

PHYSIOLOGICAL EFFECTS OF RED PHOSPHORUS SMOKE INHALATION ON PRAIRIE DOGS AND ROCK DOVES

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Abstract—Pulmonary function, blood chemistry, and hematology studies were conducted with prairie dogs (*Cynomys ludovicianus*) and rock doves (*Columba livia*) under resting conditions to assess environmental risk of exposing wildlife species to multiple applications of red phosphorus/butyl rubber smoke (0.0, 1.0, and 4.0 mg/L). Four daily 80-min smoke exposures had no significant physiological effect on prairie dogs. However, with rock doves, two daily 80-min smoke exposures resulted in elevated respiratory frequency in two males followed by death, a significant concentration \times day effect on carbon dioxide production, respiratory exchange ratio, metabolic rate, lymphocytes, and heterophiles, and a significant sex \times concentration effect on hemoglobin and methemoglobin. Although results showed that rock doves are more vulnerable to red phosphorus/butyl rubber smoke than prairie dogs, it appears that exposure to the smoke is unlikely to pose a significant risk to either species under field conditions.

Keywords—Prairie dogs Rock doves Red phosphorus smoke Inhalation
Environmental risk

INTRODUCTION

Chemical smoke obscurants are used by many of the armed forces of the world to conceal personnel, material, or installations from direct visual observation [1]. The U.S. Army, which is responsible for determining health and environmental risks associated with smoke obscurants, has stepped up research and development efforts in the past 10 years to improve field applications of smoke obscurants [2]. One product, red phosphorus/butyl rubber (RP/BR), has shown excellent potential as a smoke obscurant. It is deployed via a hand-thrown grenade or a grenade-launching system. Upon detonation, the RP/BR is ignited. The burning RP/BR produces a dense white smoke that largely consists of phosphoric and polyphosphoric acid particles [3,4].

Although it has been assumed that repeated exposure to RP/BR smoke poses no significant health hazard to personnel or threat to the environment, data to confirm such assumptions are lacking [5]. Until recently, RP/BR research has been focused on characterization [3,4,6], environmental

fate [4,7], and animal toxicity [3,8]. A literature review indicates that to date, with the exception of range-finding tests conducted by our research team [5], all RP/BR smoke studies with animals have involved only laboratory rats (*Rattus norvegicus*).

This research project stems from a need to determine the environmental risk of exposing wildlife species to RP/BR smoke. The rationale for the research is based on reports showing that RP/BR smoke administered in a wide variety of concentrations and durations causes mortality, laryngeal injury and congestion, edema, and hemorrhage of the pulmonary system in rats [1,8]. One implication of such reports is that high concentrations of RP/BR smoke could adversely impact wildlife species. This report describes the effects of multiple 80-min exposures of up to 4.0 mg/L RP/BR smoke on pulmonary function, blood chemistry, and hematology of two wildlife species, prairie dogs and rock doves. These species were chosen so that both Aves and Mammalia would be represented, because they inhabit areas of the United States where obscurant smokes are used in military training operations, and because they adapt well to use in the laboratory.

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METHODS

Selection and care of animals

Forty-eight prairie dogs (*Cynomys ludovicianus*) (24 males, 24 females) and 48 rock doves (*Columba livia*) (26 males, 22 females) were selected. Prairie dogs were captured locally near Denver, Colorado, and the birds were purchased from a local supplier of wild-trapped rock doves. Both species were obtained at a variety of times during the year and were held under a 12:12-h artificial light:dark cycle in a group housing for a minimum of two months before testing. Capture, quarantine, and handling procedures were in accordance with provisions of the Animal Welfare Act of 1966 (P.L. 89-544), plus amendments. During the time of the study, individual animals were caged and maintained under 12:12-h artificial light:dark cycle at a room temperature of 22°C. Water and food were provided *ad libitum*, Purina Pigeon Chow® Checkers for the rock doves and Purina Rabbit Chow® Checkers (Purina Mills Inc., St. Louis, MO) plus raw carrots for the prairie dogs. The animals were not fasted during the study. Conduct of research studies adhered to established National Institutes of Health (NIH) guidelines for the care and use of laboratory animals [9].

Research design

The design is illustrated in Figure 1. It consisted of four separate studies, each with three sequential periods. Each study involved 24 animals. With the exception of pulmonary function of rock doves (14 males, 10 females), study designs were balanced for sex. Studies were designed to determine immediate (<3 h out of chamber) and delayed effects (>3 h

<12 d) of multiple exposures to three target concentrations of RP/BR smoke (0.0, 1.0, and 4.0 mg/L).

Exposure chambers

Exposures were accomplished by placing animals in chambers of identical construction, either a filtered air control chamber or an RP/BR smoke chamber (stainless steel, 91.5 × 91.5 × 132.0 cm; Bertke and Young, Cincinnati, OH) for 80 min on successive days, as shown in Figure 1. Smoke concentrations, durations, and the number of days exposed were selected to minimally and maximally challenge test animals without causing mortality and were based on toxicity range-finding tests by Shumake et al. [5]. Prairie dogs were exposed to smoke four times, and rock doves were exposed twice. Both species were held for 30 d after the last smoke exposure to determine if delayed mortality would occur.

The inhalation chambers were located in adjacent rooms, each with three shelves capable of containing four cubic stainless steel wire-mesh animal cages 30.5 cm on a side. To generate smoke, RP/BR contained in segments of stainless steel pipe was extruded through an orifice by a hydraulic piston and ignited in a glass T-joint combustion chamber. Conditioned air, filtered by an HEPA filter plus a charcoal bed (Mine Safety Appliances Co., Pittsburgh, PA) and regulated to control temperature and relative humidity (Table 1), was simultaneously fed past the flame in the combustion chamber. The resultant phosphoric acid smoke was then drawn into the inhalation chamber. Air flow rate was maintained at 250 L/min.

STUDY	PERIOD													
	Pre-exp Day	Exposure Days				Post-exposure Days								
	1	1	2	3	4	1	2	3	4	5	6	7	8	
* ■ Prairie Dog Rock Dove	Pulmonary Function	*	*	*	*	*	*	*			*			
		■	■	■			■	■			■			■
* ■ Prairie Dog Rock Dove	Blood Chemistry/Hematology	*			*		*				*			
		■	■				■				■			

Fig. 1. Design for measuring the exposure effects of three concentrations of red phosphorus/butyl rubber smoke (0.0, 1.0, and 4.0 mg/L) on prairie dogs and rock doves. When measurements were taken on exposure days, they were made within 3 h after exposure.

Table 1. Inhalation chamber atmosphere for exposure of prairie dogs and rock doves to three target concentrations of red phosphorus/butyl rubber smoke (range)

Variable	Target concentration (mg/L)		
	0.0	1.0	4.0
Aerosol (mg/L) concn (total)	ND ^a	0.72–0.86	3.41–3.60
Concn (steady state)	ND	0.94–1.17	4.45–5.00
Phosphoric acid concn	ND	0.51–0.59	2.58–2.72
Particle size (MMAD, ^b μm)	ND	0.39–0.50	0.57–1.00
Respirable gases			
O ₂ (%)	17–22	18–23	17–22
CO ₂ (ppm)	48–194	169–194	48–412
Contaminant gases			
CO (ppm)	ND	2–15	8–21
PH ₃ (ppm)	ND	ND	ND
C ₆ H ₁₆ (ppm)	ND	ND	ND
Temperature (°C)	21–28	19–26	20–26
Relative humidity (%)	39–54	46–64	42–57

^aNot detected.

^bMass median aerodynamic diameter.

Air samples were collected during each smoke exposure and analyzed to characterize chamber atmosphere (Table 1). Aerosol mass and phosphoric acid concentrations were determined by gravimetric and titration techniques, respectively. Respiratory and contaminant gases were measured by a Gastec detection system (Gastec Inc., Newark, CA). Particle sizes were measured by a cascade impactor (California Measurements, Inc., Sierra Madre, CA) on alternate exposure days.

Summary values in Table 1 show that RP/BR smoke concentration was near respective target concentrations, all particles were respirable, and respiratory gases and aerosol contaminants were within acceptable limits [10]. The 4.0-mg/L dose level produced larger particle sizes than the 1.0-mg/L dose level, as would be expected from particle coalescence. This characteristic was considered as a normal part of a higher concentration, with no consideration given to how it might affect animal response.

Pulmonary function

The pulmonary data were collected on the days marked by asterisks or blocks in Figure 1 (8 d for prairie dogs and 7 d for rock doves). Measurements were obtained with a computerized Oxymax-85 pulmonary function system (Columbus Instruments, Columbus, OH). To perform measurements, animals were placed in two adjacent 43- ×

43- × 33-cm Plexiglas[®] test chambers (one animal per chamber) of the Oxymax-85 system. Measurements were made in a darkened room under stable temperature and humidity conditions between 0800 and 1700 h, with no operator present. The system was equipped with pumping units for supplying and withdrawing air. Air flow was measured by mass flow meters adjusted for standard temperature and pressure, dry. Typical values were 3,000 ml/min for prairie dogs and 1,700 ml/min for rock doves. Approximately 50% of the air supply to each test chamber was withdrawn, circulated through a drying column (Drierite, W.A. Hammond Drierite Co., Xenia, OH), and sampled for percent O₂ and CO₂ with sensors that were calibrated with calibration-grade gases. Oxygen and CO₂ measurements for each animal were determined by the difference between ambient room and test chamber air. The system was programmed to simultaneously measure oxygen consumption (V_{O_2} /kg/h), carbon dioxide production (V_{CO_2} /kg/h), respirations per minute (Rf), respiratory exchange ratio (RER, V_{CO_2}/V_{O_2}), and metabolic rate (MR, kcal/h/kg) at 2-min intervals for 2 h. Only second-hour data were analyzed because animal and system equilibration did not reach an acceptable level in the first hour. Means of the 30 observations for each of five variables per animal per test day were used in analyses.

Blood chemistry and hematology

Paired blood samples were collected on the 4 d marked by asterisks or blocks in Figure 1. On each blood-draw day, one sample was drawn for blood chemistry determinations and a second for hematology. A total of eight 0.6-ml venous samples were collected from each animal. Blood was drawn from femoral veins of prairie dogs and ulnar veins of rock doves, using one-milliliter syringes with a 25-gauge needle. Syringes were heparinized for blood chemistry determinations and treated with EDTA for hematology measurements. To collect blood samples, prairie dogs were immobilized with ketamine hydrochloride (38–50 mg/kg), with dosage held constant across treatment groups and milligram-per-kilogram dropping from the first day to the fourth as the animals required less to achieve immobilization.

An IL 1306 blood gas analyzer and an IL 282 CO-oximeter (Instrumentation Laboratory, Lexington, MA) were used for blood chemistry determinations: pH, oxygen partial pressure (P_{O_2}), carbon dioxide partial pressure (P_{CO_2}), hemoglobin (Hb), oxyhemoglobin (O₂Hb), carboxyhemoglobin (COHb), and methemoglobin (MetHb).

Table 2. Mean (\pm SD) pulmonary function measurements^a of prairie dogs exposed to three concentrations of RP/BR smoke

RP/BR smoke concn (mg/L) ^b	V _{O₂} (ml/kg/h)	V _{CO₂} (ml/kg/h)	RER (V _{CO₂} /V _{O₂})	Rf (resp/min)	MR (kcal/kg/h)
0.0	583 \pm 141	564 \pm 143	0.97 \pm 0.1	24.2 \pm 9.7	2.921 \pm 0.700
1.0	575 \pm 113	586 \pm 117	1.02 \pm 0.1	25.1 \pm 9.4	3.048 \pm 0.568
4.0	605 \pm 143	597 \pm 161	0.98 \pm 0.9	25.9 \pm 10.7	2.918 \pm 0.736

^aMeasurements are shown for illustration only, as each value represents a combination of all 64 observations.

^b $n = 8$ animals per concentration \times 8 observation days = 64 observations per parameter.

Hematology measurements of packed cell volume (PCV), total white blood count (WBC), and differential WBC were determined by using standard techniques (Colorado Veterinary Laboratory, Broomfield, CO). The automated CO-oximeter and blood gas analyzer facilitated rapid and accurate measurement of venous blood parameters. However, it should be noted that the CO-oximeter was not equipped to analyze blood from prairie dogs and rock doves by using a species-specific programmable read-only memory (PROM). Therefore, measurements of HB, O₂Hb, COHb, and MetHb are to be viewed as relative values for comparing the effects of the RP/BR smoke concentration groups, rather than as absolute values. The hemoglobin spectra for prairie dogs and rock doves were examined, and it was determined that the IL 282 CO-oximeter would be measuring the blood of the two species at acceptable points on their spectra.

Data analysis

Prairie dog data were analyzed with the PROC ANOVA program and rock dove data were analyzed by the PROC GLM program [11]. A separate analysis of variance (ANOVA) was performed on each variable of each study. With pulmonary function, ANOVA involved a design of 3 (concentra-

tions) \times 2 (sex) \times 8 or 7 (days), where "days" was regarded as a repeated measures factor. Data on blood chemistry and hematology variables were analyzed with a 3 (concentrations) \times 2 (sex) \times 4 (days) factorial ANOVA, with "days" as a repeated measures factor. Significance was determined at $P \leq 0.05$, and Duncan's multiple-range test was used to further evaluate any significant effect.

RESULTS AND DISCUSSION

Pulmonary function

Prairie dogs. Means (\pm SD) of all variables (Table 2) are in a range expected for the test species or other rodents of comparable size [12–15]. There was no mortality and only one significant sublethal effect, a day effect for RER ($P < 0.01$). The RER day effect, which was suppressed on three of four exposure days, appeared to be an experimental effect unrelated to RP/BR smoke concentration, perhaps caused by handling. The results show that prairie dogs are very resistant to RP/BR smoke and that the lethal threshold of RP/BR smoke for this species is >80-min exposure at 4.0 mg/L on each of 4 d.

Rock doves. Means (\pm SD) of all variables (Table 3) appear to be typical of pigeons [16]. However, two males died on postexposure day 5

Table 3. Mean (\pm SD) pulmonary function measurements^a of rock doves exposed to three concentrations of RP/BR smoke

RP/BR smoke concn (mg/L) ^b	V _{O₂} (ml/kg/h)	V _{CO₂} (ml/kg/h)	RER (V _{CO₂} /V _{O₂})	Rf (resp/min)	MR (kcal/kg/h)
0.0	754 \pm 126	666 \pm 128	0.88 \pm 0.8	23.5 \pm 6.4	3.697 \pm 0.624
1.0	872 \pm 202	795 \pm 205	0.91 \pm 0.09	25.6 \pm 7.0	3.675 \pm 1.009
4.0	748 \pm 102	666 \pm 148	0.88 \pm 0.1	25.9 \pm 8.5	4.398 \pm 0.588

^aMeasurements are shown for illustration only, as each value represents a combination of all 56 observations.

^b $n = 8$ animals per concentration \times 7 observation days = 56 observations per parameter.

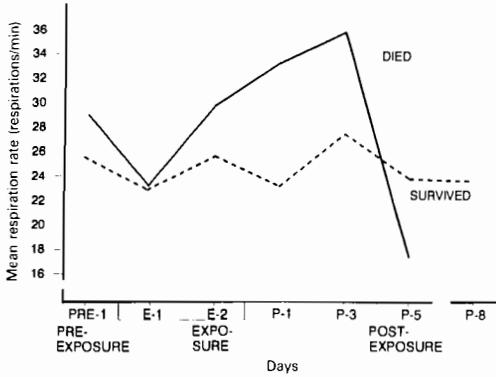


Fig. 2. Mean respiration rate (Rf) of two male rock doves that died and six rock doves that survived following exposure to 4.0-mg/L concentration of RP/BR smoke.

of 4.0-mg/L concentration. Both birds showed evidence of respiratory distress before death (Fig. 2).

There was a significant day effect on V_{O_2} ($P < 0.02$), which was expressed as a drop in V_{O_2} during the exposure days, followed by a recovery on postexposure days. A plausible explanation for the V_{O_2} day effect is that the impact of the experience in the exposure chamber, combined with the confinement in the pulmonary test chamber, resulted in less active birds that later recovered when they were placed only in the pulmonary test chamber on postexposure days.

There were also significant concentration \times day interactions on V_{CO_2} ($P < 0.01$), RER ($P < 0.01$), and MR ($P < 0.05$). Concentration \times day interaction means for V_{CO_2} , RER, and MR are depicted

in Figures 3, 4, and 5, respectively. The responses of V_{CO_2} , RER, and MR to RP/BR smoke were not always dose-response related. At 0.0 and 4.0 mg/L RP/BR smoke, V_{CO_2} , RER, and MR significantly decreased below preexposure levels by exposure day 2, whereas at 1.0 mg/L there was no significant change by exposure day 2, even though the downward movements of the values were similar to the other concentrations. By postexposure day 3 the 1.0-mg/L group showed a significant change when V_{CO_2} and MR were elevated above all other days. Although a definitive explanation for the elevated values on postexposure day 3 is not readily apparent, the upward direction of its occurrence makes it unlikely to have been caused by 1.0 mg/L smoke exposure, unless a rebound phenomenon was occurring with only the 1.0 mg/L concentration. This is supported by the fact that no significant differences in V_{CO_2} , RER, and MR means occurred between concentrations 0.0 and 4.0 mg/L across days, with the exception of RER postexposure day 8. The main cause of the concentration \times day interaction was the 1.0 mg/L concentration, as represented by significantly elevated V_{CO_2} on postexposure day 3, RER at postexposure day 3, and MR at exposure day 1 and postexposure days 1 and 3.

Although suppression of MR following exposure to 0.0 and 4.0 mg/L is indicative of elevated adrenocortical function to combat nonspecific stress [17], the similarities of MR at 0.0 and 4.0 mg/L show that handling or chamber confinement or both affected pulmonary function as much as RP/BR smoke. Another type of handling effect may be reflected in V_{CO_2} and RER at 1.0 mg/L on

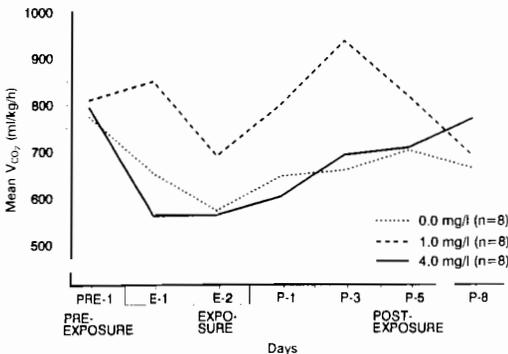


Fig. 3. Carbon dioxide production (V_{CO_2}) concentration \times day interaction means of rock doves before, during, and after exposure to three concentrations of RP/BR smoke.

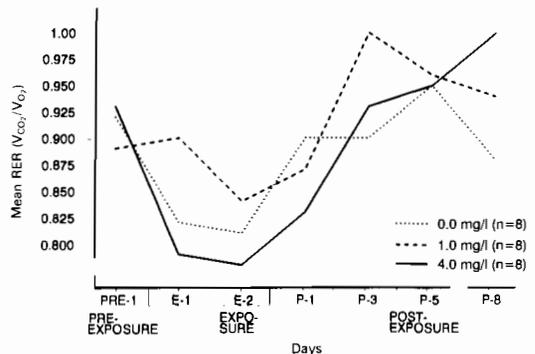


Fig. 4. Respiratory exchange ratio (RER) concentration \times day interaction means of rock doves before, during, and after exposure to three concentrations of RP/BR smoke.

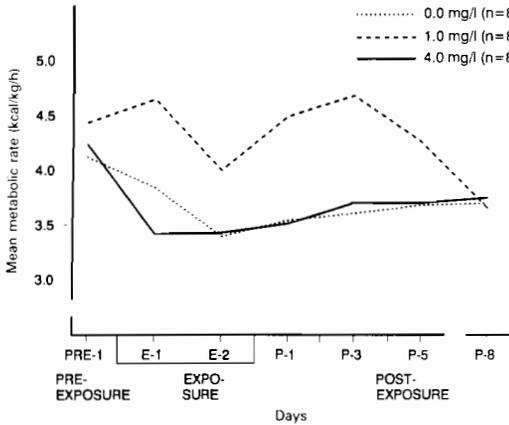


Fig. 5. Metabolic rate (MR) concentration \times day interaction means of rock doves before, during, and after exposure to three concentrations of RP/BR smoke.

postexposure day 3. For example, the RP/BR exposure scheduling resulted in birds at 1.0 mg/L being exposed and tested soon after consuming food in the morning (3–5 h), whereas birds exposed to 0.0 and 4.0 mg/L were tested later in the day, 4 to 9 h after consuming food. Because energy intake appears to be the main factor regulating concentration of thyroid hormones [18] and because half-life

of avian thyroid hormones is short (i.e., usually <8 h), it is reasonable to assume that the higher values at 1.0 mg/L were caused by diurnal variation in MR. This explanation is not inconsistent with the lack of change in Rf between RP/BR smoke concentrations ($P \geq 0.41$). V_{O_2} can change with little or no change in Rf as long as arterial P_{O_2} does not decrease below a triggering point at which demand for respiration cannot be met [19].

Blood chemistry and hematology

Prairie dogs. There was no mortality or evidence of physiological impairment among the prairie dogs. Means (\pm SD) of all variables are shown in Table 4; they appear to be within the normal mammalian range [14]. To our knowledge, this is the first report of blood chemistry and hematology values for this species. There was no significant RP/BR smoke concentration effect on blood chemistry or hematology variables ($P > 0.05$), but there was a significant day effect on P_{O_2} , Hb, O_2 Hb, COHb, MetHb, and PCV ($P < 0.05$).

The day effects probably resulted from blood collection and handling or differences in depth of anesthesia. During the study, prairie dogs became increasingly sensitive to the anesthetic, and it was necessary to reduce the dosage of ketamine hydrochloride gradually across treatments from 50

Table 4. Mean (\pm SD) blood chemistry and hematology venous values of prairie dogs exposed to three concentrations of RP/BR smoke^a

Variable	RP/BR concentration (mg/L)		
	0.0	1.0	4.0
Blood chemistry			
pH	7.28 \pm 0.08	7.26 \pm 0.08	7.30 \pm 0.04
P_{CO_2} (mm Hg)	56.9 \pm 7.2	62.8 \pm 7.9	59.6 \pm 5.6
P_{O_2} (mm Hg)	48.8 \pm 8.4	45.8 \pm 9.9	47.0 \pm 7.9
Hb (g/dl)	5.7 \pm 1.2	16.2 \pm 1.1	15.9 \pm 0.9
% O_2 Hb	80.5 \pm 10.7	75.6 \pm 14.2	80.0 \pm 9.3
% COHb	1.32 \pm 0.47	1.23 \pm 0.64	1.44 \pm 0.40
% MetHb	1.84 \pm 0.53	1.74 \pm 0.71	1.98 \pm 0.43
Hematology			
% PCV	45.4 \pm 3.2	46.8 \pm 3.2	45.7 \pm 3.1
Total WBC/ μ l	5,984 \pm 1,345	6,888 \pm 1,835	6,119 \pm 1,602
Differential WBC (%)			
Segmented granulocytes	41.9 \pm 8.8	41.8 \pm 14.3	41.0 \pm 8.2
Band granulocytes	0	0	0
Lymphocytes	53.8 \pm 9.9	52.6 \pm 13.1	54.5 \pm 8.1
Monocytes	0	0	0
Eosinophils	3.2 \pm 2.7	4.2 \pm 4.0	3.3 \pm 2.3
Basophils	0	0	0

^aEight prairie dogs per concentration; four blood collection days per animal.

Table 5. Mean (\pm SD) blood chemistry and hematology venous values of rock doves exposed to three concentrations of RP/BR smoke^a

Variable	RP/BR concentration (mg/L)		
	0.0	1.0	4.0
Blood chemistry			
pH	7.38 \pm 0.05	7.39 \pm 0.03	7.39 \pm 0.04
P _{CO₂} (mm Hg)	41.8 \pm 5.3	41.5 \pm 3.5	40.1 \pm 4.0
P _{O₂} (mm Hg)	59.9 \pm 6.2	58.9 \pm 4.9	62.1 \pm 3.5
Hb (g/dl)	14.9 \pm 1.4	15.6 \pm 1.3	15.3 \pm 0.9
% O ₂ Hb	90.2 \pm 5.0	90.1 \pm 4.0	92.4 \pm 3.5
% COHb	-2.98 ^b \pm 0.35	-2.73 \pm 0.37	-2.99 \pm 3.8
% MetHb	0.26 \pm 0.33	0.13 \pm 0.19	0.13 \pm 0.21
Hematology			
% PCV	50.4 \pm 3.9	50.9 \pm 4.0	50.5 \pm 4.3
Total WBC/ μ l	9,425 \pm 3,867	6,628 \pm 2,873	12,353 \pm 5,800
Differential WBC (%)			
Lymphocytes	55.6 \pm 14.2	53.8 \pm 11.6	49.8 \pm 17.3
Monocytes	2.4 \pm 2.3	2.1 \pm 1.9	1.7 \pm 1.5
Eosinophils	1.1 \pm 1.7	3.0 \pm 3.5	0.6 \pm 0.9
Basophils	0	0	0
Heterophils	40.3 \pm 14.1	40.2 \pm 10.9	47.1 \pm 17.6

^aEight rock doves per concentration; four blood collection days per bird.

^bAs stated in the Methods section, rock dove blood values from the IL 282 CO-oximeter are relative, not absolute, because a species-specific PROM for rock doves was not available for the instrument.

mg/kg on the preexposure day to 38 mg/kg by postexposure day 6 to ensure adequate respiration.

Rock doves. Means (\pm SD) of variables are shown in Table 5. All values appear similar to un-anesthetized pigeons [16], and there was no mortality. No significant differences among RP/BR smoke concentrations were present. However, there was a significant concentration \times sex interaction effect on Hb ($P < 0.04$) and on MetHb ($P < 0.01$). There also was a significant concentration \times day interaction effect on heterophiles and lymphocytes ($P < 0.01$).

The concentration \times sex effect on Hb was caused by the Hb of females being significantly lower than that of males, except at 4.0 mg/L (Fig. 6). The interaction shows that the Hb of females significantly increased at 4.0 mg/L, whereas the Hb of males showed no change across concentrations. The significantly higher Hb of females at the 4.0-mg/L concentration, and thus increased oxygen-carrying capacity, may help explain why they are less vulnerable to RP/BR smoke than males. With MetHb, the concentration \times sex effect was caused by the MetHb of females being significantly higher than that of males at the 0.0- and 1.0-mg/L concentrations and then declining to no significant differ-

ences at 4.0 mg/L (Fig. 7). The MetHb values appear to be too low to interfere with normal oxygen transport; however, the MetHb concentration \times sex interaction may provide additional evidence that male rock doves are more vulnerable to RP/BR smoke than females.

The lymphocyte concentration \times day interaction resulted from 4.0 mg/L RP/BR smoke causing

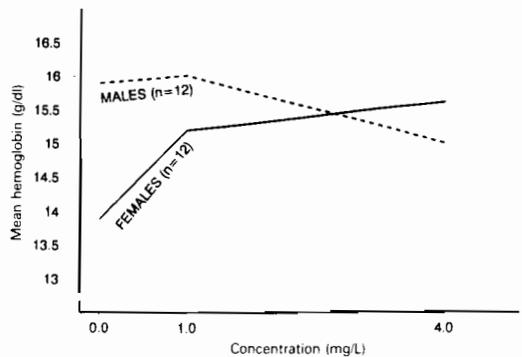


Fig. 6. Hemoglobin (Hb) concentration \times sex interaction means of rock doves exposed to three concentrations of RP/BR smoke.

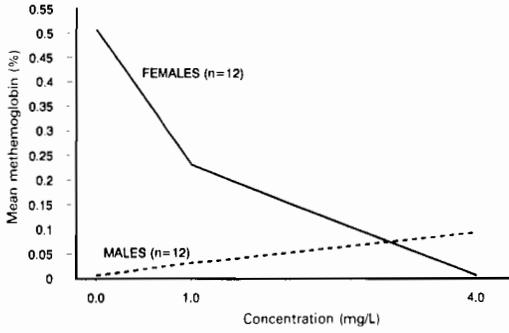


Fig. 7. Methemoglobin (MetHb) sex × concentration interaction means for rock doves before, during, and after exposure to three concentrations of RP/BR smoke.

lymphocytes to decrease from a mean preexposure level of 61.0 to 25.5% on exposure day 2 (Fig. 8). Conversely, the concentration × day interaction with heterophiles resulted from 4.0 mg/L RP/BR smoke causing heterophiles to increase from a pre-exposure mean of 35.4 to 71.4% on exposure day 2 with no corresponding change at 0.0 and 1.0 mg/L (Fig. 9). The magnitude of the shift in lymphocytes and heterophiles shows that RP/BR smoke at 4.0 mg/L was a significant environmental stressor. The magnitude of response is indicative of adrenocortical stimulation and immunological response [17].

In addition to the interaction effects, there was a significant day effect on pH, P_{CO_2} , Hb, COHb, PCV, and monocytes ($P < 0.03$). The day effects probably reflect shifts induced by blood collection and handling and are unrelated to RP/BR smoke

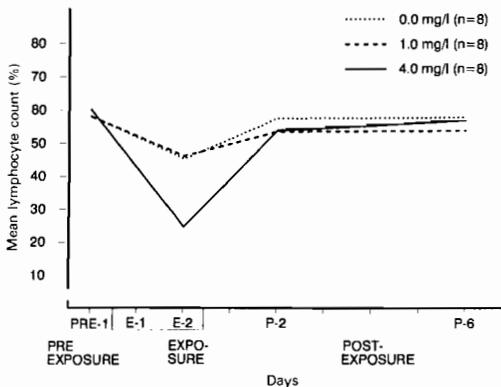


Fig. 8. Lymphocyte concentration × day interaction means of rock doves before, during, and after exposure to three concentrations of RP/BR smoke.

exposure. There was no day × concentration interaction and little variation among means (<11% from low to high mean within each variable).

SUMMARY AND CONCLUSIONS

Studies were conducted to determine the effects of RP/BR smoke (0.0, 1.0, and 4.0 mg/L) on pulmonary function, blood chemistry, and hematology of prairie dogs and rock doves under resting conditions. The results showed that four 80-min smoke exposures had no significant effect on prairie dogs. However, with rock doves, two 80-min smoke exposures resulted in elevated Rf and death of two males, a significant concentration × day effect on V_{CO_2} , RER, MR, lymphocytes, and heterophiles and a significant concentration × sex effect on HB and MetHb.

Although these findings show that rock doves are more vulnerable to RP/BR smoke than are prairie dogs and that male rock doves seem more vulnerable than females, it appears that multiple applications of RP/BR smoke are unlikely to pose a significant risk to populations of either species under field conditions. Garvey et al. [20] estimated that RP/BR smoke clouds range between 0.25 and 2.50 mg/L and that concentrations >1.0 mg/L are present only at initial RP/BR release (<1 min). In addition, prairie dogs do not demonstrate prolonged strenuous activity when threatened and can escape into burrows. Rock doves will flush in light or darkness and appear to be reluctant to remain for extended periods in an RP/BR smoke cloud. The animals in this study were exposed for a total of 80 min each session. It is very unlikely that either species would remain in the smoke, especially at high

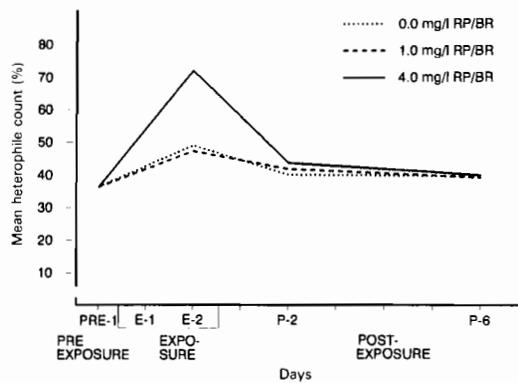


Fig. 9. Heterophile concentration × day interaction means of rock doves before, during, and after exposure to three concentrations of RP/BR smoke.

4.0-mg/L concentrations, for a period lasting more than a few minutes when they could readily escape. However, high concentrations of RP/BR smoke could have significant detrimental impacts on other species whose very nature leads to more stressful conditions (e.g., those with high Rf, inability to escape the smoke, and sex-linked vulnerabilities). As such, additional research is warranted to assess potential risk of exposing other wildlife species to RP/BR smoke under conditions likely to occur in a natural environment (e.g., strenuous and/or stressful activity during and following smoke exposure).

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