EFFECTS OF PERCHLORATE EXPOSURE ON RESTING
METABOLISM, PEAK METABOLISM, AND THYROID
FUNCTION IN THE PRAIRIE VOLE (Microtus ochrogaster)

by

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ABSTRACT

Perchlorate is the oxidizer component in most solid rocket propellant formulations. It is known to reduce the production and output of the thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4). Thyroid hormones regulate metabolism in endothermic organisms and are responsible for maintenance of body temperatures above that of the environment. Little is known about the effects of perchlorate on metabolic capacity. The objectives of this study were to 1) determine if subchronic (51 days; 0, 1, and 10 mg/kg/day) and chronic (180 days; 0.75 mg/kg/day) perchlorate exposure in adult male prairie voles (*Microtus ochrogaster*) would alter resting metabolic rates as a result of decreased circulating thyroid hormone concentrations, and 2) determine if perchlorate exposure disrupts thermogenesis in mammals challenged with cold-stress. Voles exposed to perchlorate for 51 or 180 days experienced no significant alterations in resting metabolic rates at any point during the exposure period. Additionally, there was no treatment effect on peak metabolic rates or plasma thyroid hormone concentrations. Mean peak metabolic rates were 10.17 ± 0.85 , 10.17 ± 1.50 , and 9.37 ± 0.89 mL oxygen consumed per gram of body mass per hour for 0, 1, and 10 mg/kg/day groups, respectively. Mean plasma T_4 concentrations were 3.01 ± 0.83 , 2.74 ± 1.36 , and 1.70 ± 0.91 $\mu\text{g/dL}$, and mean plasma T_3 concentrations were 109.29 ± 15.05 , 94.83 ± 13.36 , and 95.57 ± 5.92 ng/dL for 0, 1, and 10 mg/kg/day treatment groups, respectively. However, thyroid gland thyroxine concentrations, 704.62 ± 372.62 , 454.20 ± 290.52 , and

219.54 ± 196.76 ng T₄ per thyroid gland for 0, 1, and 10 mg/kg/day treatment groups, respectively, were significantly lower in perchlorate-exposed voles than controls indicating that thyroid gland thyroxine content is a more sensitive endpoint for assessing perchlorate exposure in prairie voles. This study indicates that perchlorate-induced alterations in thyroid function do not necessarily translate into alterations in metabolic rate and/or decreases in thermogenic response. Overall, this study did not provide evidence for energetic alterations associated with perchlorate exposure at concentrations that are higher than those typically found in water in the environment. It may require a longer duration of exposure or higher dose to see a significant effect on resting metabolic rate and/or cold-tolerance. This is also the first study to present data on cold-induced PMR in a helox atmosphere in prairie voles and to show that thyroid gland thyroid T₄ decreases are the most sensitive measure of thyroid function in mammals in comparison to plasma thyroid hormone concentrations.

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CHAPTER I

INTRODUCTION

Perchlorate has become a contaminant of concern for state and federal regulatory agencies since it was detected in drinking water sources in Utah, California, and Nevada in 1997 (1). Much of the contamination occurs near military bases and ammunitions manufacturing sites where perchlorate is handled (2), however contamination has become more widespread due to its persistence and high mobility in aquatic systems (3).

Ammonium perchlorate is a powerful oxidizer used in solid propellant formulations intended for fireworks and explosives. Perchlorate salts are also used in the manufacture of matches and road flares. In the past, potassium perchlorate was used as a therapeutic agent for the treatment of hyperthyroidism resulting from Grave's disease and amiodarone-induced thyrotoxicosis.

Perchlorate is a competitive inhibitor of iodide transport across the basolateral membrane of the thyroid gland (4). Because of its similarity in size and charge, perchlorate competes with iodide for uptake into the thyroid gland at the sodium-iodide symporter. At therapeutic doses, the result is reduced secretion of the thyroid hormones (thyroxine (T_4) and triiodothyronine (T_3)) and the resulting increase in secretion of thyrotropin-stimulating hormone from the pituitary gland. Prolonged exposure to perchlorate may result in histopathological effects in the thyroid gland, including hyperplasia, hypertrophy,

and colloid depletion (5, 6). In birds, high doses of perchlorate have been shown to cause a reduction in thyroid hormone content within the thyroid gland (7).

Wildlife inhabiting perchlorate-contaminated sites may be exposed via drinking water or food items derived from contaminated matrices (8, 9). Recent research has shown that in addition to aquatic organisms, terrestrial species can also be exposed to perchlorate in the environment (8, 9, 10). Since perchlorate reduces thyroid hormone production, and thyroid hormones are required for the regulation of metabolism in endothermic organisms and play an important role in adaptive heat production (11), exposed terrestrial endotherms may experience altered metabolic function and reduced capacity for thermoregulation. Many small mammals such as the prairie vole have evolved to cope with cold temperature stress, but they have a limited ability to increase their insulation; therefore, behavioral avoidance and thermogenesis become the most important means for maintaining a relatively narrow range of body temperatures (12).

Urbansky (2) found groundwater and surface water perchlorate contaminations ranging from 8 ng/mL to 3.7 mg/mL associated with military bases in several western states and Smith et al. (8) found perchlorate concentrations of 30-31 mg/L in one holding pond on the Longhorn Army Ammunition Plant (LHAAP) in Karnack, Texas. However, groundwater and surface water perchlorate contamination is typically encountered at <500 µg/mL (2). In Utah, Urbansky (2) reported perchlorate contamination in groundwater ranging from 4-200 ng/mL, Smith et al. (8) reported perchlorate concentrations in

all water, sediment, and soil samples, with the exception of one pond, less than 85 ng/mL, and Smith et al. (9) reported water perchlorate concentrations ranging from 0.15 – 1.04 µg/mL with a mean concentration of 0.45 ± 0.08 µg/mL at the Las Vegas Wash, Nevada. Two of the three previous sites are considered to be highly perchlorate-contaminated sites. Considering that a prairie vole, the species used in this study, typically consumes approximately 0.29 ± 0.02 SE g water/g body mass/day (13), has a body mass of 40 g, and then assuming a daily exposure to perchlorate in water at concentrations ranging from 0.5 – 500 µg/mL; the expected perchlorate exposure would range from 0.145 – 145 mg perchlorate/kg body mass/day.

Data collected on perchlorate contamination in aquatic and terrestrial plants suggest a different scenario. Smith et al. (8) reported perchlorate concentrations in bullrushes (*Scirpus sp.*) growing in a highly contaminated pond (water perchlorate concentrations of 30 µg/mL) ranging from 840 ± 410 – 7620 ± 1460 ng/g dry weight. Perchlorate was also detected in seeds from goldenrod (*Solidago sp.*) and crabgrass (*Digitaria sp.*) at the LHAAP at concentrations of 184 and 1880 µg/g dry weight, respectively (8). Another study reported perchlorate in aquatic and terrestrial broadleaf and grass at concentrations ranging from below detection limits (100 ng/g) to 4460 µg/g, with a mean of 290 ± 94 µg/g dry weight (9). Considering that a prairie vole consumes approximately 0.13 g rodent chow/g body mass/day, and assuming a daily exposure to perchlorate in plant food items at concentrations ranging from 300 – 5000 µg/g

dry weight, the expected perchlorate exposure would range from 39 – 650 mg perchlorate/kg body mass. However, this calculation assumes the vole is consuming dry rodent chow versus wet plant food items (grasses, roots, and seeds) that are likely to be consumed in the environment. Therefore, food consumption will likely increase and plant perchlorate concentrations will likely decrease because previous perchlorate concentrations were reported on a dry weight basis. Other factors, such as perchlorate bioavailability in water versus food items, may alter the exposure scenario.

Perchlorate-induced alterations in thyroid function may be significant in organisms inhabiting regions with wide seasonal temperature ranges and/or daily temperature fluctuations. Individuals that are susceptible or responsive to decreased thyroid hormones may be placed at a disadvantage when faced with cold ambient temperatures. Disturbances in an animal's overall metabolic and thermogenic capacity may have the potential to influence whether the individual can survive, grow, and reproduce, as well as where it can colonize. Ultimately, these disturbances may set limitations on sensitive individuals and affect the abundance of a species.

To our knowledge, no studies have assessed the impact of perchlorate on metabolism in endothermic organisms. In general, overcoming the effects of toxic chemicals is metabolically costly, and there is a prediction that metabolic rate should increase with increasing levels of toxicants until irreversible pathological effects impair metabolism itself (14). However, perchlorate is

excreted virtually unchanged in both the rat and in man (15). Therefore, it is possible that there are very little or no energetic costs associated with biotransformation of the toxicant, and any alterations in metabolic rate will most likely be attributed to perchlorate-induced reductions in thyroid hormones.

The purpose of this study was to examine the effects of perchlorate on resting metabolic rates, facultative thermogenesis as measured by peak metabolic rate, and thyroid function in prairie voles. We hypothesized that perchlorate exposure would result in a decrease in thyroid gland hormone content and circulating thyroid hormone concentration, therefore reducing the resting metabolic rates of voles. We further hypothesized that perchlorate exposure would disrupt thermogenesis in voles, ultimately decreasing their ability to cope with cold-stress. Another objective of our study was to investigate the relative sensitivity of the different thyroid variables, plasma thyroid hormone and thyroid gland T₄ concentrations, for detecting an alteration in thyroid function.

CHAPTER II

MATERIALS AND METHODS

Drinking Water Solutions

Drinking water solutions containing ammonium perchlorate (NH_4ClO_4 , CAS No. 7790-98-9) were prepared in milli-Q water on a weekly basis during the study. The concentration of perchlorate in the drinking water was adjusted weekly for each treatment group, based on daily measured body weights and water consumption, to achieve desired dosage levels. Weekly dosage was calculated by using weekly mean treatment group values for body mass and treatment group means for water consumption. Dilutions (1:10) of the dosing solutions were prepared and passed through 0.45 μm Acrodisc® filters (Pall Gellman, Ann Arbor, MI). Filtered samples were then analyzed for perchlorate by ion chromatography using a preconcentration/preelution method (16).

Experimental Groups

Twenty-one adult male prairie voles were acquired from a breeding colony located at Texas Tech University and individually housed in acrylic Nalgene rodent cages (25 x 15 x 11 cm) partially filled with Aspen shavings. All animal activities were performed under protocols approved by the Texas Tech Animal Care and Use Committee. Prolab rabbit chow (5P26; Purina Mills, Richmond, IN, USA) and water were provided ad libitum. Voles were maintained in a

temperature controlled room at 25-26°C and 30-40% relative humidity on a 12h:12h light:dark photoperiod. All animals were weighed daily between 1200 and 1500 hours and food and water consumption were monitored on a daily basis during pre-exposure (one week) and exposure periods. Seven voles received continuous exposure to ammonium perchlorate via the drinking water at target dosage levels of 0, 1, or 10 mg perchlorate/kg body mass/day for 51 days. Five voles received continuous exposure via the drinking water at target dosage levels of 0.75 mg perchlorate/kg body mass/day for 180 days.

Prior to perchlorate exposure experiments, twelve voles were acquired from the same breeding colony and assigned to one of three dosing groups to take part in a short-term experiment studying the effects of thyroxine on vole resting metabolic rate. L-thyroxine hormone releasing pellets (1.5 mm diameter) were subcutaneously implanted into the lateral side of the neck (between ear and shoulder) of each vole. The pellets were designed to release thyroxine at a constant rate over 21 days (Innovative Research of America, Sarasota, FL, USA). In order to implant the pellets, voles were sedated and the pellet implanted before the animal awakened. Pellets were implanted with a 10 gauge trochar. Trochars were appropriately sterilized by dipping them into a 2% chlorhexidine solution and then rinsing the trochar with milli-Q water to remove any residual disinfectant. Voles in the control group received placebo pellets, voles in the low dose group received a 0.5 mg pellet (daily dose = 0.0238 mg/day

or 0.437 mg/kg body mass/day), and the high dose group received a 1.5 mg pellet (daily dose = 0.0714 mg/day or 1.29 mg/kg body mass/day).

Resting Metabolism

All animals were allowed free access to food and water prior to being placed into metabolic chambers. For all resting metabolic rate (RMR) measurements, voles were weighed and then placed in gas-tight metabolic chambers (modified plastic rodent cages; volume 2.1 L). Chambers were fitted for inlet and outlet tubes. Gas-cylinder air (dry grade; 20.95% oxygen) was metered into an incubator, where it was then channeled through a mass flow controller and a gas multiplexer (G245 and G244, respectively; Qubit Systems, Ontario, Canada). Gas then flowed into the animal chambers and excurrent air was rendered dry by passing it through a dessicant-filled column (magnesium perchlorate). A subsample of air (60 mL/min) from the excurrent air stream was rendered carbon dioxide free by passing through a soda lime column, and then routed into a differential oxygen analyzer (DOX) (S104; Qubit). Before reaching the soda lime columns, half of the subsample of air was pulled through a carbon dioxide analyzer (S154; Qubit) so that the sample reached the analyzers simultaneously. Metered air was directed to a single animal chamber for measurement, while simultaneously flushing air through 3 other unmeasured chambers. Air flow rates were adjusted to 850 mL/min (standard temperature and pressure conditions) for all vole chambers. During each trial, the automated

respirometry system was programmed to measure oxygen consumption and carbon dioxide production for each vole at 1 second intervals for 15 minutes per chamber and then switched to the next chamber in series. All data were collected by Labview 6.0 (National Instruments, Austin, TX) and imported into separate Microsoft Excel files for each gas channel. Before beginning measurements on the next vole in series, chambers were flushed with air for 15 minutes to ensure that residual gases had been removed from the system. Gas concentrations were measured in an empty chamber to obtain baseline levels passing through experimental chambers. Behavioral criteria and a video camera inside the temperature cabinet were used to determine whether animals were inactive during measurement of oxygen consumption. RMR testing occurred weekly for 4 weeks for thyroxine pellet experiments and 7 weeks for voles in the 0, 1, and 10 mg perchlorate/kg body mass treatment groups. Voles in the 0.75 mg perchlorate/kg body mass treatment group were tested on exposure days, 55, 90, 135, and 170. Resting metabolic rate was designated as the metabolic rate of an inactive vole measured during the rest phase of its daily cycle. It was calculated as the lowest measured rate of oxygen consumption averaged over a continuous 5-minute period at 28°C between 0730 and 1500 h.

Peak Metabolic Rate (cold-stress)

Maximal oxygen consumption during cold stress was determined after exposing voles to a respiratory gas mixture of approximately 80% helium and

20% oxygen (helox) (17). For sliding cold exposure tests, individual animals were exposed to a series of declining temperatures in helox. Cold stress temperatures were held at 12°C for 20 minutes followed by a continuous drop in temperature (~0.5°C per minute) until reaching 5°C. Helox tests were conducted for one hour or until the vole became hypothermic. These tests were conducted between 1200 and 1600 h on voles which were allowed free access to food and water prior to metabolic tests. Flow rates were maintained at 1000-1050 mL/min for measurements of peak metabolic rate. The mass flow monitor was calibrated for helox gas with the use of a wet cell calibrator (Gilian Gilibrator 2; Sensidyne; Clearwater, FL). Voles were weighed to the nearest 0.01 grams before and after testing. Metabolic chambers (volume 850 mL) consisted of a borosilicate glass tube capped on both ends fitted with an inlet and outlet. One end of the tube contained an electrical fan (velocity of 6.3 ft³/min) that aided in circulating air inside the metabolic chamber. One animal from each group was tested at a time. Before animals were tested, all chambers (2 blanks and 1 animal) were flushed with helox for at least 5 minutes at flow rates of 1000-1050 mL/min. Colonic temperature was measured before cold exposure and within 60 seconds after being taken out of the chamber. Peak metabolic rates (PMR) were calculated as the average of the highest rate of oxygen consumption over a 2-min period. Voles in the 0, 1, and 10 mg/kg body mass treatment groups were tested following 51 days of perchlorate exposure, while voles in the 0.75 mg/kg body mass group were tested on exposure day 180.

Animal Euthanasia and Sample Collections

One day after peak metabolic rate experiments, animals were weighed and then anesthetized in a saturated carbon dioxide chamber. Blood samples were then collected from each vole, placed into microcentrifuge tubes, and centrifuged until plasma had been separated. Plasma was then transferred to labeled tubes and frozen at -80°C until analysis. Following blood sampling, all animals were euthanized and necropsied. Livers, kidneys, and thyroid glands were collected and frozen at -80°C until analysis.

Plasma Hormone Analysis

Vole plasma total thyroxine (TT₄) and total triiodothyronine (TT₃) were measured using clinical radioimmunoassay (RIA) kits (Diagnostic Products Coat-A-Count; TKT4X and TKT3X, respectively). The assay procedures for plasma that accompanied the kits were followed, except for the inclusion of additional calibration points. Calibration points for the T₄ standard curve were 0, 0.2, 0.4, 0.6, 1, 4, 10, 16, 24 µg/dL. Plasma samples (35 µL) were analyzed in duplicate. Calibration points for the T₃ standard curve were 0, 5, 10, 15, 20, 50, 100, 200, and 600 ng/dL. Plasma samples (125 µL) were analyzed in duplicate. Both TT₄ and TT₃ radioimmunoassay kits were validated for prairie vole plasma by testing various volumes of plasma against the standard curve for parallelism, and

spiking plasma samples of known T₄ or T₃ concentration with standards from the RIA kit.

Thyroid Gland T₄ Content

Total T₄ content in vole thyroid glands was also measured by the method of McNabb and Cheng (7, 18). In brief, this involved radioimmunoassay of ethanol extracts of thyroid gland homogenates that had been digested with Pronase solution. The digestion mixture consisted of Tris-base (1.21 g), glutathione (153 mg), propylthiouracil (42.55 mg), Triton-X (1 mL), and milli-Q water (100 mL). All chemicals were obtained through Sigma Chemical Co., St. Louis, MO. Protease (*Streptomyces griseus*; Sigma) was added to the digestion mixture in an amount that equaled five times the mass of each vole thyroid gland. From this Pronase digestion solution, 350 µL was pipetted into each 2.0-mL microcentrifuge tube containing gland tissue from one vole. Each sample was vortexed and then incubated in a 37°C water bath for 24 hours. Following incubation, 1 mL of ice cold 100% ethanol was added to each tube, samples were vortexed, and tubes stored at -20°C for 24 hours. The following day, samples were centrifuged at 13,500 g for 5 minutes in a Beckman centrifuge (Allegra 21R; Beckman Coulter, Inc., Fullerton, CA). The supernatant was decanted into 1.5-mL microcentrifuge tubes and the pellet discarded. Extracts were stored at -20°C until RIA analysis.

The same clinical RIA kit used for plasma thyroid hormone analysis was used to determine total thyroid gland T_4 . Calibration standards were made in 100% ethanol and points for the standard curve were 0, 1.0, 5.0, 10.0, 15.0, and 25.0 $\mu\text{g/dL}$. Samples from extracts (25 μL) were analyzed in triplicate. The T_4 RIA kit was validated for gland extracts by testing various dilutions of extract samples against the standard curve for parallelism, and spiking sample extracts of known concentration with T_4 standards prepared in ethanol. Thyroxine spike recoveries from vole thyroid gland extracts were 97.38%.

Tissue Perchlorate Analysis

Ion chromatography was used to analyze vole liver and kidney tissues for perchlorate. Tissue samples were allowed to thaw, and a wet weight for each was recorded. Samples were allowed to air-dry for approximately 48 hours and reweighed. Perchlorate was extracted from the samples (entire tissue) using an accelerated solvent extractor (ASE; ASE 200, Dionex Corporation). The following operating conditions were used: milli-Q water as extraction solvent, 1500 psi, 1 cycle, 60% flush, 5 minute preheat, 5 minute static, and 100°C oven. Total extraction time was 15 minutes per sample. Total volume of extract collected (~22 ml) was measured and recorded. A 1:10 dilution of each sample extract was prepared. Samples were then cleaned using silica and C^{18} solid phase extraction (SPE) cartridges. Extracts were then filtered through 0.45 μm Acrodisc® filters (Pall Gellman, Ann Arbor, MI) and then analyzed via a

preconcentration/preelution ion chromatography method (16). Samples were concentrated on a Dionex TAC-LP1 with 10 mM sodium hydroxide eluent for 2.5 minutes and then injected into the separation system. Ion separation occurred on an analytical column (Dionex IonPac AS16) using 100 mM sodium hydroxide. Total run time was 12.5 minutes with a 0.92 mL/min flow rate and 1000 μ L injection volume. Retention times were used to identify perchlorate and the peak area was used for quantification. A standard curve was generated from calibration standards of 4, 50, 100, 150, and 300 ng/mL to determine sample concentrations. The tissue detection limit was 133 ppb in liver tissue and 980 ppb in kidney tissue on a wet tissue weight basis.

Statistical Methods

Measures of central tendency are expressed as mean $\pm$ standard error. Effects of exposure on thyroid hormone concentrations and thyroid hormone content were tested using analysis of variance (ANOVA). Peak metabolic rates were analyzed using ANOVA after log transformation. Any differences between group means were further analyzed using Duncan's multiple-range test. The effect of thyroxine or perchlorate dose on resting metabolic rates over time was tested with repeated measures analysis of variance using a general linear model procedure. Kidney mass (wet weight) was analyzed using analysis of covariance, using body mass at time of euthanasia as the covariate. Body mass was not a significant covariate for kidney mass; thus, the effects of exposure on

kidney mass were analyzed with ANOVA. When nongaussian distribution or heterogeneity of variance was observed, nonparametric tests were chosen for subsequent analyses. Liver mass (wet weight), mean body mass per week, and mean water and food consumption per week were analyzed using Wilcoxon's signed-ranks test. Weekly mean water and food consumption values per treatment group were calculated from an average weekly value per individual. Pearson's correlation coefficient and linear regression analysis were used to assess potential relationships between plasma T₄ or thyroid gland content and peak metabolic rate in voles, as well as plasma T₄ and thyroid gland content concentrations. All statistical analyses were performed with SAS 8.02 (SAS Institute, Cary, NC, USA). Statistical tests were considered significant when $p < 0.05$.

CHAPTER III

RESULTS

Perchlorate Intake

Following ion chromatography analysis of dosing solutions, actual mean perchlorate dose rates were calculated on a mg/kg/day basis. Mean perchlorate intake rates were 0 , 0.93 ± 0.21 , 1.33 ± 0.10 , and 11.68 ± 1.28 mg/kg/day/vole for the nominal 0 , 0.75 , 1 , and 10 mg/kg/day/vole groups, respectively (Table 3.1; Figure 3.1). Mean perchlorate concentrations in water were 0 , 2.16 ± 0.04 , 2.91 ± 0.07 , and 19.79 ± 0.99 mg/L for 0 , 0.75 , 1 , and 10 mg/kg/day treatment groups, respectively.

Thyroxine Pellet Effects on Metabolism

Mean resting metabolic rates were not significantly different between any of the treatment groups during the pre-implant measurement period or post-implant periods (Table 3.2; Figure 3.2). However, there was a noticeable dose-response relationship between thyroxine dose and metabolic rate at day 7 post-implantation. The dose-response diminished on weeks 2 and 3, but both the 0.5 and 1.5 mg T_4 pellet groups continued to have higher resting metabolic rates than control voles. Although differences between groups were not significant on any of the days post-implantation, the trend was apparent that thyroxine administration increased metabolic rate in a dose-dependent fashion.

Perchlorate Effects on Metabolism

There were no significant differences in RMR between any of the treatment groups during pre-exposure measurements ($n=20$, $F=0.53$, $p=0.6049$). There was no treatment effect on RMR during any of the 6 weeks of exposure (Table 3.3; Figure 3.3), nor was there a trend in RMR to decrease or increase for the long-term exposure group (0.75 mg/kg for 180 days). Mean RMR of the low (1 mg/kg) and high (10 mg/kg) exposure groups were higher than mean RMR of the control group through the first 4 weeks of exposure. Following that period, there was an increase in mean RMR of the control, low, and high exposure groups for the fifth week, and even further increases in mean RMR for control and high exposure groups during the sixth week. The reason for the increase was unknown. Mean RMR of voles in the 0.75 mg/kg group remained relatively constant throughout the 180 day exposure duration, with means of 1.66 ± 0.11 , 1.58 ± 0.11 , 1.72 ± 0.09 , and 1.48 ± 0.06 mL/g/h for exposure days 55, 90, 135, and 170, respectively (Figure 3.4). There were also no significant differences in PMR among treatment groups on exposure day 51 ($n=20$, $F=0.19$, $p=0.827$; Table 3.3; Figure 3.5), and the mean PMR of the 0.75 mg/kg treatment group was comparable to the 10 mg/kg group (Figure 3.5).

Thyroid Hormone Concentrations

Mean plasma T_4 concentrations from perchlorate-exposed voles decreased in an apparent dose-dependent manner, however there were no significant differences among the perchlorate treatment groups ($n=20$, $F=2.83$,

$p=0.0871$; Table 3.4; Figure 3.6A). Thyroxine concentrations from the long-term exposure group were comparable with concentrations from the 1 mg/kg treatment group (Table 3.4; Figure 3.6A). Likewise, plasma T_3 levels did not differ among the treatment groups following 51 days of exposure ($n=15$, $F=0.40$, $p=0.678$; Table 3.4; Figure 3.7). Plasma volumes were not sufficiently large for both T_4 and T_3 analysis for all 0.75 mg/kg treatment voles, two voles from the control and 10 mg/kg groups, and one vole from the 1 mg/kg group.

Mean thyroid gland T_4 concentrations from perchlorate-exposed voles decreased in a dose-dependent manner (Table 3.4; Figure 3.6B). There were significant differences in mean thyroid gland T_4 concentrations among 51-day exposure treatment groups (Fig. 3.6; $n=20$, $F=4.50$, $p=0.0268$). Post-hoc analysis revealed that thyroid gland thyroxine concentrations from the 10.0 mg/kg treatment group were significantly lower than control group concentrations. Thyroid gland thyroxine concentrations from the long-term exposure group were comparable with concentrations from the 1.0 mg/kg treatment group (Table 3.4; Figure 3.6B). Circulating T_4 levels and thyroid gland T_4 concentrations were not significantly correlated with peak metabolic rate ($n=20$, $r^2=0.099$, $p=0.177$; Figure 3.8; $n=20$, $r^2=0.023$, $p=0.520$; Figure 3.9, respectively). There was a significant relationship between circulating T_4 levels and thyroid gland T_4 concentrations from 51-day exposure treatment group voles ($n=20$, $r^2=0.621$, $p<0.0001$; Figure 3.10).

Other Physiological Indices

Mean body mass did not differ among treatment groups for any of the monitored weeks (Table 3.5; Figure 3.11). Voles from the 0, 1, and 10 mg/kg/day treatment groups had a mean decrease in body mass from the time period between first perchlorate-exposure and animal euthanasia, while voles from the 0.75 mg/kg/day group had a mean increase in body mass (Table 3.5; Figure 3.12). Analysis of vole kidney and liver masses from the 6-week exposure study indicated that there were significant differences in kidney mass among treatment groups ($n=20$, $F=4.00$, $p=0.038$; (Table 3.6; Figure 3.13), however no significant differences in liver mass among treatment groups ($n=20$; $\chi^2=4.212$, $p=0.122$; Table 3.6; Figure 3.14). Both control and 1 mg/kg treatment groups had significantly higher kidney wet weights than those of the 10 mg/kg group. Vole livers and kidneys contained no detectable levels of perchlorate (concentrations <133 and <980 ng perchlorate/g tissue, respectively). Both mean liver and kidney masses from the long-term exposure group were comparable to control or 1.0 mg/kg treatment groups (Table 3.6; Figures 3.13, 3.14). There were no significant differences in food or water consumption among groups for any of the monitored weeks (Table 3.1; Figures 3.15, 3.16).

Table 3.1. Mean ($\pm$ SE) perchlorate intake (mg/kg/day/vole) and food and water consumption (g/day/vole) for subchronic (0, 1, and 10 mg/kg/day/vole) and long-term (0.75 mg/kg/day/vole) treatment groups. Values presented for daily perchlorate intake are calculated as the overall treatment group means of the individual means as determined from 51 or 180 data points. Values presented for daily food and water consumption are calculated as the overall treatment group means of the weekly means per individual. Means for subchronic dosing groups are over a 51 day period, while means for the long-term dosing group are over a 180-day period.

Treatment	ClO ₄ ⁻ intake (mg/kg/day/vole)	Food consumption (g/day/vole)	Water consumption (g/day/vole)
0 mg/kg/day	0	8.52 (0.16)	19.86 (0.64)
0.75 mg/kg/day	0.93 (0.21)	8.38 (0.38)	21.66 (3.44)
1 mg/kg/day	1.33 (0.10)	8.55 (0.17)	23.22 (0.48)
10 mg/kg/day	11.68 (1.28)	7.97 (0.10)	20.15 (0.29)

Figure 3.1. Mean ($\pm$ SE) perchlorate intake (mg/kg/day) for 0.75, 1, and 10 mg/kg/day treatment groups. The 0.75 mg/kg/day treatment group was exposed for 180 days, while 1 and 10 mg/kg/day treatment groups were exposed for 51 days. Values presented for daily perchlorate intake are calculated as the overall treatment group means of the individual means as determined from 51 or 180 data points.

Table 3.2. Weekly mean ($\pm$ SE) oxygen consumption rates of prairie voles implanted with 21-day thyroxine releasing pellets. Units of oxygen consumption rates are expressed as mL of oxygen consumed per gram of body weight per hour (mL/g/h).

Treatment	Week			
	0	1	2	3
placebo	1.49 (0.09)	1.62 (0.07)	1.55 (0.12)	1.57 (0.22)
0.5 mg	1.52 (0.05)	1.76 (0.17)	1.72 (0.11)	1.72 (0.16)
1.5 mg	1.47 (0.07)	1.93 (0.10)	1.72 (0.01)	1.66 (0.24)

Figure 3.2. Mean oxygen consumption rates (mL/g/h) of prairie voles implanted with 21-day thyroxine releasing pellets. Values are means $\pm$ standard error for the 4 individuals each measured once during the indicated week.

Table 3.3. Weekly mean resting metabolic rates (mL/g/h; days 0-42) and peak metabolic rates (mL/g/h; day 51) of prairie vole treatment groups. Metabolic rates are in units of mL oxygen consumption per gram of body mass per hour (mL/g/h). Standard errors are given in parentheses.

Exposure day	Control	1 mg/kg	10 mg/kg
0	1.68 (0.08)	1.78 (0.07)	1.77 (0.12)
7	1.80 (0.14)	2.08 (0.23)	1.92 (0.10)
14	1.50 (0.12)	1.77 (0.11)	1.75 (0.09)
21	1.68 (0.14)	1.94 (0.13)	1.87 (0.14)
28	1.54 (0.13)	1.60 (0.07)	1.58 (0.06)
35	1.67 (0.12)	1.76 (0.12)	1.70 (0.11)
42	1.93 (0.21)	1.71 (0.06)	1.86 (0.06)
51	10.13 (0.85)	10.17 (1.50)	9.37 (0.89)

Figure 3.3. Weekly resting metabolic rates (mL/g/h) of prairie voles exposed to perchlorate in drinking water for 6 weeks at target doses of 0, 1.0, and 10 mg/kg/day, with sample sizes of 6, 7, and 7, respectively. Values are means $\pm$ standard error.

Figure 3.4. Resting metabolic rates of prairie voles (n=5) exposed to perchlorate at a target dose of 0.75 mg/kg/day for 180 days. Pre-exposure metabolic rate was not measured. Values are means $\pm$ standard error.

Figure 3.5. Peak metabolic rates (mL/g/h) of prairie voles in helox atmosphere following 51 days (0, 1.0, and 10.0 mg/kg; n=7/treatment) or 180 days (0.75 mg/kg; n=5/treatment) of perchlorate exposure in drinking water. Temperature in metabolic chamber held at 12°C for 20 minutes followed by a drop in temperature to 5°C for a maximum duration of 60 minutes. Values are means $\pm$ standard error. One vole in the control group died prior to peak metabolic rate experiments.

Table 3.4. Mean ($\pm$ SE) prairie vole plasma T₄ (μ g/dL), T₃ (ng/dL) and thyroid gland T₄ (ng T₄/thyroid gland) following 51 days of exposure (0, 1, 10 mg/kg/day) or 180 days exposure (0.75 mg/kg/day) to perchlorate in the drinking water.

Treatment	Plasma T ₄ (μ g/dL)	Plasma T ₃ (ng/dL)*	Thyroid Gland T ₄ (ng T ₄ / thyroid gland)
0 mg/kg/day	3.01 (0.83)	109.29 (15.05)	704.62 (372.62) ^a
0.75 mg/kg/day	2.69 (0.41)	NA	464 (182.05)
1 mg/kg/day	2.74 (1.36)	94.83 (13.36)	454.20 (290.52) ^{ab}
10 mg/kg/day	1.7 (0.91)	95.57 (5.92)	219.54 (196.76) ^b

*n=15

Values with different letters differ significantly (p<0.05).

Figure 3.6. Plasma T_4 concentrations ($\mu\text{g/dL}$) (A) and thyroid gland T_4 content (ng T_4 /thyroid gland) (B) per treatment group after 51 days of exposure to 0, 1.0, and 10.0 mg/kg ($n=7$ /treatment) or 180 days to 0.75 mg/kg ($n=5$) in drinking water. Values are means $\pm$ standard error. One vole in the control group died prior to peak metabolic rate experiments. Bars with different letters differ significantly ($p<0.05$).

Figure 3.7. Plasma T₃ (ng/dL) concentrations of prairie voles exposed to perchlorate in the drinking water at doses of 0, 1, and 10 mg/kg/day. Values are means $\pm$ standard error.

Figure 3.8. Regression analysis expressing changes of peak metabolic rate (mL oxygen consumed/g of body mass/hour) in prairie voles exposed to perchlorate as a function of plasma T₄ concentration (μg/dL).

Figure 3.9. Regression analysis expressing changes of peak metabolic rate (mL oxygen consumed/g of body mass/hour) in prairie voles exposed to perchlorate as a function of thyroid gland T₄ concentration (ng T₄/thyroid gland).

Figure 3.10. Regression analysis expressing changes of plasma T_4 concentrations ($\mu\text{g/dL}$) in prairie voles exposed to perchlorate as a function of thyroid gland T_4 concentrations (ng T_4 /thyroid gland).

Table 3.5. Mean ($\pm$ SE) body mass(g) and body mass change for subchronic (0, 1, and 10 mg/kg/day) and long-term (0.75 mg/kg/day) treatment groups. Means for subchronic dosing groups are over a 51 day period, while means for the long-term dosing group are over a 180 day period.

Treatment	Body mass (g)	Body mass change (g)
0 mg/kg/day	49.96 (0.62)	-2.71 (1.71)
0.75 mg/kg/day	53.02 (4.68)	+1.51 (1.04)
1 mg/kg/day	43.20 (0.24)	-1.78 (1.04)
10 mg/kg/day	42.28 (0.28)	-3.18 (0.76)

Figure 3.11. Mean ($\pm$ SE) body mass (g) of voles during subchronic perchlorate exposure (0, 1, and 10 mg/kg/day; 51 days) or long-term exposure (0.75 mg/kg/day; 180 days).

Figure 3.12. Mean body mass change as a percent of initial of voles during subchronic perchlorate exposure (0, 1, and 10 mg/kg/day; 51 days) or long-term perchlorate exposure (0.75 mg/kg/day; 180 days). Initial body mass was calculated as the mean of one-week of pre-exposure measurements. Values are means $\pm$ standard error.

Table 3.6. Mean ($\pm$ SE) prairie vole tissue masses (g; wet weight) following 51 days of exposure (0, 1, 10 mg/kg/day) or 180 days exposure (0.75 mg/kg/day) to perchlorate in drinking water.

Treatment	Tissue Mass (g)	
	Liver	Kidney
0 mg/kg/day	2.27 (0.20)	0.51 (0.03) ^a
0.75 mg/kg/day	2.16 (0.19)	0.52 (0.08)
1 mg/kg/day	2.01 (0.27)	0.50 (0.03) ^a
10 mg/kg/day	1.84 (0.16)	0.41 (0.03) ^b

Values with different letters differ significantly ($p < 0.05$).

Figure 3.13. Kidney mass (g; wet weight) of voles following perchlorate exposure at target concentrations of 0, 1, and 10 mg/kg/day for 51 days and 0.75 mg/kg/day for 180 days. Sample sizes are 6, 7, 7, and 5 for 0, 1, 10, and 0.75 mg/kg/day treatment groups. Values are means $\pm$ standard error. Bars with different letters differ significantly ($p < 0.05$).

Figure 3.14. Liver mass (g; wet weight) of voles following perchlorate exposure at target concentrations of 0, 1, and 10 mg/kg/day for 51 days and 0.75 mg/kg/day for 180 days. Values are means $\pm$ standard error.

Figure 3.15. Food consumption (g/day/vole) of prairie voles during subchronic perchlorate exposure (0, 1, and 10 mg/kg/day; 51 days) or long-term perchlorate exposure (0.75 mg/kg/day; 180 days). Values presented for daily food consumption are calculated as the overall treatment group means of the weekly means per individual. Values are means $\pm$ standard error.

Figure 3.16. Water consumption (g/day/vole) of prairie voles during subchronic perchlorate exposure (0, 1, and 10 mg/kg/day; 51 days) or long-term perchlorate exposure (0.75 mg/kg/day; 180 days). Values presented for daily water consumption are calculated as the overall treatment group means of the weekly means per individual. Values are means $\pm$ standard error.

CHAPTER IV

DISCUSSION

This study indicates that perchlorate-induced alterations in thyroid function do not necessarily translate into alterations in metabolic rate and/or decreases in thermogenic response. Initially we expected to see an increase in vole metabolic rate after first exposure, followed by a gradual drop in metabolic rate during the last weeks of exposure. We hypothesized that initial exposure would cause the hypothalamus-pituitary-thyroid (HPT) axis to compensate for any reductions in thyroid hormone secretion from the thyroid gland. An increase in circulating thyroid hormones usually results in increased metabolic rate, as numerous studies have documented among thyroid hormone-administered rodents and birds (19, 20, 21). With severe and/or prolonged perchlorate exposure and/or iodine deficiency in the thyroid, HPT activation will be unable to maintain euthyroid status, causing decreased levels of circulating thyroid hormones and eventually reduced metabolic rate. However, despite reductions in thyroid hormone concentrations in exposed groups, the trend of decreased RMR was not evident in our data over the six-week exposure period.

We also hypothesized that it might take a longer time than 6-7 weeks to see perchlorate-induced changes in plasma or thyroid gland T_4 and/or T_3 concentrations. The mammalian thyroid gland has an ability to store large amounts of hormone (22); therefore, physiological effects of thyroid hormone

deficiency may not be observed for weeks or months, depending on the organism's capacity to store hormone. Additionally, it may take a longer exposure duration to see any potential perchlorate-induced reductions in RMR. Although we did observe slight reductions in both plasma and thyroid gland thyroxine concentrations in the long-term exposure group (0.75 mg/kg/day for 180 days) there were no alterations in RMR throughout the exposure period.

RMR values for prairie voles from this study are consistent with data from other studies (23, 24). Mean RMR of prairie voles collected from the field during summer and winter ranged from 1.40 ± 0.15 to 2.16 ± 0.34 mL/g/h, respectively, with mean summer body masses of 48.5 ± 8.7 and winter body masses of 38.5 ± 4.5 g (23). The overall mean RMR of control voles calculated from 7 weeks of data from this study was 1.68 ± 0.06 mL/g/h, with a mean body mass of 49.96 ± 0.62 g. Therefore, the absolute RMR data collected from this study likely reflect the true RMR of prairie voles.

Perchlorate exposure had no effect on RMR in this study, but that did not necessarily imply that there would be no effect on metabolic rate under conditions of cold exposure. Peak aerobic performance under cold-challenge was used as an ecologically relevant indicator of thermogenic response in perchlorate-exposed organisms. Since there have been no reports of any species of *Microtus* showing torpor either on a seasonal basis or for a shorter term, they must always expend energy for thermoregulation. Therefore, thermoregulation is a major maintenance cost for wild voles, as well as other

rodent species with similar behavior (25). Our data did not demonstrate a marked reduction in the ability of perchlorate-exposed animals to deal with cold-stress tests. Again, it may require a longer duration of exposure or higher dose to see such an effect on cold-tolerance. Some of the voles in the high exposure group showed signs that they were able to deal with cold temperatures as well as control animals. Three of the seven voles in the high exposure group were able to sustain peak metabolic rate until completion of the 60 min cold exposure, and following removal from the chamber, their internal body temperature had only decreased 2°C. These voles also had metabolic rates higher than those of the mean control vole metabolic rate; however, other voles in the high dose group had substantially lower peak metabolic rates than control or low dose voles. This is most likely due to variability in individual response to perchlorate exposure and variability in fur thickness and subcutaneous fat reserves, which may ultimately influence thermal conductance and sensitivity to cold temperatures. It also appears that long-term perchlorate exposure may have little effect on a prairie vole's capacity for facultative thermogenesis.

This is the first study to present data on cold-induced PMR in a helox atmosphere in prairie voles. Other studies have presented data for norepinephrine (NE)-induced non-shivering thermogenesis in prairie voles, as well as oxygen consumption of prairie voles exposed to 5°C in air in a laboratory setting (23, 25). It was not determined whether those values represented PMR in prairie voles. One study reported NE-induced non-shivering thermogenesis

metabolic rates ranging from approximately 1.80 – 4.20 mL/g/h (25). Oxygen consumption of prairie voles exposed to 5°C in air was reported as 2.72 ± 0.40 mL/g/h (23). Mean PMR of prairie voles from our study was approximately six-times higher than mean RMR, while NE-induced and cold air temperature-induced thermogenesis metabolic rate values were approximately 42% and 27% of PMR values from our study, respectively. Therefore, it is likely that PMR was not achieved in these other studies, and our study presents data on the true thermogenic capacity of prairie voles.

Several studies have suggested that an increase in thermogenic capacity may be expected with an increase in brown adipose tissue deposits (23, 26, 27). Although, vole carcasses were not investigated for presence of brown adipose tissue deposits, several carcasses among treatment groups contained high levels of intraperitoneal white fat deposits. White adipose tissue functions as a heat insulator, as its thermal conductance is only approximately one-third of that of other tissues, as well as an energy source, whereas brown adipose tissue primarily serves in heat generation for mammalian non-shivering thermogenesis. An increase in brown adipose tissue may coincide with the growth of white adipose cells; therefore it may be expected that some voles with higher levels of brown adipose tissue could have increased thermoregulatory capabilities. In most scenarios, insulation will suffice to meet most summer cold stress since these should be moderate relative to winter cold stress. In winter at the lowest temperature stresses, insulation needs met by white adipose tissue alone may

not suffice to combat heat loss; therefore, prairie voles have increased levels of thermogenesis and must have high energetic turnover.

Voles in our study were provided with a formulated rabbit chow, which should provide them with sufficient nutrients to maintain or exceed the daily energy needs of a caged rodent. Past studies have shown that animals that are well fed in the laboratory may maintain high body temperatures as a result of increase metabolic turnover rate (ie. turnover rate of chemical energy in an animal's body) (23, 27). However, wild voles are primarily grass feeders and food quality and perhaps food quantity may vary seasonally, thus shifting metabolic turnover rate and demanding higher total energy needs for winter animals over summer animals. Given that voles do not have the important adaptive pattern of torpor, which allows an animal to store energy and reduce foraging activity during coldest conditions, that other rodents express, they must continue to forage through some of the coldest conditions. Foraging in times of cold temperatures and reduced food quality may place additional stress on an animal that may be expensive in terms of energy. This may ultimately influence compensatory mechanisms to cold-stress, such as the growth of white and brown adipose tissue, as well as an animal's sensitivity to cold temperatures and thermogenic response.

Our data did not show a marked reduction in plasma T_3 . Small sample size ($n=15$) for T_3 analysis may explain why there were no statistically significant differences. Although the results were not statistically significant, we did observe

a dose-response pattern in plasma T_4 levels (Figure 3.6A). We also found statistically significant reductions in thyroid gland thyroxine content in high dose treatment groups compared to controls (Figure 3.6B). Despite a reduction in T_4 , T_3 is the biologically active thyroid hormone and any reduction in T_4 may not have an impact on T_3 concentrations. A change in T_4 concentration that does not have an impact on deiodination processes would not impact the levels of T_3 (28). Cellular metabolism and the generation of ATP are tied to concentrations of T_3 , which were not found to be affected by perchlorate exposure. It is logical that resting metabolic rates as well as peak metabolic rates would not be affected either.

Alterations in plasma thyroid hormone concentrations could have been ameliorated by the HPT axis, which tends to restore circulating thyroid hormones to euthyroid levels. To our knowledge, there have been no other studies investigating perchlorate effects on metabolic rate in endothermic organisms; however, another endocrine disruptor acting on the thyroid gland, Tapazole, yielded decreases in serum T_4 concentrations, but had no effect on standard metabolism (29).

Reductions in thyroid hormones are often related to perchlorate exposure and thyroid hormones are key players in metabolic capacity, however, we found no relationship between peak metabolic rate and plasma thyroxine concentrations or thyroid gland content. Therefore, despite reductions in thyroid

hormone concentrations, it may be possible that these animals were able to overcome any potential effects of exposure.

It is also important to note that iodine was not limited in the rodent diet, as the rodent chow contains an average value of 0.48 ppm iodine (www.labdiet.com). With sufficient or high iodine or iodide in the diet, the HPT axis may be able to maintain a euthyroid status (30).

Although results were not statistically comparable, a 180-day exposure to perchlorate at 0.93 mg/kg caused similar effects on thyroid function as did a 42-day exposure at 1.33 mg/kg. It is noteworthy that such a strong relationship existed between plasma and thyroid gland T_4 concentrations. With sufficient competition between iodide and perchlorate at the sodium-iodide symporter, there may be reduced T_4 production within the gland and therefore less secretion into the blood. Perhaps it may be important to investigate various duration and dose combinations to determine how long and what doses it would take to cause a biologically meaningful depletion of thyroid gland thyroid hormone. Such an effect may have severe implications for an animal's thermogenic capacity and response to cold temperatures.

One of the most often used indices of exposure has been plasma thyroid hormone level, which is probably the most variable measure of thyroid function. This is most likely due to the dynamic adjustment ability of the HPT axis (7, 30). This study showed that glandular T_4 content may be a more sensitive indicator of perchlorate-induced alterations in thyroid function in prairie voles than plasma

hormone concentrations. This is the first study to document such a finding in mammals. Current research is also proving that this technique may be a better indicator of perchlorate exposure than plasma thyroid hormone concentrations, at least in avian species (7), and perhaps in rodents as well, pending further research.

The indication of no difference in RMR among treatments groups and the large variability among weeks within a treatment group may also have been attributable to an increase in activity within the metabolic chamber. High variability of peak metabolic rate between animals of the same treatment and low sample sizes may have obscured differences as well. The configuration of the respirometry system and confounding factors such as long wash times (time taken for recirculation of gas in a chamber) made the process of collecting metabolic rates relatively difficult. The number of animals that could be run simultaneously (three per day) was low due to arrhythmic activity of voles, and thus limited the sample sizes at each metabolic rate sampling period. In some cases, test voles may have rested for a total of 30 minutes in the metabolic chamber per 7-8 hour test period. Other species, such as *Peromyscus* sp., may be better models to use when investigating contaminant effects on metabolic function (31, 32). Future studies of this nature must find a balance between designing experiments that incorporate larger sample sizes, while still making the procedures of measuring oxygen consumption a relatively time and cost efficient process.

Energy expenditure, as measured by oxygen consumption, has been a useful endpoint in linking environmental contaminant exposure to a measure of individual performance. For example, terrestrial organisms exposed to contaminants such as polychlorinated biphenyls incur greater maintenance costs and may be adversely affected by cold-stress (32, 33, 34). However, this study did not provide evidence for energetic costs associated with perchlorate exposure at the individual level. It also did not provide evidence that rodents exposed to perchlorate in water at concentrations that were much higher than those typically found in surface or groundwater are adversely affected by short-term cold exposure. The perchlorate concentrations used in our study may reflect those of highly perchlorate contaminated sites, such as the LHAAP in Texas and Las Vegas Wash in Nevada.

There are issues that must be taken into consideration before drawing any conclusions on whether perchlorate exposure can induce alterations in metabolic rate, and more importantly thermoregulatory capabilities. First, we must continue to investigate how the HPT axis responds to perchlorate exposure at varying levels. Another issue concerns the complex hormonal control of thermogenesis in endothermic organisms. Although thyroid hormones are key players in heat production, adaptive thermogenesis is primarily controlled by the sympathetic nervous system via norepinephrine (35). Because animals often have the ability to adapt behaviorally or physiologically under conditions of toxicant stress, there should be an emphasis on the need for further evaluation of

the interaction between perchlorate exposure and adaptive strategies. For instance, small mammals in temperate climates often compensate for thermal stress in winter by increasing their resting metabolic rate and activity of nonshivering thermogenesis (36). Rodents can also respond to cold exposures by inducing growth of brown adipose tissue (37). Prairie voles also show a higher weight-specific rate of oxygen consumption in winter than in summer which may allow tolerance to lower thermal exposures (23). Given that rodents have these physiologically controlled adaptive responses to cold-conditions, it is possible that low, environmentally-relevant concentrations of perchlorate exposure will have little or no effect on an animal's ability to deal with cold-stress or cause alterations in metabolic rate.

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APPENDIX

Body Mass, Body Mass Change, Water Consumption and Food Consumption of Prairie Voles Implanted with 21 Day Thyroxine- Releasing Pellets

Table 5.1. Mean ($\pm$ SE) body mass (g), body mass change (g), and water and food consumption (g/day/vole) over a 3- week period of prairie voles implanted with 21-day thyroxine-releasing pellets. Values represent 3 weeks of data.

Treatment	Body mass (g)	Body Mass Change (g)	Water consumption (g/day/vole)	Food consumption (g/day/vole)
placebo	51.81(3.30)	+2.70 (0.45)	18.68 (3.01)	7.96 (0.54)
0.5 mg	53.39(2.19)	+1.55 (0.41)	23.24 (3.65)	9.90 (1.07)
1.5 mg	53.83(3.89)	+2.56 (0.80)	17.00 (1.60)	9.17 (0.67)

Figure 5.1. Mean ($\pm$ SE) body mass (g) of prairie voles that received 21-day thyroxine-releasing pellets. Values represent 3 weeks of data.

Figure 5.2. Mean ($\pm$ SE) increases in body mass (g) for prairie voles that received 21-day thyroxine-releasing pellets. Values represent 3 weeks of data.

Figure 5.3. Mean ($\pm$ SE) water consumption (g/day/vole) of prairie voles that received 21-day thyroxine-releasing pellets. Values presented for daily water consumption are calculated as the overall treatment group means of the weekly means per individual. Values represent 3 weeks of data.

Figure 5.4. Mean ($\pm$ SE) food consumption (g/day/vole) of prairie voles that received 21-day thyroxine-releasing pellets. Values presented for daily food consumption are calculated as the overall treatment group means of the weekly means per individual. Values represent 3 weeks of data.

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