after Exposure) and acute ($\leq$12 days after Exposure) effects were assessed in each study. Chamber atmosphere conditions were monitored in

each study for aerosol mass, phosphoric acid, aerosol opacity, particle sizes, respiratory and contaminant gases, temperature and relative humidity. The results showed that RP/BR caused several immediate and acute behavioral and physiological effects; the most pronounced effects resulted from 4.0 mg/ℓ exposures, and were transient (lasting 1 to 3 days). Rock doves were more affected than prairie dogs. No mortality occurred in prairie dogs but 4 of the 96 rock doves died at 4.0 mg/ℓ. As in Task 2, male birds appeared to be more vulnerable to the smoke than females.

The findings of these studies show that multiple exposures to RP/BR smoke, up to 4.0 mg/ℓ, are unlikely to pose a significant threat to populations of prairie dogs and rock doves under passive conditions. However, smoke concentrations up to 4.0 mg/ℓ could have significant detrimental impacts in other species (e.g., those with higher respiratory frequencies and sex linked vulnerabilities, and species in which vocalization is important for survival). Additional research is warranted to assess the potential environmental risks of exposing wildlife species to various concentrations of RP/BR smoke under stressful or strenuous conditions.



FOREWORD

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R D Thompson 8/2/90

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 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 Project Order was initiated with the support of U.S. Fish and Wildlife Service, Department of Interior; DWRC transferred to APHIS on March 3, 1986.

Research comprising the Project Order was divided into 3 tasks: Task 1 -- Inhalation Equipment Development/Ambient Carbon Monoxide (CO) Evaluation/ Aerosol Distribution and Air Quality Study (Sterner, Shumake, Johns and Thompson, 1988), Task 2 -- Effective Smoke Concentration Range-finding Determinations (Shumake, Sterner, Johns and Thompson, 1989) 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) (Sterner, Shumake, Thompson and Johns, 1990). Technical reports describing the research efforts involved in each of these tasks are available from: Defense Technical Information Center, Cameron Station, Alexandria, VA 22304-6145.

The current report is the last of 4 reports needed to fulfill the Project Order. It is essentially a summary of the earlier reports, with conclusions and research recommendations added.

Authors' address: USDA/APHIS/S&T, Denver Wildlife Research Center, Building 16, Federal Center, Denver, CO 80225-0266.

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Mr. Henry S. Gardner, Jr., the Contracting Officer's Representative, Health Effects Research Division, USABRDL, provided helpful guidance and advice regarding numerous technical and administrative details related to the research effort.

We also thank The Directorate, DWRC, for allowing us to engage in this research.

Of course, numerous individuals contributed to the technical and administrative activities of this project. Their efforts were recognized in previous Task Reports. We sincerely appreciate their efforts.

Finally, we are most grateful to Ms. Barbara Healzer for her careful typing of this manuscript.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION

A. Background and Objectives

In the past decade, the U.S. Army has stepped up research and development efforts to improve battlefield applications of smoke and obscurant products. Improved products are sought not only as traditional visual masking agents for soldiers but also as electromagnetic masking agents for armor and equipment. In this regard, a red phosphorus butyl rubber (RP/BR) product has shown excellent potential as an improved smoke (Yon, Wentzel and Bane, 1983).

The RP/BR product is dispatched using a hand-thrown canister or a grenade-launching system (Burton, Clark, Miller and Schirmer, 1982). The canister produces a limited cloud, whereas the grenade-launching system fires a salvo of grenades that, upon detonation, produces a dense white smoke from the burning of the dispersed product over a wide area. The smoke consists almost entirely of phosphoric (H_3PO_4) and polyphosphoric acids (e.g., $H_4P_2O_7$, $H_5P_3O_{10}$), with traces of hydrogen (H_2) and carbon monoxide (CO) (Brazell, Moneyhun and Holmberg, 1984; Yon et al., 1983).

The U.S. Army has a regulatory responsibility to ensure that the repeated use of smokes and obscurants does not pose undue health risks to personnel or undesirable environmental impacts on (or nearby) military training reservations. In particular, The Health Effects Research Division, U.S. Army Biomedical Research and Development Laboratory (USABRDL) has been given the mission of determining health and environmental risks associated with munitions and munitions by-products. Smokes and obscurants are a class of munitions.

Although environmental hazards posed by detonations of RP/BR smoke canisters and grenades have been assumed to be negligible, painstaking research is required to fully assess the myriad of variables that reflect environmental and health-effects risks from chemicals. 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, 1983b; Burton et al., 1982), animal behavior-physiology (Aranyi, 1984, 1986), and environmental fate (Van Voris, Cataldo, Ligote, Garland, McFadden, Frederickson, Li, Bean, Thomas and Carlile, 1986). To date, all of these studies involving animals have used laboratory rats as the test model.

The objectives of Project Order 85PP5847 were to determine gross toxicological and sub-lethal behavioral-physiological effects of RP/BR-smoke exposure in 2 wildlife species -- Black-tailed Prairie Dogs (Cynomys ludovicianus) and Rock Doves (Columba livia). The research involved inhalation-chamber studies. Symptomatological variables (plus gross pathology and histopathology examinations) were used to assess toxicity; whereas, spontaneous activity, startle

response, pulmonary function and blood chemistry/hematology variables were used to assess immediate and acute sub-lethal effects of smoke exposure.

B. Wildlife Species

Figure 1 is an illustration of a black-tailed prairie dog and rock dove. Use of wildlife models was viewed as expanding the Army's comparative database of RP/BR-smoke effects in animals/birds. These species were chosen for 4 reasons: (1) their widespread distribution and habitation of areas traditionally used for armor and artillery training exercises (see Figure 1), (2) their representativeness of wild mammalian and avian orders of fauna, (3) their adaptability to laboratory procedures and (4) their familiarity and availability to staff of Denver Wildlife Research Center (DWRC).

1. Black-tailed Prairie Dog

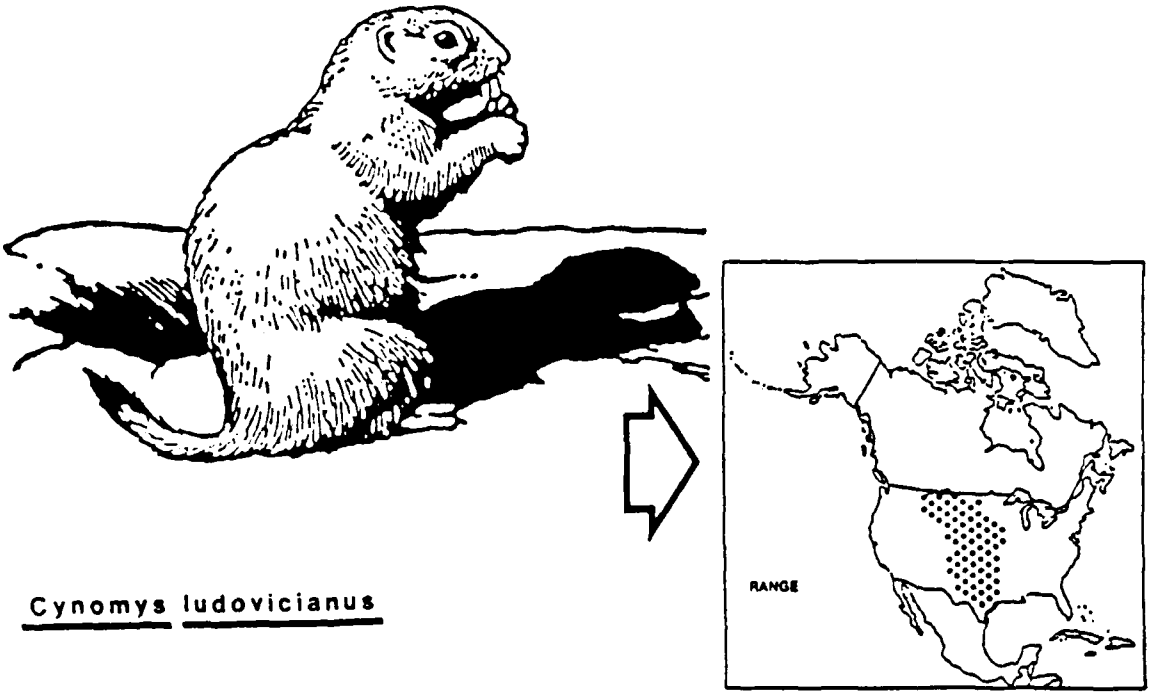
The Black-tailed Prairie Dog is a large, diurnal, herbivorous, burrowing, colonial ground squirrel that inhabits the short-grass prairieland of the North American Great Plains (Jones, Armstrong, Hoffman and Jones, 1983). The species is a member of Order Rodentia and Family Scuiridae (i.e., squirrel). Coloration varies from tan to brown, with lighter ventral hair and black tail. Typical measurements are: 30.5-41.9 cm length and 0.5-1.4 kg weight (Palmer, 1954). The average lifespan of captive males and females is 10.0 and 8.5 years, respectively (Jones et al., 1983).

Behaviorally, prairie dogs are gregarious, but territorial. Hoogland (1985) reported on a typical colony (town) that covered 6.6 hectares (i.e., 500 by 130 m). The colony's average population size was 132.8 (SD $\pm$ 8.9) adults and yearlings (plus 84.1 juveniles); it was comprised of 23.2 (SD $\pm$ 2.3) coteries (i.e., contiguous territorial and breeding groups) annually. Each coterie included 1 adult breeding male ($\geq$ 2 years), 3 or 4 adult females, and several yearling and juvenile offspring -- 8 to 10 animals.

Communication involves a distinct set of bark-like sounds. Commonly recognized communications include the "alarm bark" (i.e., predator warning cry), "all clear" and various snarls, growls and chatters related to territorial and reproductive activities (Jones et al., 1983).

2. Rock Dove

The rock dove is the common feral pigeon. All pigeons belong to the Order Columbiformes. Adult birds typically measure 30-36 cm in length and weigh between 300 and 600 g (Goodwin, 1983; Reilly, 1968). Coloration is mainly gray (except for the rump) with a purplish sheen on the head, neck and breast feathers.



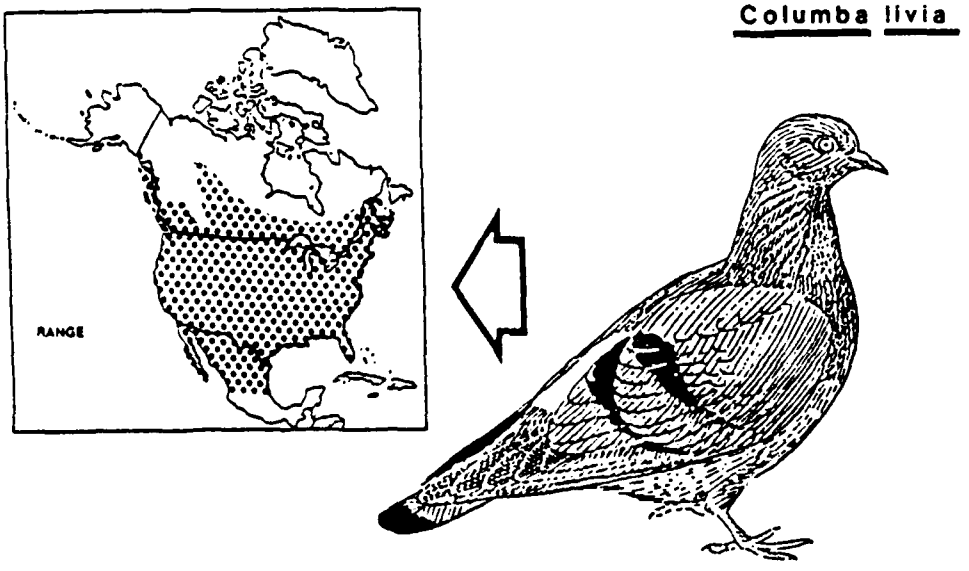


Figure 1. Illustrations of a black-tailed praire dog (Cynomys ludovicianus) and a rock dove (Columba livia), with distributional range maps for each species shown as inserts.

Behaviorally, rock doves cluster in flocks of a few to several hundred. The doves scavenge grain and other foods discarded by man, hence the large urban and farm populations.

Vocalizations are soft and muted coos, with communication limited to only several m distant.

C. Project Timeline

Research comprising Project Order 85PP5847 was divided into 3 tasks: Task 1 -- Inhalation Equipment Development/Ambient Carbon Monoxide (CO) Evaluation/Aerosol Distribution and Air Quality Study (Sterner, Shumake, Johns and Thompson, 1988), Task 2 -- Effective Smoke Concentration Range-finding Determinations (Shumake, Sterner, Johns and Thompson, 1989) 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) (Sterner, Shumake, Thompson and Johns, 1990).

II. TASK 1: INHALATION EQUIPMENT DEVELOPMENT/AMBIENT CO EVALUATION/AEROSOL DISTRIBUTION AND AIR QUALITY STUDY

Task 1 involved 3 objectives: (1) installation and modification of an inhalation chamber and RP/BR-smoke generating system, (2) evaluation of ambient levels of CO within and nearby the research site and (3) evaluation of the spatial and temporal uniformity of RP/BR aerosol and air quality within the chamber (Sterner et al., 1988).

A. Installation and Modification of the RP/BR Extruder and Inhalation Chamber Systems

1. RP/BR Extruder and Inhalation Chamber System

Figure 2 is a technical illustration of the RP/BR Extruder and Inhalation Chamber System. The System was virtually the same as that described by Holmberg et al. (1985) and used by Aranyi (1983a, 1983b, 1984, 1986), with the addition of several features to adapt the System for current studies. These additions were: (1) humidity-control subsystem for the intake air supply to maintain $\geq$ 40 percent relative humidity (RH), (2) HEPA-filter for the intake air, (3) acid-resistant tubing between the RP/BR burn chamber and the inhalation chamber and (4) temperature-control subsystem to cool the intake aerosol.

a. Formulation of RP/BR Product

The RP/BR product was prepared by staff of the Bio/Organic Analysis Section, Analytical Chemistry Division, ORNL. The mixture was formulated from 2.05 kg lots of a 95 percent RP and 5 percent BR product. Following mixing of the dry RP and BR

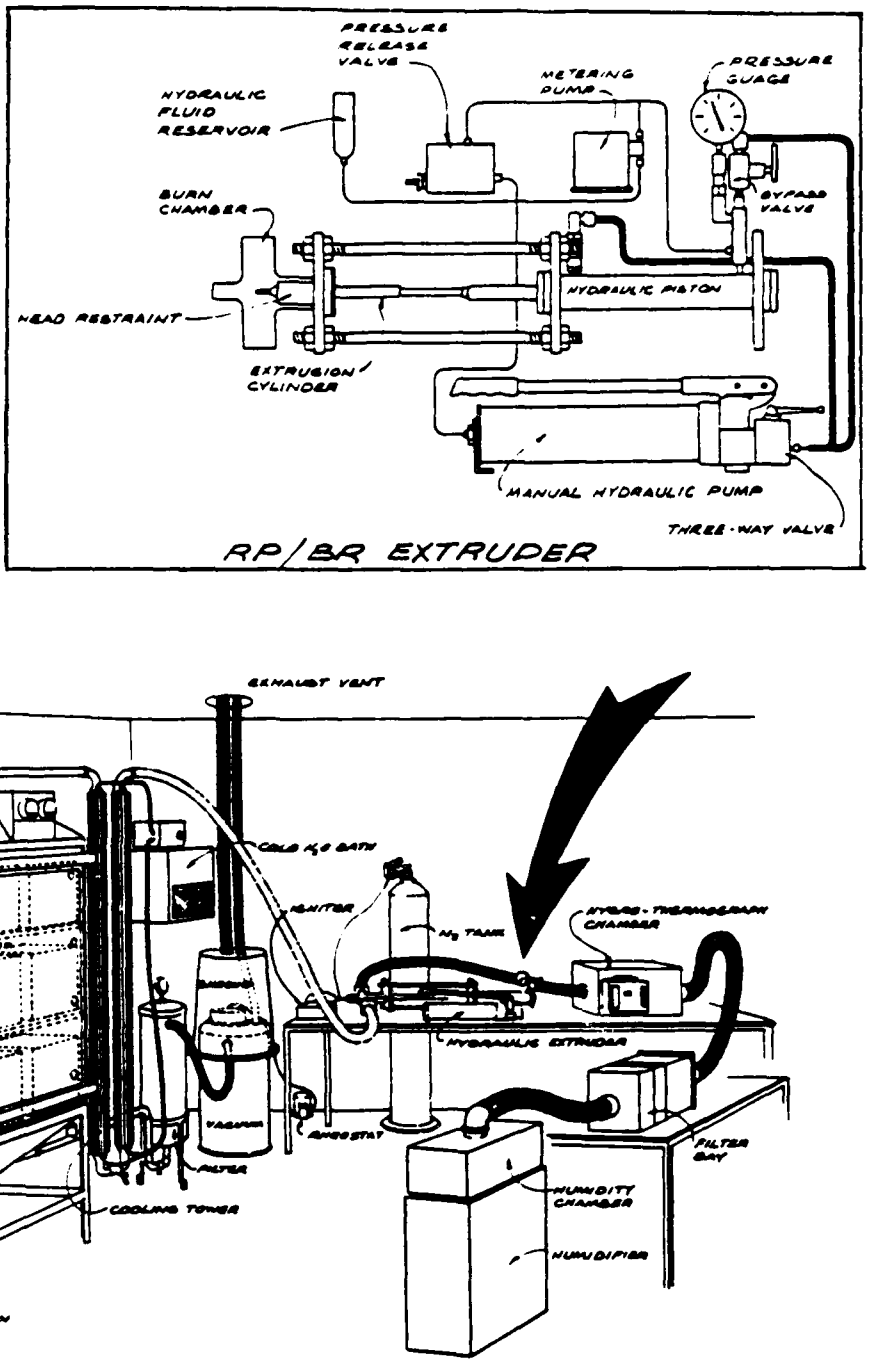


Figure 2. Technical illustration of the RP/BR Extruder and Inhalation Chamber System, with a schematic diagram of the RP/BR extruder-generator subsystem shown as an insert (from Holmberg et al., 1985). (Note. - Components of the System are scaled relative to the perspective, i.e., 1 cm equals 0.236 m, but the locations of some components have been drawn to improve the visual display.)

substances, the product was placed in a vacuum desiccator, and hexane (C_6H_{14})was introduced until 7 to 8 percent (wt/wt) was absorbed. This "softened product" (40g) was then loaded into 11.45 cm, Teflon-sealed sections of stainless steel pipes (billets) and shipped to DWRC. The product was not allowed to age more than 90 days prior to use.

b. Operation of the System

The insert of Figure 2 is a schematic drawing of the RP/BR extruder/generator subsystem. Operation required loading the extrusion cylinder with the formulated RP/BR mixture. This material was then extruded under pressure into a 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. Next, the RP/BR aerosol moved from the glass burn chamber to the apex of the inhalation chamber via flexible, stainless steel tubing.

The chamber was a standard stainless steel unit with autoclave door (Bertke and Young, Cincinnati, OH). The internal chamber had 3 shelves, each holding 4 stainless steel wire mesh animal cages. A polyvinylchloride (PVC) drain and valve was plumbed to the bottom of the chamber and to a floor drain for flushing residues from the interior.

At the base of the chamber, aerosol was exhausted via a standard PVC pipe to a DX-grade filter unit. This filter removed over 99 percent of the aerosol and associated contaminants which exited the chamber (Holmberg et al., 1985). Finally, the scrubbed air flowed via flexible PE tubing to the vacuum source and from there to a ceiling vent. A 30-gallon PVC shroud covered the vacuum source to prevent any residual smoke products from entering the room.

2. Filtered-air Inhalation Chamber System

The Filtered-air Inhalation Chamber System was used for the 0.0 mg/ℓ (control) exposures. This System was located in a separate test room, and was essentially identical to the RP/BR Extruder and Inhalation Chamber System. It was built and installed just prior to Task 2.

B. Evaluation of Ambient CO Levels at the Research Site

Because of the close proximity of DWRC to a heavily traveled highway (U.S. Rt. 6), ambient CO levels both within and nearby the Center were checked at the start of the Project. Numerous CO measurements were made using an Ecolyzer Carboxyhemoglobin Analyzer System (Energetics Science, Hawthorne, NY). No detectable ambient CO was observed at any of the DWRC measurement sites.

C. Spatial and Temporal Uniformity of RP/BR Aerosol and Air Quality Study

Assessment of aerosol uniformity (distribution) within an inhalation chamber is a prerequisite to exposure studies involving humans and animals (Griffiths, Wolff, Beethe, Hobbs and McClellan, 1981; Phalen, 1984). Specification of the in-chamber aerosol distribution ensures that variation in respired lung burdens of the aerosol are due to biological factors, rather than chamber locations.

1. Approach

The study involved 48 RP/BR burns at target concentrations of 0.4, 1.5 and 3.0 mg/ℓ and 3.0, 4.5 and 6.0 mg/ℓ with air-flow rates of 500 and 250 ℓ/min, respectively. Each burn lasted approximately 1 h and 45 min. Spatial uniformity of RP/BR concentration was assessed by sampling aerosol from 12 animal cage sites and the center of chamber. Temporal uniformity was determined by comparing 3, 10-min collections of aerosol during each burn. Homogeneity/heterogeneity of smoke was assessed based upon measurements of aerosol mass, phosphoric acid (H_3PO_4), aerosol opacity and particle size; air quality was determined by routine checks for oxygen (O_2), carbon dioxide (CO_2), phosphine (PH_3), hexane (C_6H_{14}), and CO.

2. Results and Discussion

Results of the spatial and temporal aerosol uniformity study indicated that the RP/BR Extruder and Inhalation Chamber System was acceptable for the exposure of prairie dogs and rock doves to controlled concentrations of RP/BR smoke.

Figure 3 shows the main effect of different air flow rates (250 and 500 ℓ/min) and RP/BR extrusion settings on in-chamber aerosol mass and H_3PO_4 deposition. This graph indicates the expected "target concentrations" of RP/BR aerosol and H_3PO_4 deposition to be associated with selected extrusion (burn) settings.

Several additional specific findings of note included: (a) Cage Site 12 adjacent to the sampling port used for Gastec Analyzer Tube measurements yielded 10 percent lower aerosol mass values than other sites, (b) an overall "dilution effect" occurred for mean aerosol mass and H_3PO_4 titration values collected from the Bottom Shelf of the chamber (i.e., about -3 percent), and (c) a slight gradient of larger aerosol particle sizes occurred from the Top to Bottom Shelf of the chamber --0.52 to 0.55 μm (MMAD), respectively. The lower mass and acid values reflected aerosol dilution from periodic bursts of air entering the chamber in the vicinity of the Bottom Shelf during insertion and withdrawal of Gastec Analyzer Tubes -- an artifact of the air quality assessments. Although these data did not violate the imposed heterogeneity criterion, (i.e,≤ 20% deviation between center of

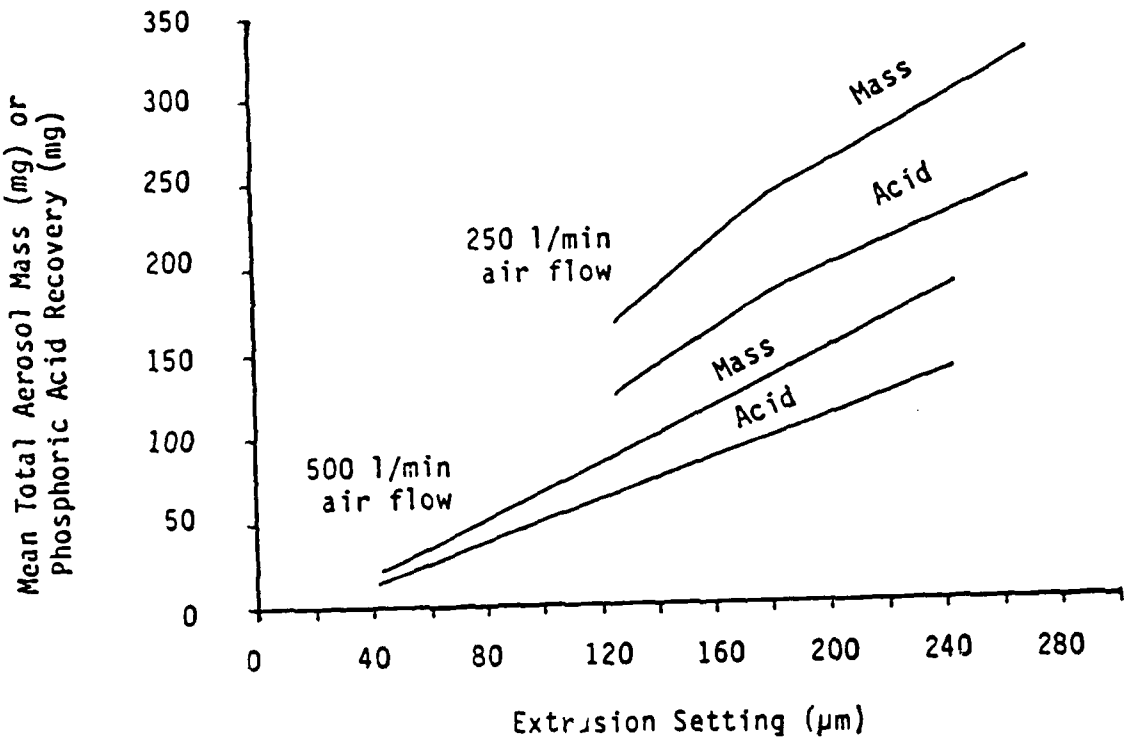


Figure 3. Mean aerosol mass weights (gravimetric analysis) and mean phosphoric acid depositions associated with the RP/BR burns conducted at each of the 3 extrusion settings at 500 ℓ/min (i.e., 43, 123, and 243 μm) and 250 ℓ/min (i.e., 125, 180, and 270 μm) air flow rates.

chamber and cage sites) only Cage Sites 1 to 11 (i.e., exclude Cage Site 12) were used for animal exposures in Tasks 2 and 3. The particle size gradient was of no concern because the range of all particles was small ($\leq$1.0 μm) -- particles with similar pulmonary deposition characteristics (see Phalen, 1984).

Regarding air quality, O_2 and CO_2 levels within the chamber were always at sufficient levels for normal respiratory functions. The presence of PH_3 and C_6H_{14} contaminants was extremely rare and occurred in only negligible quantities. Carbon monoxide posed the only concern. That is, measurable amounts of CO occurred during practically all burns. To avoid the buildup of high levels of CO during behavioral-physiological studies (Task 3), RP/BR-aerosol concentrations were limited to 4.0 mg/ℓ target levels at 250 ℓ/min air flow.

III. TASK 2: EFFECTIVE SMOKE CONCENTRATION RANGE-FINDING DETERMINATIONS

Task 2 was to determine gross toxicological and sub-lethal symptomatological effects of RP/BR-smoke exposure in prairie dogs and rock doves (see Shumake et al., 1989). The goal was to select appropriate RP/BR-smoke-exposure schedules for the behavioral-physiological studies (Task 3).

A. Approach

Task 2 was comprised of 3 range-finding studies -- 2 involved prairie dogs and 1 involved rock doves.

Each study involved a sequential 7-day Pre-exposure, 1- to 4-day Exposure and 30-day Post-exposure Phase. Attempts were made to balance animals by sex within groups. This worked perfectly with prairie dogs, but mis-sexing of several rock doves using a cloacal examination procedure (Miller and Wagner, 1955) led to an unbalanced design with this species.

Initially, a pair of similar studies was conducted with 48 prairie dogs and 48 rock doves. Prairie dog groups were (n=6/group) exposed to 2.0, 4.0 or 6.0 mg/ℓ target concentrations of RP/BR aerosol or filtered-air (0.0 mg/ℓ) over 1 to 4 successive sessions. Rock dove groups (n=6/group) were exposed to 3.0 or 6.0 mg/ℓ target concentrations of RP/BR-aerosol or filtered-air also over 1 to 4 successive sessions.

The third study involved 24 prairie dogs. This was necessitated by the lack of mortality and overt symptomatology observed for this species in the first study. Two groups (n=6/group) received either 3 or 4 successive 80-min daily exposures to a 6.0 mg/ℓ concentration of RP/BR aerosol and 2 groups received 4 successive 80-min daily exposures to 0.0 mg/ℓ (i.e., 1 received minimal handling and 1 received regular handling during symptom examination periods).

Mortality was assessed daily throughout the Pre-exposure, Exposure and Post-exposure Phases. Number of surviving animals and number of days until death were key variables.

Symptomatology assessments were conducted daily during the Pre-exposure and Exposure Phases and then again on the first 7 days plus Days 10, 13, 16, 19, 22, 25 and 28 of the Post-exposure Phase. Specific operational definitions of the symptoms included in the examination of each species were prepared. Qualitative symptoms were: body posture (rest and post-exercise), respiratory congestion (rest and post-exercise), coat or plumage condition, aggression, and vocalization (occurrence and quality). Quantitative symptoms were: water consumption (mℓ/day) and body weight (g).

Symptom examinations took 4 to 6 min per animal. This involved: a visual inspection and determination of body posture and coat or plumage condition, removal of the animal from cage and determination of aggression and respiratory congestion (resting), weighing of the animal, placement of the prairie dog in a large rotating drum (30 cm/sec) for 60 sec or placement of the rock dove in a large flight enclosure and inducing of the bird to make 6 flight crossings of the approximately 3-m cage, removal of the animal from exercise area with determination of congestion/aggression and return of the animal to home cage with determination of post-exercise body posture. Vocalizations were observed throughout the examination period.

On Day 31 post exposure, each animal was euthanized. Post-mortem necropsy examinations included assessments of the following organs by APHIS Veterinary Services personnel: nasal passages, trachea, larynx, epiglottis, bronchi, lungs, heart, liver, spleen and kidneys.

Sections of the nasal passages, bronchi, lungs and liver, plus the entire trachea, larynx and epiglottis, from each animal were preserved jointly in a specimen jar containing formalin solution. The specimens were then shipped to the National Veterinary Services Laboratory, Ames IA for histological evaluation by USDA/APHIS veterinary pathologists.

Regarding data analyses, mortality, qualitative symptoms, gross necropsy and histological variables were analyzed descriptively by comparing frequencies or percentages of scores in different groups of animals. The quantitative symptoms of body weight and water consumption within each study were analyzed using analysis of variance (ANOVA). All ANOVAs were computed using the PROC GLM Program of the SAS package of programs (SAS Institute, Inc., 1985) and Type III sums of squares to determine effects. Designs were multifactor ANOVAs, with Days considered a repeated measures factor (Winer, 1971). Effects were tested at the 0.05 level of significance. Where

significant effects were found, post-hoc Duncan Multiple Range Tests were used for pair-wise comparisons of all means (Waller and Duncan, 1969).*

B. Results and Discussion

Table 1 presents a brief list of the main results gleaned from the toxicity range-finding studies.

1. Mortality Effects

Results of the toxicity range-finding studies indicated that mortality effects of RP/BR-smoke exposure were more pronounced in rock doves than prairie dogs. No prairie dogs died; whereas, 11 of 42 (26%) rock doves succumbed following the diverse aerosol-schedules used in this Task. Moreover, of the 11 deaths for doves, 91 percent were males. These data demonstrate that rock doves are less resistant to the effects of the aerosol than prairie dogs, but more resistant than laboratory rats (see Aranyi, 1983b; Burton et al., 1982).

2. Qualitative Symptomatology

Qualitative symptomatology following RP/BR-aerosol exposures was somewhat similar for both species, but often subtle and difficult to measure. The most pronounced effects were lost or affected vocalization, increased respiratory congestion and abnormal body posture (doves listed forward when severely affected).

Lost or raspy bark for prairie-dogs was prevalent in groups that received multiple 6.0 mg/ℓ exposures; whereas, lost or muted coos characterized almost all aerosol groups of doves.

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* 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).

Table 1. List of important research outcomes obtained from the toxicity range-finding studies.

Category	Description
Mortality	1. Black-tailed prairie dogs were less sensitive to RP/BR-aerosol as compared to rock doves. No RP/BR-smoke-related mortality was observed for prairie dogs; whereas, 11:42 (26%) of rock doves given 1 to 4 daily 80-min exposures to approximately 3.0 and 6.0 mg/ℓ steady-state concentrations of RP/BR smoke died within 8 days of the last exposures (i.e., controls omitted). 2. A significant sex difference in mortality of rock doves was observed, with 10:24 males but only 1:18 females receiving a lethal dose of RP/BR aerosol under the aforementioned exposure schedules.
Qualitative Symptomatology	1. Lost and altered vocalizations (i.e., hoarseness, decreased volume, lowered pitch, decreased frequency) characterized the post-exposure communications of sizable numbers of animals, with prairie dogs showing the most dramatic effects. 2. Increased respiratory congestion characterized the post-exposure respirations of both species, with congestion especially pronounced following brief exercise. 3. Rock doves that succumbed displayed a number of qualitative symptoms (i.e., listing forward posture, congestion, altered vocalization and unkempt plumage), but these were subtle (hard to distinguish without close inspection) and apparent only 24-48 h prior to death.
Quantitative Symptomatology	1. Based on analyses that compared the pre- and post-exposure body weights of prairie dogs and rock doves, only the doves displayed significant depression of body weight after RP/BR-smoke exposure(s). Prairie dogs showed only a slight (1.2%) loss in weight gain post exposure. Rock doves showed direct concentration- and exposure-related losses in weight during Days 1-7 post exposure, with recovery of lost weight nearly complete by Day 28 post exposure. The mean body weights of male doves were more depressed than those of female doves (i.e., sex effect). 2. The water consumption of both prairie dogs and rock doves increased between the 10th to 28th day of the Post-exposure Phase as a function of the number of RP/BR-aerosol exposures. 3. Except for slight evidence of congestion in some prairie dogs and doves, plus a whitish exudate in the trachea of some doves exposed to 6.0 mg/ℓ smoke for 3-4 sessions, no consistent, gross necropsy or histopathological effects attributable to RP/BR aerosol were found.

Respiratory congestion was detected in high numbers of prairie dogs that received 6.0 mg/ℓ concentrations of smoke. Although congestion characterized the post-exposure symptoms of doves, it was difficult to measure; a more pronounced symptom for doves was the listing forward posture often displayed by birds given 2, 6.0 mg/ℓ exposures of RP/BR aerosol. This listing posture also became a predictive sign of death in lethally-dosed doves, typically becoming very pronounced 24 to 48 h prior to death.

3. Quantitative Symptomatology

Regarding quantitative symptomatology, body weight was much more affected in the rock doves than prairie dogs. Rock doves showed a sex difference in their body weight changes from pre-exposure levels after RP/BR-aerosol exposures. Males had much more depression of body weights especially in the 6.0 mg/ℓ Groups given 2 to 4 exposure sessions. In general, male rock doves at the 6.0 mg/ℓ level showed high mortality, and survivors did not recover to their pre-exposure mean body weight levels for the duration of the 28-day post-exposure period. The 6.0 mg/ℓ Groups, compared to the 3.0 mg/ℓ Groups, were more severely depressed and did not return to their pre-exposure body weight levels until 22 days postexposure. Prairie dogs, on the other hand, generally showed only a 1-day loss in weight gain followed by a rapid recovery to pre-exposure levels by the third day post-exposure. Also, the 1-Exposure Groups gained weight slightly faster than the 2-Exposure Groups during post exposure.

Water consumption measures for the 2 species yielded results in partial agreement. In prairie dogs, groups given multiple RP/BR-aerosol exposures consistently drank more post exposure than did the 1-Exposure Group, and this effect was most evident during Days 10 through 28. Similarly, rock dove groups given 1, 2 or 3 exposures displayed increasingly greater water consumption on Days 7 through 25 post exposure.

4. Gross Necropsy

Necropsy findings for both prairie dogs and rock doves were limited. In only a few cases were there excessive amounts of mucus or exudate found in the nasal passages or larynges of doves exposed to the 6.0 mg/ℓ concentration for 3 to 4 exposure sessions.

5. Histopathology

Despite the detection of several pathological tissues in rock doves, it was concluded that "no lesions, attributable to red phosphorus/butyl rubber smoke exposure, were found in the tissues examined." Of course, lack of standard histology slides for both

species, the pathologists' limited familiarity with acid-aerosol insults and the delayed collection of tissue samples (i.e., 31 days) could have contributed to the lack of detected pathology.

IV. 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).

Task 3 sought to assess sub-lethal effects of multiple RP/BR-aerosol exposures upon selected behavioral and physiological variables in prairie dogs and rock doves (Sterner et al., 1990).

A. Approach

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

1. 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 effects of RP/BR-aerosol inhalation was based on changes in responses that occurred across these Phases.

2. Prairie Dogs and Rock Doves

Altogether, 96 prairie dogs (48 males, 48 females) and 96 rock doves (49 males and 47 females) were used in Task 3, plus approximately 30 animals of each species that participated in pilot studies. A total of 24 prairie dogs and 24 rock doves were used in the respective behavioral-physiological studies.

During all non-test portions of the Startle Response, Pulmonary Function and Blood Chemistry/Hematology Studies, animals were housed in the main prairie dog or rock dove holding areas. Prairie dogs were held individually in stainless steel cages (61 X 62.5 X 41 cm); whereas, rock doves were held individually in galvanized wire mesh cages (51 X 27 X 38 cm). Prairie dogs and doves involved in the Spontaneous Activity Study were acclimatized in the holding areas, but were moved to an activity test room for

study; measurements involved housing animals individually in special Plexiglas cages (42.2 X 43.2 X 31.7 cm) during conduct of this Study.

3. Inhalation-exposure Systems

The RP/BR Extruder and Inhalation Chamber System and the Filtered-air Inhalation Chamber System were described in Tasks 1 and 2 (see Sterner et al., 1988; Shumake et al., 1989).

4. RP/BR-aerosol and Filtered-air Characterizations

Characterization of the chamber atmosphere present for a sample of RP/BR-aerosol or filtered-air exposures during the behavioral-physiological studies was accomplished using variables cited for Tasks 1 and 2 (see Sterner et al., 1988; Shumake et al., 1989).

5. Experimental Designs and Statistical Analyses

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 and (c) sex of animals.

All designs involved 3 separate groups of prairie dogs or rock doves which received 4- or 2-successive 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).

Figure 4 presents a schematic diagram showing the behavioral-physiological sampling sequences during Task 3. Except for the Blood Chemistry/Hematology Studies, all designs involved measurements during 1 to 3 Pre-exposure Sessions, either 4 (prairie dogs) or 2 (rock doves) Exposure Sessions and 2 to 6 Post-exposure Sessions. Sessions refer to the collection of particular measurements (e.g., 12 min startle response session) during a portion of a day; whereas, the Day Factor in the ANOVAS refers to the respective Pre-exposure, Exposure and Post-exposure Day when sessions occurred. Blood chemistry/hematology measurements were limited to 1 Pre-exposure, 1 Exposure and 2 Post-exposure Session(s) to reduce stress upon animals during blood-sampling procedures. The longest chronological period encompassed by any study was 18 consecutive days (i.e., Prairie Dog Startle Response Study).

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/group). Designs with rock doves in the Spontaneous Activity and Pulmonary Function Studies were unbalanced; designs for the Startle Response and Blood

	STUDY	PHASE																
		Pre-			Exposure				Post-									
		3	2	1	1	2	3	4	1	2	3	4	5	6	7	8	9	10	11	12
Prairie Dog	Spontaneous Activity		*	*	*	*	*	*	*	*	*	*	*	*						
	Startle Response		*	*	*	*	*	*	*	*									*	*
	Pulmonary Function	*	*	*	*	*	*	*	*		*			*						
	Blood Chemistry/ Hemotology			*				*		*				*						

	STUDY	PHASE																
		Pre-			Exposure		Post-											
		3	2	1	1	2	1	2	3	4	5	6	7	8	9	10	11	12
Rock Dove	Spontaneous Activity		*	*	*	*	*	*	*	*	*	*						
	Startle Response		*	*	*	*	*	*			*	*					*	*
	Pulmonary Function	*	*	*	*	*	*		*		*			*				
	Blood Chemistry/ Hemotology			*		*		*				*						

Figure 4. 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.)

Chemistry/Hematology Studies with doves were balanced. 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 bird's reproductive organs.

Data for each experiment were analyzed separately using ANOVA. Balanced data sets were analyzed with the PROC ANOVA Program of the SAS Package of programs (SAS Institute, Inc., 1985); whereas, unbalanced data sets were analyzed with the PROC GLM Program of this Package and Type III sums of square (SAS Institute Inc., (1985). Designs were multifactor ANOVAs, with Day(s) generally treated as a repeated measures factor (see Winer, 1971). The 0.05 level of significance was used with all tests. Duncan Multiple Range Tests were used to compare means of significant ANOVA effects (Waller and Duncan, 1969).*

6. Specific Procedures

a. Spontaneous Activity

Measurements of activity were obtained using the Opto-varimex Activity System. This consisted of 8 infrared-type movementdetection units (Columbus Instruments International Corp., Columbus, OH), with auxiliary computer peripheral devices adapted to provide for the automatic storage and transmission of data (AEON Electronics, Denver, CO). Each unit consisted of a rectangular base (51.1 X 69.2 X 9.5 cm) with a large open area (43.2 X 44.4 cm) for insertion of a Plexiglas housing box (42.2 X 43.2 X 31.7 cm). Along 2 adjacent edges of the base were 15 infrared sources, with matching infrared detectors located and aligned along the opposite sides. Supplying 110 Vac to the units caused 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 were tallied as spontaneous activity.

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* See footnote on Page 11.

Two types of beam interruptions were discriminated: horizontal and ambulatory activity. Horizontal breaks of the light beams reflected all movements of an animal (e.g., grooming, scratching, walking). Ambulatory interruptions of the beams reflected only lateral movements of an animal (e.g., walking, jumping) -- no output resulted from repeated interruptions of the same beam.

System hardware provided for the automatic acquisition of activity measurements from each Opto-varimex Unit. All hardware and software functions of the System were governed by a Horizon II Computer, with activity counts during 46 continuous, 30-min daily periods (i.e., 23 h) automatically stored on a floppy diskette.

All activity measurements were conducted in a separate "activity test room" at DWRC; 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 the room during the studies was about 20 C^{O} (19-24) and 33 (21-88) percent, respectively. No visual isolation of animals was provided among activity units.

Procedures for the collection of horizontal and ambulatory activity were essentially the same throughout the Pre-exposure, Exposure and Post-exposure Phases. Each day involved a standard 23 h activity period (i.e., 0900-0759 h) and a 1-h maintenance period (i.e., 0800-0859 h). The daily body weight, food intake and water intake of each animal was determined during the 1-h maintenance period.

b. Startle Response

The SR-LAB San Diego Instruments Startle Response System used in these studies was a computerized test apparatus designed to monitor and record 3 measures of startle: maximum peak response (mV), average response (mV) and latency-to-peak response (msec). Animal response information was recorded after each startle stimulus presentation in terms of mV changes over each msec of recording. All stimulus parameters (i.e., duration, intensity, frequency and inter-trial interval) were computer-controlled. White noise prepulses (40 msec at 76 to 81 dB), pure tone pulse stimuli at 7.8 and 15 kHz (both at 5 msec and approximately 109 dB), along with brief, mild foot shock (45 msec at 1.5 ma or 200 VAC) were the startle stimuli used with prairie dogs. White noise prepulses (40 msec at 76 to 81 dB) and electronic photoflashes (F18 to F22) were the startle stimuli used with rock doves.

Twenty stimulus presentation trials were conducted within 2 h of each smoke exposure and on each of the 2 Pre-exposure and 4 Post-exposure Days with prairie dogs. Each 14 min test session consisted of 4 presentations of 5 types of trials: (1) electrical foot shock, (2) a 40 msec white noise pulse followed after 40 msec by a brief electrical foot shock, (3) no shock (control), (4) a 15 kHz tone pulse for 5 msec, and (5) a 7.8 kHz tone pulse for 5 msec. The mean values for the 3 measures of startle response for each animal were calculated for each test session.

Twelve stimulus presentation trials were conducted within 2 h of each smoke exposure and on each of the 2 Pre-exposure and 6 Post-exposure Days with rock doves. The 12 min test sessions were comprised of 3 presentations each of 4 types of trials (1) a brief electronic photoflash (2) no flash (control) (3) a 40 msec white noise pulse followed in 40 msec by the photoflash and (4) the 40 msec white noise prepulse alone. Mean values for the 3 measures of startle for each dove were calculated for each session.

Sham trials with no prairie dogs or rock doves in each of the 2 test chambers were also conducted after each daily test session to detect and check for stimulus artifact effects. Artifacts refer to spurious voltage changes that occur due to miscellaneous factors (e.g., power surges, platform vibrations). Data for these trials were also averaged for each session and analyzed for significant effects. Whenever stimulus artifacts were detected, those specific trial types or sessions that were significantly different from others in the empty chamber condition were omitted from statistical interpretations of the actual startle data.

c. Pulmonary Function

Five variables were used to characterize RP/BR-aerosol effects upon pulmonary function of the animals: respiration frequency (Rf), volume of O_2 consumed (Vo_2), volume of CO_2 exhaled (Vco_2), the average respiratory exchange rate (RER) and metabolic rate (MR).

Respiratory measurements were obtained in a darkened room with a computer-controlled Oxymax-85 Pulmonary Function System (Columbus Instruments, Columbus, OH). The System was equipped and programmed to simultaneously measure Rf, Vo_2, Vco_2, RER, and MR of 2 animals held in adjacent Plexiglas chambers (43 X 43 X 33 cm). A visual barrier separated these chambers. Measurements were recorded for 2h, with the data for the second h used in analyses. Measurements of each chamber required 1 min and were alternated every other min (30/h).

d. Blood Chemistry/Hematology

An IL 1306 Blood Gas Analyzer and an IL 282 CO-Oximeter (Instrumentation Laboratory, Lexington, MA) were used to determine the 7 blood chemistry variables for the animals: pH, O_2 partial pressure (Po_2), CO_2 partial pressure (Pco_2), hemoglobin (Hb), percent oxyhemoglobin (O_2Hb), percent carboxyhemoglobin (COHb) and percent methemoglobin (MetHb). The blood gas analyzer was used to determine pH, Po_2 and Pco_2; whereas, the co-oximeter was used for Hb, O_2HB, COHb and MetHb. The hematology variables of percent packed cell volume (PCV), total white blood cell count (WBC) and percent differential WBC were determined by a commercial laboratory using standard techniques.

Blood was drawn from the femoral veins of prairie dogs and the ulnar veins of rock doves. Prairie dogs were immobilized with ketamine hydrochloride for blood collections, but no anesthesia was used for rock doves.

B. Results and Discussion

Results of the behavioral-physiological studies confirmed certain of the mortality/symptomatology findings noted for the toxicity range-finding studies and revealed that multiple exposures to RP/BR smoke produces a number of subtle, immediate and acute behavioral-physiological effects mainly in rock doves.

1. Mortality/Symptomatology

No prairie dogs died during the course of Task 3. The most troublesome symptom concerned the raspy and hoarse bark displayed by a number of the animals exposed to 4.0 mg/ℓ RP/BR aerosol. These 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) of RP/BR smoke could alter vocalizations and interfere with communication systems of these rodents.

Altogether, 4 male rock doves died (4:49 males) following 2, 80-min RP/BR-smoke exposures; all lethally-dosed birds were in the 4.0 mg/ℓ RP/BR-aerosol Groups. Specifically, deaths and days-to-death of these doves within specific studies were as follows: Spontaneous Activity (1 at 3 days post exposure), Startle Response (1 at 6 days post exposure), Pulmonary Function (2 at 5 days post exposure) and Blood Chemistry/Hematology (0 deaths).

Although antecedent respiratory, postural and vocalization symptoms were difficult to discern for doves, lethally-dosed birds almost invariable displayed congestion, forward listing posture and raspy cooing, especially within 48 h of death. Necropsy

examinations also revealed a high incidence of a "white exudate material" in the trachea of the 4.0 mg/ℓ birds -- data in agreement with Shumake et al. (1989).

Analyses of auxiliary measurements to corroborate RP/BR-aerosol-induced, sub-lethal toxicosis effects upon consummatory and body weight variables in the 2 species were monitored during the Spontaneous Activity Study. These confirmed and extended findings of Task 2. Essentially, results demonstrated that the current exposure schedules produced acute decreases in body weight, food and water intake of both prairie dogs and rock doves -- with recovery generally completed by 3 days post exposure.

2. Behavior/Physiology

a. Spontaneous Activity

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). Preache, as reported in Aranyi (1984), observed increased locomotor activity in laboratory rats following RP/BR-aerosol exposure. Groups of rats received 4 to 16, 2.25-h/day inhalation-chamber exposures of 0.75 to 1.2 mg/ℓ RP/BR-aerosol concentrations. Separate 20-min measurements of rat movements through a "figure-8 maze" were made immediately after the final inhalation exposure and 14 days later. 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 locomotor activity was noted.

The current studies were to assess the effects of sub-lethal RP/BR-aerosol exposure upon the spontaneous activity of black-tailed prairie dogs and rock doves. Mild irritation from the deposition of H_3PO_4 on mucosal tissue at the lower concentration (1.0 mg/ℓ) was predicted to cause greater horizontal activity (grooming, preening) immediately after exposure. Conversely, the general malaise caused by H_3PO_4 burns of respiratory pathways and mucosal tissues at the high (4.0 mg/ℓ) concentration was expected to yield temporary lethargy and decreased ambulatory activity during the Post-exposure Phase.

(1) Prairie Dogs. -- No effects for the horizontal activity counts were found when measured within 2h of exposure (immediate effects). Thus, the hypothesis that the potential irritation caused by H_3PO_4 on the mucosal tissue of RP/BR-aerosol-exposed prairie dogs would lead to increased grooming immediately after exposure is unsupported. This lack of effects could have other implications. No change in grooming-

type activity can be viewed as indicative of the benign nature of the aerosol or that continued grooming would allow prairie dogs to ingest considerable H_3PO_4.

Figure 5 is a plot of the Concentration X Day interaction which characterized the ambulatory counts for prairie dogs during the Exposure Phase. The graph shows that the interaction was 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 counts of control animals (0.0 mg/ℓ) following the last 2 exposures. Essentially, this finding is contrary to the earlier report by Preache (see Aranyi, 1984) which noted increased locomotor activity in laboratory rats immediately after prolonged bouts of RP/BR-aerosol exposure to about 1.0 mg/ℓ concentrations. The current interaction shows that exposure to RP/BR aerosol (4.0 mg/ℓ) causes immediate post-exposure lethargy, not hyperactivity, in prairie dogs.

Regarding acute effects, only the Light On/Off main effects for the horizontal and ambulatory activity counts were significant. Light On mean activity counts were roughly double those during Light Off. For a diurnal species such as the black-tailed prairie dog, this pattern of means gives validation to the activity readings.

(2) Rock Doves. -- Mean horizontal and ambulatory activity counts of doves for the immediate sessions (i.e., E-1 and E-2) yielded identical results. Both variables yielded significant Concentration X Day interactions and Day main effects. Because of the similarity of these statistical effects, interpretations are presented jointly.

Figures 6a and 6b 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. Duncan Tests for mean horizontal and ambulatory counts indicated that doves in the 4.0 mg/ℓ-aerosol Group made significantly fewer preening and lateral movements immediately following exposure than doves in the remaining conditions. Conversely, doves in the 0.0 mg/ℓ Group made more movements immediately after exposure than 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. In short, a general chamber-confinement stress caused significantly greater preening, wing-flapping, strutting movements for doves immediately after the second chamber-confinement session (E-2) as compared to the 2-h period after the first session. Analogous to observations for prairie dogs, RP/BR aerosol produced brief periods of hypoactivity in doves (not hyperactivity) immediately after exposure.

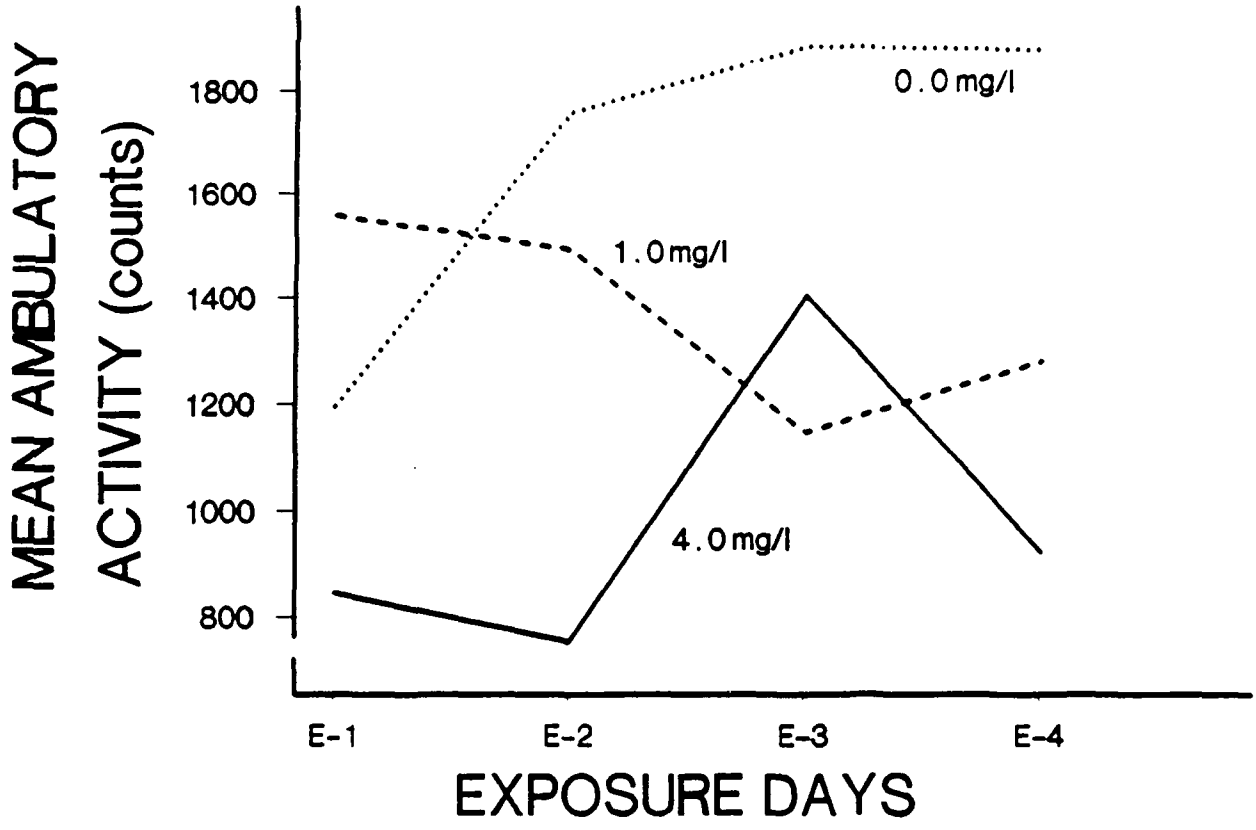
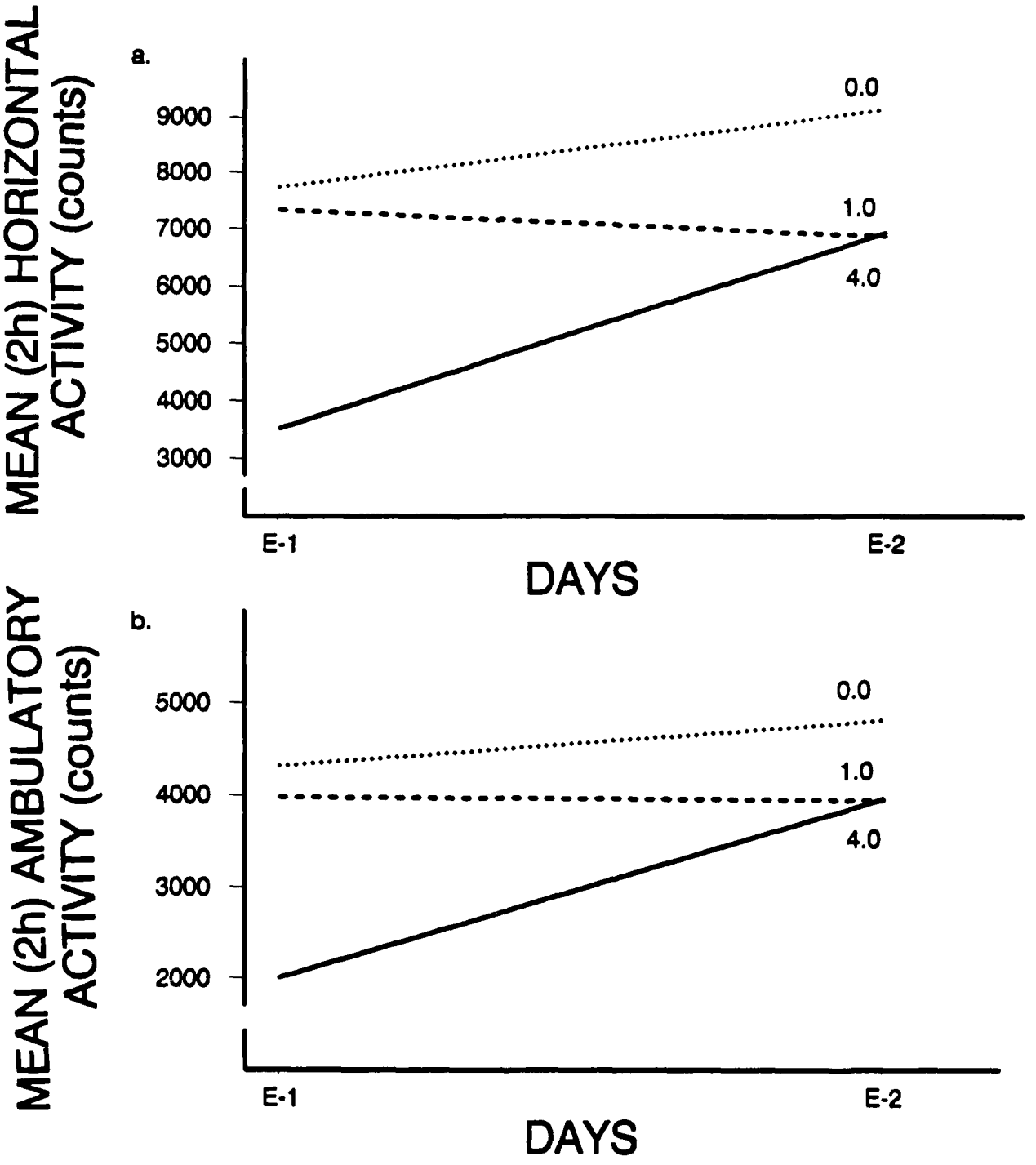


Figure 5. Graph of the mean ambulatory activity counts for prairie dogs in the 0.0 (Filtered-air), 1.0, and 4.0 mg/ℓ RP/BR-aerosol Groups during the 2 h immediately after chamber confinement on each of the Exposure Days (E-1 to E-4) -- the Concentration X Session interaction effect.



Figures 6a and 6b. 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 acute effects of mean daily "light on and off" activity yielded a relatively complex set of results. Sex X Day X Light On/Off and Sex X Day interactions, plus the Light On/Off main effect, were significant sources of variance for horizontal activity; whereas, the Sex X Day X Light On/Off interaction and Light On/Off main effect were significant for ambulatory activity. Data indicated that male and female birds differed in their daily diurnal and nocturnal activity, but that these effects were induced by chamber-confinement stressors, rather than RP/BR-aerosol exposures, per se. Duncan Tests showed that: (a) Post-exposure (Day P-1) diurnal means for female doves were significantly less than all other diurnal means and (b) all diurnal versus nocturnal sub-group comparisons were significantly higher within male and female groups (i.e., the Light On/Off effect).

Together, the findings for doves indicated that a general chamber-confinement stress operated to lower both the horizontal and ambulatory activity of females during the diurnal portion (Light On) of the light cycle. This acute effect lasted about 24 h after the last exposure (P-1). Two successive daily 80-min sessions of either 1.0 or 4.0 mg/ℓ RP/BR-aerosol exposure produced minimal acute effects (1 day) upon the spontaneous activity of rock doves.

b. Startle Response

Numerous reports have demonstrated 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 $\geq$ 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 direct accounts of prairie dog startle reactions to RP/BR aerosol exist, measures of peak amplitude and average response amplitude were expected to decrease, whereas time-to-peak amplitude was expected to increase, following exposure. These changes were 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 was predicted to produce reduced inhibition of peak and average response

amplitudes, and increased response latency after RP/BR-smoke exposures in both species.

A summary table of the main results obtained for the startle response variables with prairie dogs and rock doves is shown in Table 2.

(1) Prairie Dogs. -- A number of general findings related to the effectiveness of the startle stimuli were noted. Neither male nor female prairie dogs showed reliable or sustained startle responses to the pure tone stimuli. Only females responded consistently to the electrical foot shock stimuli, with peak amplitude, average response and latency-to-peak measures significantly altered. The white noise prepulse was not effective in reliably suppressing startle response to foot shock.

The startle response behavior of prairie dogs was generally unaffected by exposures to RP/BR smoke. Nevertheless, a significant Concentration X Trial Type interaction was detected for the latency-to-peak measure. This 2-way effect was due to different latency patterns for Trial Types (No Shock, Shock, Pre-pulse Shock) in the 2 RP/BR-smoke Groups (1.0 and 4.0 mg/ℓ) throughout the 10 test sessions (i.e., days).

(2) Rock Doves. -- Rock doves of both sexes showed reliable responses to brief photoflash stimuli on peak amplitude, average response and latency-to-peak variables. In addition, the rock doves in all groups showed a suppression of peak amplitude and average startle response to photoflash stimuli when the brief white noise pre-pulse preceded each of the flashes by 40 msec.

The latency-to-peak data for female doves yielded a significant Concentration X Trial Type X Session interaction effect. This 3-way interaction indicated that : (1) birds in the 4.0 mg/ℓ Group had the greatest differences in latencies among the 4 Trial Types compared with the 0.0 and 1.0 mg/ℓ Groups, (2) all rock doves showed sharp decreases in mean latencies on the first exposure day (E-1) and (3) decreased latencies were mainly associated with photoflash-type trials. Doves in the Filtered-air (0.0 mg/ℓ) Group showed a pattern of average decreased startle latencies across sessions (days) that was similar to, and intermediate between, the 1.0 and 4.0 mg/ℓ Group birds. This indicated that only a subtle and weak change in latency of startle was generated by the RP/BR-smoke exposures. This change was superimposed upon a significant chamber-confinement effect (i.e., all groups displayed decreased latencies to startle following chamber confinement on Day E-1).

Table 2. Summary of main results for the Startle Response Studies with prairie dogs and rock doves.

Stimuli	Prairie Dog (Males)	Prairie Dog (Females)	Rock Dove (Males)	Rock Dove (Females)
Pure Tone	7.8 and 15 KHz-tone stimuli not effective after first Pre-exposure Session (Day Pre-1).	7.8 and 15 KHz-tone stimuli not effective after first Pre-exposure Session (Day Pre-1).		
Electrical Foot Shock	Shock levels not sufficient to elicit reliable responses.	Shock levels sufficient to elicit reliable responses, but no pre-pulse suppression effect observed. Concentration X Trial Type interaction observed, but found unrelated to smoke exposures (msec latency-to-peak).		
Photoflash			Reliable startle responses elicited, but no direct RP/BR-smoke effects found.	RP/BR-smoke effect super-imposed upon a stronger chamber-confinement effect (msec latency-to-peak).
Prepulse & Photoflash			White noise prepulse effectively suppressed peak amplitude (mv) and average response voltage (mv).	RP/BR-smoke effect super-imposed upon a stronger chamber-confinement effect (msec latency-to-peak.)

c. Pulmonary Function

This section describes the effects of RP/BR-aerosol exposure on pulmonary function variables of each species. The rationale for the research was based on a report showing that RP/BR smoke causes sclerosis, irritation and alveolar lung tissue scaring in rats (Burton et al., 1982). The hypothesis tested was that RP/BR smoke would adversely impact pulmonary function variables in the 2 species.

(1) Prairie Dogs. -- All pulmonary function values were in a range expected for prairie dogs or other rodent species of comparable size (Altman and Dittmer, 1974; Fowler, 1986; Rugh, 1968). Results of ANOVA for each variable revealed that only 1 of 28 p values was significant, the Day main effect for RER. In short, there was no mortality, evidence of toxicity or pulmonary impairment due to RP/BR smoke in prairie dogs.

(2) Rock Doves. -- All pulmonary values, excluding the 2 birds that died, were typical of those reported for pigeons (Abs, 1983). Results of ANOVA showed that there were no significant effects for Rf; however, there was a significant Day effect for Vo_2 and significant Concentration X Day interaction effects for Vco_2 and MR.

The Concentration X Day interaction means for Vco_2, RER and MR are depicted in Figures 7, 8 and 9, respectively. These figures show that the pattern of response across Days was similar for each variable. At 0.0 and 4.0 mg/ℓ RP/BR-smoke concentrations, Vco_2, RER and MR were significantly decreased below Pre-exposure levels during the Exposure Phase; whereas, at 1.0 mg/ℓ there was no significant change in any variable until Post-exposure Day P-3 when $Vco_2$and MR were elevated above all other days. 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 7). With RER, there was no difference between 0.0 and 4.0 mg/ℓ smoke exposures until Post-exposure Day P-8 (Figure 8); thus, this 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 higher metabolic value for the 1.0 mg/ℓ Group than for 0.0 or 4.0 mg/ℓ Group on Day E-1 and Days P-1 and P-3(see Figure 9). 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 values at 0.0 and at 4.0 mg/ℓ shows that chamber confinement or handling probably affected pulmonary function as much as the 4.0 mg/ℓ smoke exposure in rock doves.

Even though Sex was not a statistically significant factor in this study, it appeared that male rock doves were more vulnerable to RP/BR-smoke than female doves. Evidence for this

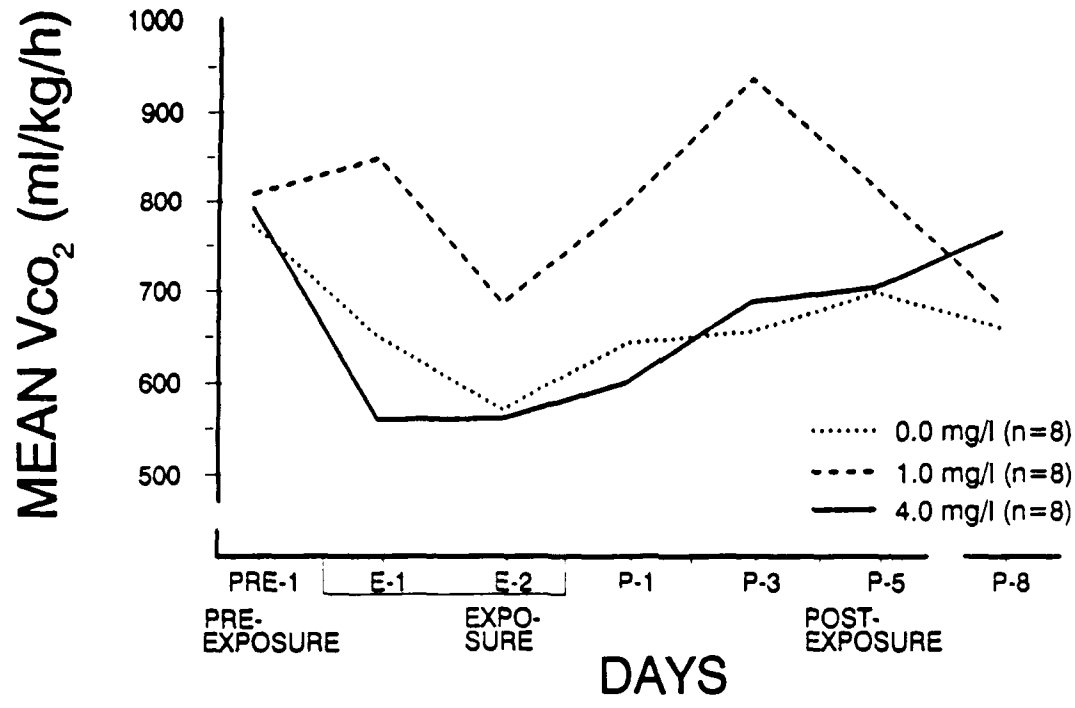


Figure 7. Carbon dioxide production (Vco_2) Concentration X Day interaction means of rock doves before, during and after exposure to 3 concentrations of RP/BR smoke.

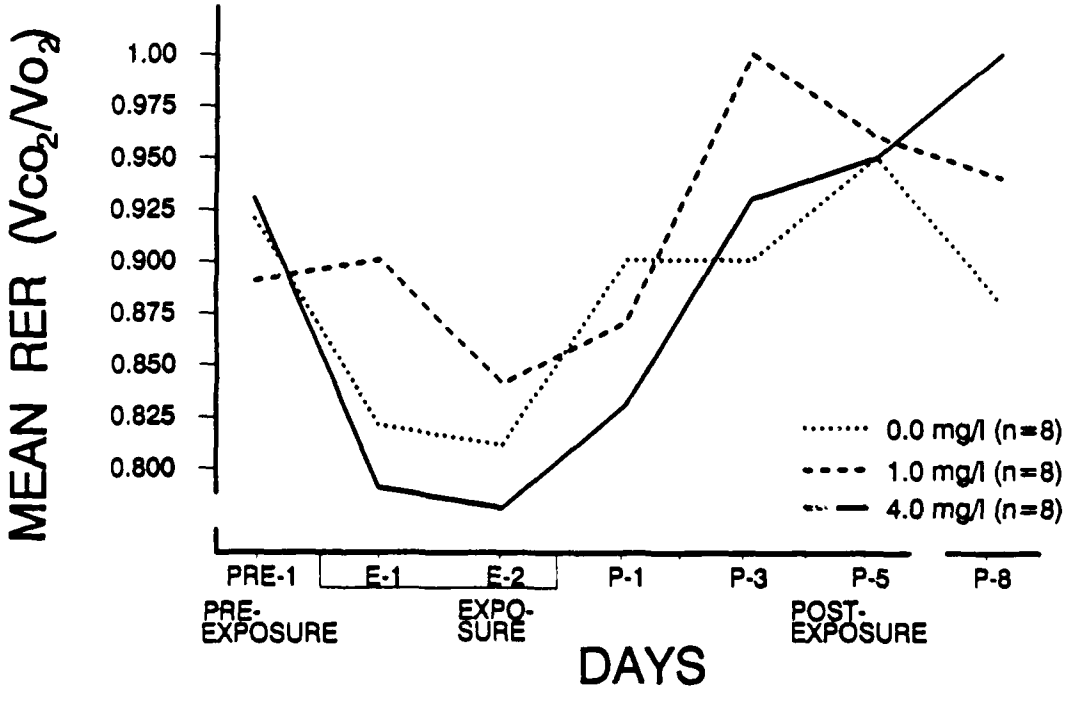


Figure 8. Respiratory exchange ratio (RER) Concentration X Day interaction means of rock doves before, during, and after exposure to 3 concentrations of RP/BR smoke.

can be seen in Figure 10, where mean Rf on Day E-2 for the 2 males that subsequently died was 16 percent higher than the corresponding Rf of surviving 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 (4.0 mg/ℓ) and suppression of Vco_2 on Day E-1 (4.0 mg/ℓ), the results provide no evidence that RP/BR smoke caused any effects on pulmonary function of rock doves.

d. Blood Chemistry/Hematology

The following results present findings of the Blood Chemistry/ Hematology Studies for the 2 species.

(1) Prairie Dogs. -- The Hb, O_2Hb, MetHb and COHb values were within the normal mammalian range for venous blood. The pH, Pco_2 and Po_2 values also fell within the range of expected values for rodent venous blood. The PCV, WBC and differential WBC values were typical of other rodents (Jain, 1986).

Results of ANOVA for blood chemistry variables showed no significant RP/BR-aerosol concentration effects; however, a significant Day effect was found for 5 of the variables: PO_2, Hb, O_2Hb, COHb, and MetHb. The hematology variables showed only 1 significant effect, the Day effect for PCV. These Day effects were thought to reflect different levels of stress resulting from collecting blood samples or differences in depth of anesthesia. The blood chemistry and hematology results clearly show that prairie dogs are very resistant to multiple exposures of low and high concentrations of RP/BR smoke.

(2) Rock Doves. -- Blood values for doves in the 0.0 mg/ℓ RP/BR smoke Group were in the typical range for unanesthetized pigeons (Abs, 1983).

Results of the PROC ANOVAs for blood chemistry variables revealed a significant Concentration X Sex interaction effect for Hb and MetHb and a significant Day effect for pH, Pco_2, Hb and COHb.

The Concentration X Sex effect for Hb was caused by the female doves having significantly lower Hb than males at 0.0 mg/ℓ and 1.0 mg/ℓ RP/BR smoke, but equivalent Hb to males at 4.0 mg/ℓ RP/BR smoke. Moreover, females reacted to the RP/BR smoke at the 4.0 mg/ℓ concentration with increased Hb; whereas, the males showed no significant change among RP/BR-smoke concentrations. The ability of females to significantly increase Hb at 4.0 mg/ℓ RP/BR smoke may help to explain why

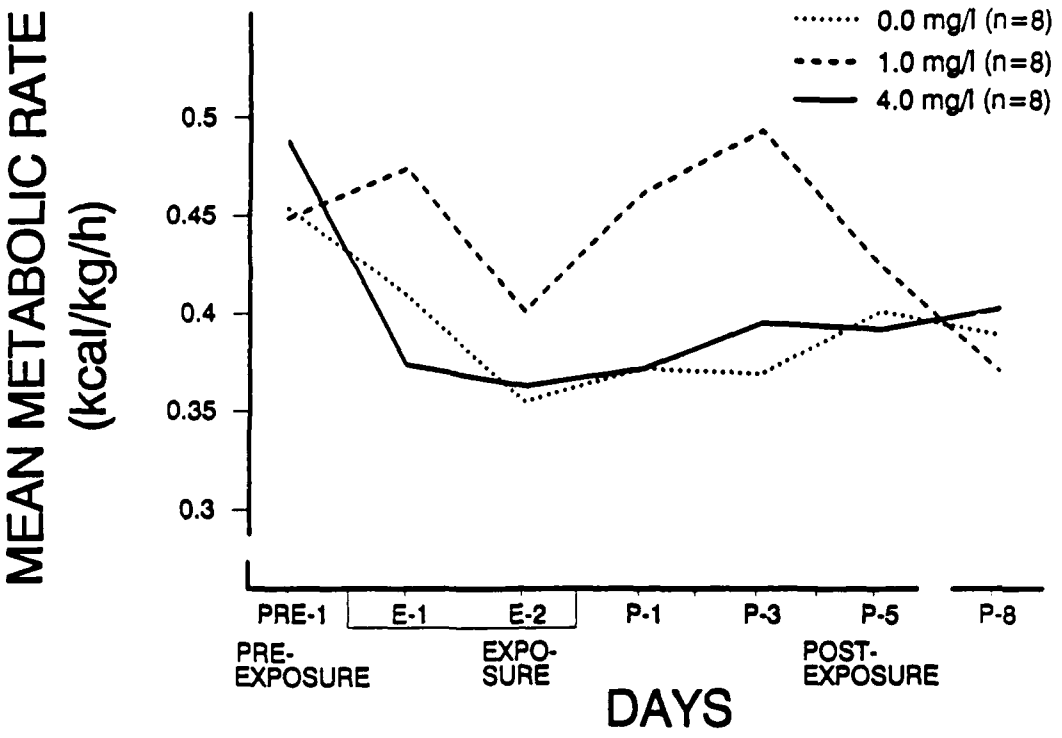


Figure 9. Metabolic rate (MR) Concentration X Day interaction means of rock doves before, during and after exposure to 3 concentration of RP/BR smoke.

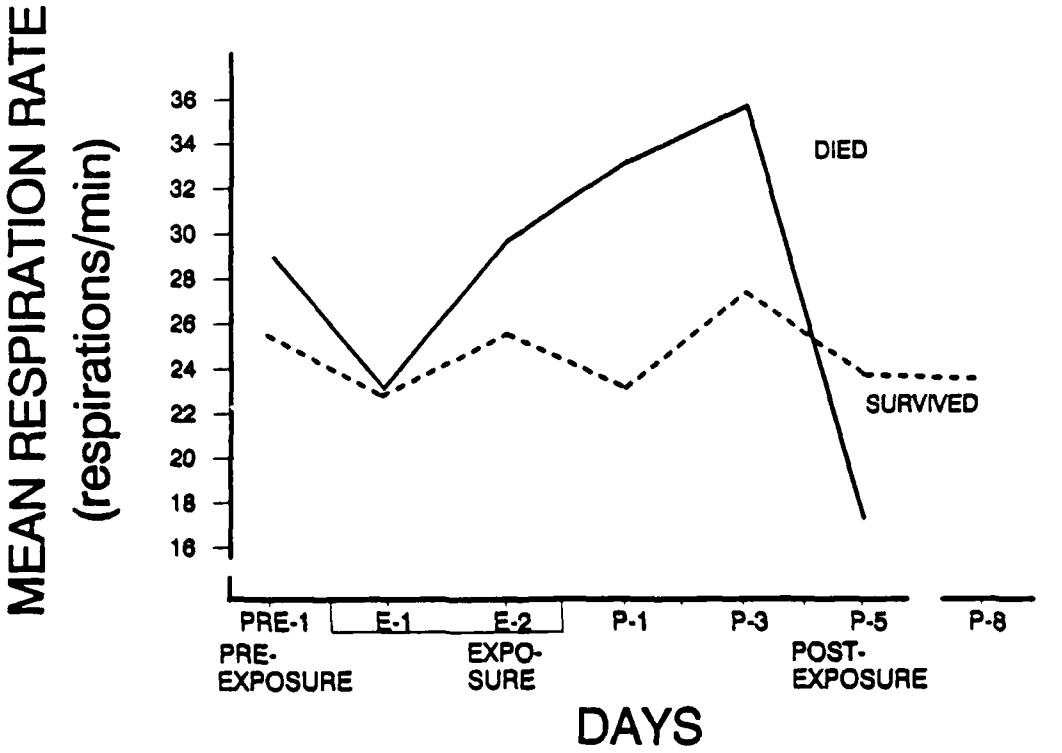


Figure 10. Mean respiration rate (Rf) of 2 male rock doves that died and of 6 rock doves that survived following exposure to a 4.0 mg/ℓ concentration of RP/BR smoke.

they were less vulnerable than males. The MetHb values appear to be too low to interfere with normal oxygen transport but may be an indicator that points to males being more vulnerable to the smoke than females.

The Day main effects appeared to be unrelated to RP/BR aerosol. These seemed to be associated with the stress of being handled, bled and confined in the chamber.

The ANOVAs for the hematology variables revealed 4 significant effects in rock doves: Concentration X Day interactions for lymphocytes and heterophiles plus Day main effects for PCV and monocytes.

The Concentration X Day interaction for lymphocytes resulted from the 4.0 mg/ℓ RP/BR smoke causing lymphocytes to decrease from a mean Pre-exposure level of 61.0 to 25.5 percent on Day E-2 of Post-exposure and return to 57.9 percent by Day P-6 (see Figure 11).

The Concentration X Day interaction for heterophiles resulted from the 4.0 mg/ℓ concentration 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 level by Day P-6 (see Figure 12). 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).

While there was a significant Day main effect for PCV and monocyte variables, this probably reflects changes caused by the stress of handling and blood-sampling.

V. CONCLUSIONS AND RECOMMENDATIONS

Regarding the conclusions derived from our work, several preliminary comments are in order.

Conduct of Project Order 85PP5847 called for acute inhalation-chamber studies. While such studies afford excellent control and delineation of exposure conditions, they do not yield directly field-relevant data of either acute or chronic aerosol effects. At best, these studies are useful for range-finding and identifying sensitive response systems.

Previous researchers have estimated the concentration of an "actual RP/BR-smoke cloud" to range between 0.25 and 2.50 mg/ℓ, with mass loadings greater than 1.0 mg/ℓ present only at the time ($\leq$1 min) of initial RP/BR release (Garvey, Fernandez, Bruce and Pinnick, 1981). The

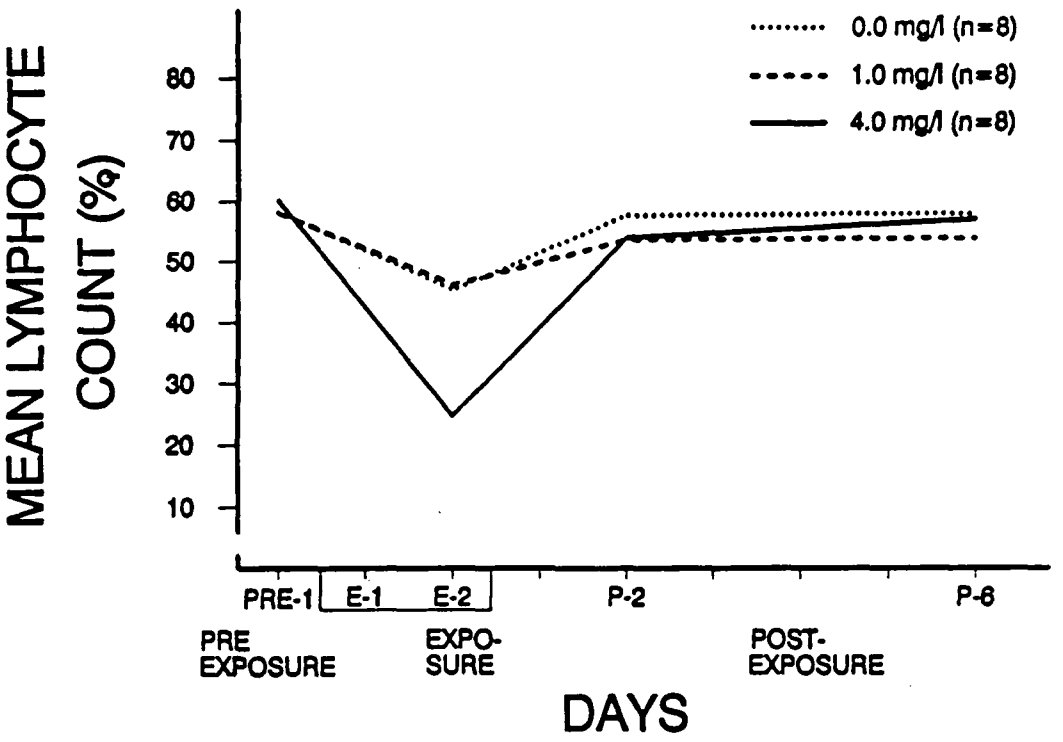


Figure 11. Lymphocyte Concentration X Day interaction means of rock doves before, during and after exposure to 3 concentrations of RP/BR smoke.

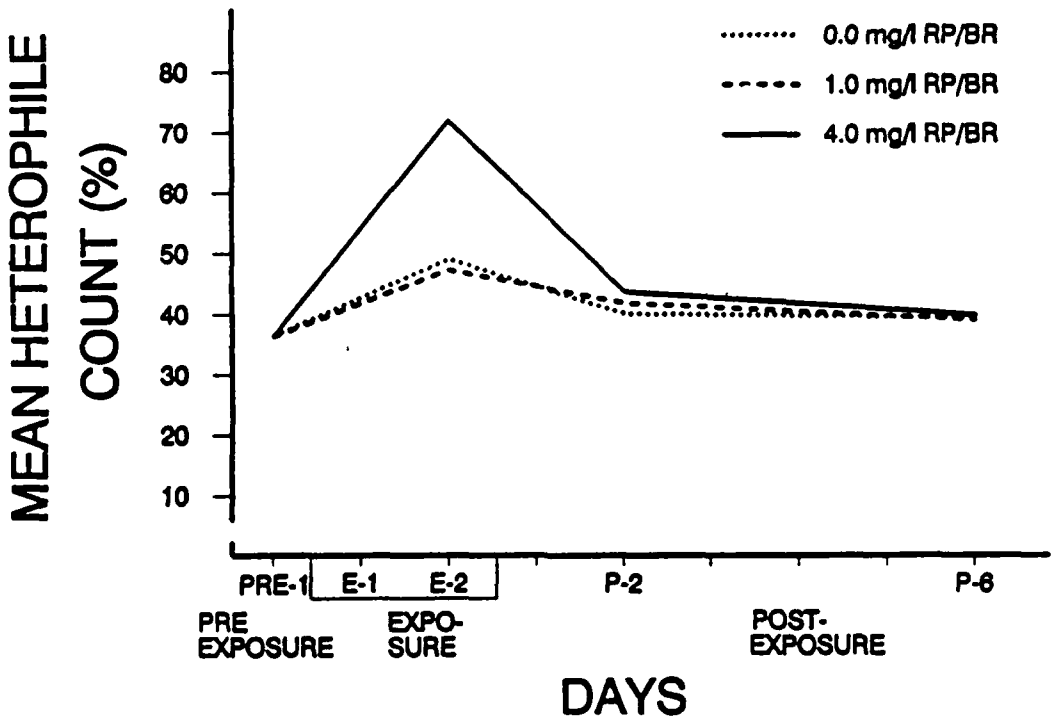


Figure 12. Heterophile Concentration X Day interaction means of rock doves before, during, and after exposure to 3 concentrations of RP/BR smoke.

1.0 to 6.0 mg/ℓ "steady-state concentrations" involving 1 to 4 successive daily 80-min exposures used in the current tests represent improbable field conditions.

The present studies involved measurements of both species while at rest or following minimal exertion. This complicates the discussior of scientific-environmental implications. Exertion should aggravate most RP/BR-smoke-induced effects.

Finally, considerations relevant to ecological assessments of military smokes have been discussed by Novak, Porcella, Johnson, Herricks and Schaffer (1985). They suggest that a series of diverse data collections is needed for an "ecotoxicological or ecoepidemiological assessment" of smoke-caused effects. Such a series includes: (a) characterization of the chemical and physical properties of the agent, (b) identification of sensitive biological test or response systems, (c) specification of the variables and measurements needed to quantify biological effects and (d) conduct of the appropriate studies to generate the final ecological impact data. Within this framework, the current research is perhaps best viewed as a preliminary identification of major mammalian and avian response systems for ecotoxicological assessments of RP/BR-smoke effects. Thus, while the research afforded a comparative perspective on the consequences of RP/BR-smoke exposure, inferences from our results to actual impacts upon wildlife and military lands are tenuous. It is unlikely that any burrow-dwelling rodent or flight-prone bird would receive doses of RP/BR smoke close to the experimental schedules.

Listed below are conclusions and recommendations from the current series of studies.

A. Conclusions

1. Lethal Effects

a. Prairie dogs are more resistant to RP/BR smoke than rock doves. This was demonstrated in toxicity range-finding studies in which exposure to RP/BR (4 exposures of 80-min each; up to 6.0 mg/ℓ) caused no mortality in prairie dogs but 26 percent mortality in rock doves (11 of 42).

b. Male rock doves are more vulnerable to RP/BR smoke than females. This was demonstrated in range-finding studies where there was 42 percent mortality in males (10 of 24) and 6 percent mortality in females (1 of 18). A sex-linked vulnerability to RP/BR smoke was also indicated in behavioral-physiological studies in which 4 male birds died following 2 exposures at 4.0 mg/ℓ.

c. It appears that the lethal threshold of RP/BR smoke for prairie dogs is greater than 4, daily 80-min exposures at 6.0 mg/ℓ; whereas, in rock doves it is between 2 and 3, daily, 80-min exposures at 3.0 to 4.0 mg/ℓ.

2. Sub-lethal Effects

   a. Multiple exposures of RP/BR smoke at 3.0 mg/ℓ or higher caused several symptomatological effects. There was respiratory congestion in both species and weight loss in rock doves. There was a significant increase in water consumption by both species 10-28 days after exposure. Vocalization was affected or lost in both species. The most pronounced effects on vocalization in prairie dogs were at 6.0 mg/ℓ and to a minor degree at all concentrations in rock doves.

   b. Multiple smoke exposures at 4.0 mg/ℓ caused several immediate and acute behavioral and physiological stress effects. Most of the effects were in rock doves and lasted only 1 to 3 days after exposure. These effects included decreased ambulatory activity in both species and decreased preening activity of rock doves immediately after initial smoke exposures. Also, latency of startle responses of female rock doves showed hypersensitivity to photoflash stimuli immediately after smoke exposures. Pulmonary function values of rock doves were suppressed in immediate periods but elevated in acute periods after exposures (e.g., carbon dioxide production, respiratory exchange ratio and metabolic rate). Also, respiration rates of 2 male rock doves that died were elevated in the acute period. Hematology values showed a sharp transient increase in heterophiles and marked decrease in lymphocytes immediately following 4.0 mg/ℓ smoke exposures.

B. Recommendations

   a. Conduct additional laboratory studies to further assess the possible environmental risk of exposing wildlife species (e.g., small avians with high respiration rates) to multiple applications of various RP/BR-smoke concentrations under more stressful and/or strenuous conditions.

   b. Conduct additional laboratory studies to determine the nature and significance of RP/BR-smoke effects on vocalization and sex differences. Such research should include spectrographic analyses of vocalizations and studies to determine the basis of any sex-linked vulnerability to inhalants.

   c. Conduct laboratory and field studies with selected sentinel species of wildlife to compare toxicity of RP/BR smoke with that of other military obscurants (e.g., fog oil).

VI. REFERENCES

Abs, M. 1983. Physiology and Behavior of The Pigeon. Academic Press: New York, NY. 360 pp.

Altman, P. L. and D. S. Dittmer. 1974. Biology Data Book: Biological Handbooks. (2nd Ed. Vol III). Federation of American Societies for Experimental Biology: Bethesda, MD. 2123 pp.

Aranyi, C. 1983a. Research and Development on Inhalation Toxicologic Evaluation of Red Phosphorus/Butyl Rubber Combustion Products. Phase I Report, Contract DAMD17-82-C-2121 (AD-A157686). IIT Research Institute: Chicago, IL. 88 pp.

_____. 1983b. Research and Development on Inhalation Toxicologic Evaluation of Red Phosphorus/Butyl Rubber Combustion Products. Phase II Report, Contract DAMD17-82-C-2121 (AD-A158323). IIT Research Institute: Chicago, IL. 113 pp.

_____. 1984. Research and Development on Inhalation Toxicologic Evaluation of Red Phosphorus/Butyl Rubber Combustion Products. Phase III Report, Contract No. DAMD17-82-C-2121 (AD-A17549). IIT Research Institute: Chicago, IL. 529 pp.

_____. 1986. Research and Development on Inhalation Toxicologic Evaluation of Red Phosphorus/Butyl Rubber Combustion Products. Final Report, Contract No. DAMD17-82-C-2121 (AD-A189254). IIT Research Institute: Chicago, IL. 330 pp.

Boche, R. D. and J. J. Quilligan. 1960. Effect of synthetic smog on spontaneous activity of mice. Science. 131:1733-1734.

Brazell, R. S., J. H. Moneyhun, and R. W. Holmberg. 1984. Chemical Characterization and Toxicologic Evaluation of Airborne Mixtures. Final Report. Oak Ridge National Laboratory: Oak Ridge, TN. DE-AC05-840R21400. 61 pp.

Buelke-Sam, S. J., C. A. Kimmel, J. Adams, C. J. Nelson, C. V. Vorhees, D. C. Wright, V. St. Omer, B. Korol, R. E. Butcher, M. A. Geyer, J. F. Holson, C. L. Kutscher and M. J. Wayner. 1985. Collaborative behavioral teratology study: results. Neurobehavioral Toxicology and Teratology. 7:591-624.

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

Davis, M. 1980. Neurochemical modulation of sensory-motor reactivity: acoustic and tactile startle reflexes. Neuroscience and Biobehavioral Reviews. 4:241-263.

Finger, F. W. 1972. Measuring behavioral activity. Pages 1-19 in R.D. Myers (Ed), Methods in Psychobiology (Vol. 2.). Academic Press: New York, NY.

Fowler, M. E. 1986. Zoo and Wild Animal Medicine. W. B. Saunders Co.: Philadelphia, PA. 1127 pp.

Garvey, D. M., G. Fernandez, C. W. Bruce and R. G. Pinnick. 1981. In situ measurements of phosphorus smokes during Smoke Week III. Volume I of the Proceedings of the Smoke/Obscurants Symposium V. Adelphi MD, April 28-30, 1981. Office of the Project Manager Smoke/Obscurants, Aberdeen Proving Ground, MD. 1:183-213.

Griffiths, L. C., R. K. Wolff, R. L. Beethe, C. H. Hobbs and R. O. McClellan. 1981. Evaluation of a Multitiered Inhalation Exposure Chamber. Fundamental and Applied Toxicology. 1:8-12.

Goodwin, D. 1983. Pigeons and Doves of the World. Cornell University Press: Ithaca, NY. Pgs. 59-62.

Holmberg, R. W., J. H. Moneyhun and T. M. Gayle. 1985. A System for the Continuous Generation of Phosphorus Aerosols from Red Phosphorous-Butyl Rubber. Final Report, ORNL/TM-9649 (AD-AI57342). Oak Ridge National Laboratory, Oak Ridge, TN. 37 pp.

Hoogland, J. L. 1985. Infanticide in prairie dogs: lactating females kill offspring of close kin. Science. 230:1037-1040.

Ison, J. R. 1984. Reflex modification as an objective test for sensory processing following toxicant exposure. Neurobehavioral Toxicology and Teratology. 6:437-445.

Jain, N. C. 1986. Schalm's Veterinary Hematology. (4th Ed.) Lea and Febiger: Philadelphia, PA. 1221 pp.

Jones, J. K., Jr., D. M. Armstrong, R. S. Hoffman and C. Jones. 1983. Mammals of the Northern Great Plains. University of Nebraska Press: Lincoln, NB. 379 pp.

Kellog, C.,D. Tervo, J. Ison, T. Parisi and R. K. Miller. 1980. Prenatal exposure to Diazepam alters behavioral development in rats. Science. 207:205-207.

Lock, S., W. Dalbey, T. Gayle and R. Schmoyer. 1985. A method for studying startle reflex in rats and its application in quantitating some acute effects of aerosolized diesel fuel. (Unpublished manuscript).

Miller, W. J. and F. H. Wagner. 1955. Sexing mature Columbiforms by cloacal characters. Auk. 72:279-285.

Novak, E. W., D. B. Porcella, K. M. Johnson, E. E. Herricks and D. J. Schaffer. 1985. Selection of test methods to assess ecological effects of mixed aerosols. Ecotoxicology and Environmental Safety. 10(3):361-381.

Palmer, R. S. 1954. The Mammal Guide: Mammals of North America North of Mexico. Doubleday and Co.: Garden City, NY. 384 pp.

Petrinovich, L. F. and C. D. Hardyck. 1969. Error rates for multiple comparison methods: some evidence concerning the frequency of erroneous conclusions. Psychological Bulletin. 71:43-54

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

Reilly, E. M., Jr. 1968. The Audobon Illustrated Handbook of American Birds. McGraw-Hill Co.: NY. Pgs. 227-228.

Rugh, R. 1968. The Mouse: Its Reproduction and Development. Burgess Publishing Co.: Minneapolis, MN. 430 pp.

Ruppert, P. H., K. F. Dean and L. W. Reiter. 1984. Trimethyltin disrupts acoustic startle responding in adult rats. Toxicology Letters. 22:33-38.

SAS Institute, Inc. 1985. SAS User's Guide: Statistics Version 5 Edition. SAS Institute, Inc.: Cary, NC. 956 pp.

Selye, H. 1973. Stress in Health and Disease. Butterworth: Woburn, MA.

Shumake, S. A., R. T. Sterner, B. E. Johns and R. D. Thompson. 1989. Behavioral-physiological Effects of Red Phosphorus Smoke Inhalation on Two Wildlife Species. Task 2 Report (Effective Smoke Concentration Range-finding Determinations), Project Order No. 85PP5847. USDA/APHIS/S&T, Denver Wildlife Research Center: Denver, CO. 124 pp.

Siegel, H. S. 1980. Physiological stress in birds. Bioscience. 30(8):529-534.

Squibb, R. E. and H. A. Tilson. 1982. Neurobehavioral changes in adult Fisher 344 rats exposed to dietary levels of chlordecone (Kepone®): a 90-day chronic dosing study. Neurotoxicology. 3(2): 59-65.

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 (ADA196753), USDA/APHIS/S&T, Denver Wildlife Research Center: Denver, CO. 64 pp.

Sterner, R. T., S. A. Shumake, R. D. Thompson and B. E. Johns. 1990. 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), Project Order No. 85PP5847. USDA/APHIS/S&T, Denver Wildlife Research Center: Denver, CO. 164 pp.

Stinson, S. F. and C. G. Loosli. 1979. The effect of synthetic smog on voluntary activity of CD-1 mice. Pages 233-240 in Animals as Monitors of Environmental Pollutants. National Academy of Science: Washington, DC. 421 pp.

Turner, C. D., 1955. General Endocrinology. (2nd Ed.) W. B. Saunders Co.: Philadelphia, PA. 553 pp.

Van Voris, P., D. A. Cataldo, M. W. Ligotke, T. R. Garland, K. M. McFadden, J. K. Fredrickson, S. W. Li, R. M. Bean, B. L. Thomas and D. W. Carlile. 1986. Transport, Transformation, Fate and Terrestrial Ecological Effects of Red Phosphorus-Butyl Rubber and White Phosphorus Obscurant Smokes. Draft Final Report, Project Order No. 84PP4819. Pacific Northwest Laboratory: Richland, WA. 212 pp.

Waller, R. A. and D. B. Duncan. 1969. A Bayes rule for the symmetric multiple comparison problem. Journal of the American Statistical Association. 64:1489-1499.

Winer, B. J. 1971. Statistical Principles in Experimental Design. McGraw Hill: New York, NY. Pgs. 559-571.

Yon, R. L., R. W. Wentsel and J. M. Bane. 1983. Programmatic Life Cycle Environmental Assessment for Smoke/Obscurants: Red, White and Plasticized White Phosphorus. Vol. 2 of 5. ARCSL-EA-83004. U.S. Army Armament, Munitions and Chemical Command, Chemical Research and Development Center: Aberdeen Proving Ground, MD. 73 pp.

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