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Captive Canada Geese Acceptability and Toxicity Trials with Two Formulations of 0.005% Diphacinone Rodenticide Baits

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ABSTRACT: The 0.005% diphacinone rodenticide pellets used in this study have been proposed for use in field applications to control introduced rodents on conservation lands in the state of Hawaii. Introduced rodents (especially *Rattus* spp.) cause a wide array of conservation problems in the Hawaiian Islands and on other islands. We assessed the acceptability and toxicity (should the pellets be consumed) of two rodenticide baits to Canada geese, a surrogate species for the endangered Hawaiian goose. Based on these trials with captive, wild Canada geese, it appears that neither the whole nor the chopped (to simulate broken or weathered baits) pellets pose a significant risk to the Hawaiian goose, a species considerably smaller than the Canada goose. The pellets (whole or chopped) were not accepted by the Canada geese during this study despite their having only a small amount of green grass sod as an alternative food. There were no mortalities of geese during the feeding trials and all geese remained healthy, based on body weights and packed blood cell volumes. The endangered status of the Hawaiian goose precluded using it as the target study species.

KEY WORDS: anticoagulant, *Branta canadensis*, Canada goose, diphacinone, Hawaii, nontarget hazard, pesticide hazards, rodenticide

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INTRODUCTION

Introduced rodents (especially *Rattus* spp.) cause a wide array of conservation problems in the Hawaiian Islands and on other islands (Stone and Anderson 1988, Buckle and Fenn 1992, Ohashi and Oldenburg 1992). Damage to native flora and fauna have been documented, including several cases of contribution to species endangerment.

Rodenticides are an important tool in the management and eradication of rodents introduced to islands (Witmer et al. 1998). The use of rodenticide baits in Hawaii could contribute to the management of introduced rodents on conservation lands, but caution must be used to reduce non-target hazards. Two relatively large (6-8 g per pellet) pelleted rodenticide baits are being proposed for use on conservation lands in Hawaii. The size of the pellets ensures that, when dropped from aircraft, they will fall through the forest canopy to the forest floor where they can be consumed by foraging rodents. Nontarget hazards to native, wild mammals are virtually nonexistent in Hawaii, where there are no native terrestrial mammals except the hoary bat (*Lasiurus cinereus*), which feeds on insects captured in flight (Tomich 1986). Although a number of non-native, mammalian game species are actively hunted by humans, the proposed bait would be used only in fenced areas not open to hunting or in areas that are closed to hunting during and following the application of bait.

Many native species of birds, however, cannot be excluded from baited areas. One of the more notable of these is the endangered Hawaiian goose ["NeNe"; *Branta* (= *Nesochen*) *sandvicensis*], the state bird of Hawaii (Berger 1988). The Hawaiian goose feeds on the buds, flowers, seeds, fruits, and leaves of a variety of plants (Scott et al. 1986). Although anticoagulants such as diphacinone are believed to pose little secondary hazard (e.g., Mendenhall and Pank 1980, Radvanyi et al. 1988), it is important to assess the primary hazard that would result

from the direct consumption of rodenticide baits (e.g., Banko et al. 1999). Waterfowl seem relatively resistant to diphacinone, based on the LD₅₀ of 3,158 mg/kg body weight for mallards (Anas platyrhynchos) (World Health Organization 1995). A review by Colvin et al. (1988) reported that the primary hazard of diphacinone to gallinaceous birds appears to be low. In a trial with captive quail (Callipepla californica), Blus et al. (1985) observed no increase in mortality or in prothrombin times after consumption of chlorophacinone pellets, but that several quail died from impaction of the gizzard by paraffin from baits that contained paraffin. In a review of anticoagulant poisoning of wildlife in New York, Stone et al. (1999) reported 51 cases, none involving waterfowl and only four involving diphacinone. However, considering the wide variation in species-specific responses to anticoagulants (Timm 1994), pen trials, as conducted in this study, with captive, wild geese would provide important data for decision-makers considering the registration of diphacinone baits for use on conservation lands in Hawaii.

The design of this study closely followed the EPA's (1996) Ecological Effects Test Guidelines: OPPTS 850.2200, Avian Dietary Toxicity Test. The study was conducted under the approved NWRC Project: Integrated Pest Management Strategies for Rodent Damage to Agriculture. The 1996 Research Needs Assessment (Bruggers 1996) of the Wildlife Services Program identified the need for new and improved methods to reduce damage by rodents as a high priority research area. This study was conducted in compliance with the EPA's Federal Insecticide, Fungicide, and Rodenticide Act under Good Laboratory Practices (GLP; 40 CFR Pt. 160), the USDA's Animal Welfare Act, and the National Environmental Policy Act (NEPA).

The objectives of the study were to assess the acceptability and toxicity of two 0.005% diphacinone rodenticide bait formulations to geese, using the Canada

goose (B. canadensis) as a surrogate for the Hawaiian goose.

METHODS

This study was conducted in north-central Colorado, Larimer County, near Fort Collins, using outdoor pens located at a Colorado Division of Parks and Wildlife research facility.

Two manufacturers (HACCO, Inc., Madison WI, and J. T. Eaton & Co., Inc., Twinsburg, OH) formulated and supplied the 0.005% diphacinone (CAS 82-66-6) pelleted HACCO Bait (EPA Reg. No. HI-980006) and Eaton Wax Bait (EPA Reg. No. HI-970007) used in this study. Fiftythree free-ranging Canada geese were live-trapped, using a cannon net, at College Lake, Fort Collins, Larimer County, on February 8, 2000. These geese were group-housed in a fenced enclosure until the feeding trials began. An additional seven free-ranging Canada geese were captured, using alpha-chloralose-treated bread baits, at the Colorado State University (CSU) Veterinary Teaching Hospital, Fort Collins, on August 4, 2000. Geese were captured, transported, and handled under state and federal permit authority (State of Colorado Permit 00-TR060 and Federal Fish and Wildlife Service Permit MB019065-8). Prior to the feeding trials, birds were maintained in a group pen, measuring about 10×22 m, with sides and top completely enclosed by wire mesh sides and top.

For the feeding trials, individual, chain-link panel, outdoor pens were designed and built for the study. Each pen measured about 2.1×4.3 m and 1.8 m high. A durable, but flexible, nylon mesh was used to cover all individual pens. Geese were individually housed in pens for two weeks prior to the study to undergo the necessary quarantine and to allow acclimation to the pens and the maintenance diet of pelleted duck grower chow and cracked corn. About 455 ± 1 g (1 lb.) of maintenance diet was provided to each goose every morning, approximately between 8-10 am, using a food bowl placed on a sheet of composite board so that spillage could be gathered and weighed each day. Water was provided ad libitum to all birds. Any bird not eating or appearing to be in poor health was not used in the feeding trials.

Trial 1

Five groups (10 geese/group) of geese (50 total) were randomly assigned to 1 of 5 treatment groups: Group 1, Eaton Wax Pelleted Bait Diet (EP); Group 2, HACCO Pelleted Bait Diet (HP); Group 3, Eaton Wax Pelleted Bait Diet, chopped pellets (EPC); Group 4, HACCO Pelleted Bait Diet, chopped pellets (HPC); and Group 5, Maintenance Diet Only (control). Pellets were chopped to simulate weathering or breakage under field conditions. Pellets were chopped on a wooden chopping block in the laboratory, using a butcher's knife.

Birds were hand or net captured from the group pen for processing and placed in one of 10 adjacent individual pens representing one treatment until that treatment group had up to a maximum of six birds of one sex; subsequent captures were added of the opposite sex until each treatment group of 10 pens included 4-6 birds of each sex. Three of the groups had five birds of each sex, while two

groups had four males and six females (i.e., each treatment group of 10 geese was composed of 5-6 female geese and 4-5 male geese).

Prior to Trial Day 1, weights of geese were recorded to the nearest 0.1 kg by placement in a pre-weighed cloth bag (weighing 0.35 kg) and suspending from a hand-held spring scale. The goose weight was the observed scale weight minus the bag weight. The spring scale was not calibrated, but the same spring scale was used throughout the study. A blood sample was then taken from a wing vein of each goose for determination of the percentage packed blood cell volume. We used a standard technique, involving centrifuging blood-filled microhematocrit tubes at 12,000 rpm for about five minutes (Campbell 1988). The upper and lower levels of the blood in the tube were marked with a permanent marker. After centrifuging, the upper level of the packed blood cells in the bottom of the tube was marked. The percentage packed blood cell volume was determined by taking two measurements with a millimeter ruler: the total blood height in the tube and the height of the packed blood cells in the bottom of the tube. The latter number (mm) was divided by the former (mm) and multiplied by 100 to give the percentage packed blood cell volume. Usually, two microhematocrit tubes were processed per bird and the average percentage packed blood cell volume was used for the analysis. Blood clotting time (prothrombin time) was not used as a measure of animal health because a necessary reagent, avian freezedried prothrombin, is no longer available on the commercial market (Dr. Terry Campbell, CSU, pers. commun.; also, Becton, Dickerson, and Company, Product Information).

On the morning of Trial Day 1, remaining maintenance food was removed from each pen in Groups 1-4, approximately between 8-10 am, and replaced with 454-456 g (about 1 lb.) of the appropriate test diet (EP, EPC, HP, or HPC). A consistent beginning weight could not be easily achieved because the variation in whole pellet and chopped pellet weights. Treatment diets were randomly assigned to a group of 10 pens by a roll of a six-sided die. Each pen was provided with a mat (about 0.3×0.3 m) of fresh sod (Kentucky blue grass, Turf Master, Ltd., Fort Collins, CO). The fresh grass provided an alternative food similar to what would occur in a natural setting complete with some of the nutrients, such as Vitamin K or its precursors, that birds would obtain by foraging on green vegetation (Robbins 1993). Each day, remaining food in the bowl was removed along with any visible spillage and weighed. A Mettler PE3600 balance was used for weighing food before and after placement. The accuracy of the balance was confirmed with a certified set of weights. Food bowls were cleaned, as necessary, prior to addition of the newly allotted ration. The food bowls were then filled with 454-456 g of fresh diet and returned to the appropriate pens. The grass sod mats were watered and replaced as needed. The Group 5 (control) geese continued to receive the daily allotment of maintenance diet throughout the trial along with the sod mat.

Each day, two bowls each of the five treatment diets were placed near the goose pens and covered with a wire mesh to exclude animals. These samples were processed daily in the same manner as the pen samples and were used to monitor moisture loss or gain so that the trial food weights could be adjusted accordingly. Daily bait consumption was determined by weighing the food presented before and after each 24-h period, corrected for moisture loss or gain.

According to the Study Protocol, the treatment geese were to receive their respective daily ration for seven trial days unless they were not consuming a substantial portion of the treated diet. If the treated diet was not consumed, or only minimally consumed (i.e., S 10%), by the geese in any of Group 1-4 by the end of Trial Day 3, the treated diet for that group was replaced with the maintenance diet for the duration of the 7-day trial. That treated diet was then declared unacceptable to the geese in that group. In Trial 1, the treatment diets were only minimally consumed, and all geese were returned to the maintenance diet at the start of Day 4.

At the beginning of Trial Day 4, all geese were returned to the maintenance diet for a post-exposure period. One bird (ID#974) escaped during the post-exposure period and, hence, that treatment group (HP) only had nine birds for data analysis. After a 14-day post-exposure period, all geese were weighed, a blood sample drawn to determine packed cell volume, and then euthanized by lethal injection (1.5-2.0 cc of Euthansol injected into a brachial vein). If any geese had died during the Trial, including the post-exposure period, the post-exposure period would have been extended until two consecutive days passed with no deaths of treatment group geese (EPA 1996); however, no geese died during the Trial. After euthanasia, the carcasses were frozen for later necropsy.

Six birds (three females and three males) of each treatment group (a total of 30 geese) were randomly selected (by rolls of a six-sided die) for necropsy. Necropsies were conducted by a board-certified veterinary pathologist, Dr. Terry Spraker (DVM, Ph.D.) of CSU, with experience in avian necropsy. During necropsy, birds were examined for signs of anticoagulant poisoning: bloody excretions, bruising, subcutaneous and internal hemorrhaging, and other abnormalities as described by Stone et al. (1999). Gizzards were examined for paraffin impaction as described by Blus et al. (1985).

Trial 2

Trial 2 was conducted because of a raccoon (*Procyon lotor*) problem that affected one treatment group (J. T. Eaton wax pelleted bait diet, chopped; EPC) during Trial 1. Three raccoons were captured and removed from the area on August 25, 2000, and the problem of missing food from some pens was resolved. Trial 2 was conducted similar to Trial 1, using a control group of 10 geese and a treatment group (EPC) of 10 geese, with differences noted below.

Plans to use the same 20 geese (10 control geese and 10 EPC-treatment geese) from Trial 1 had to be modified somewhat because of subject health concerns. One goose (ID #13; in the EPC group of Trial 1) had consistently lost weight during Trial 1 and died (on September 6, 2000) after the Trial's post-exposure period and before Trial 2 was begun on September 18, 2000. The bird was replaced

by a goose randomly chosen from the remaining geese in the group pen. Two other birds (ID#970; in the control group of Trial 1 and ID#998; in the EPC group of Trial 1) had lost substantial weight (0.35 kg) as of the start of Trial 1. These two birds were also replaced by two random selections from the group pen.

The cloth bag (used to weigh the geese prior to the start of Trial 2) weighed 0.3 kg, so that weight was subtracted from the total goose-in-bag weight to get the starting weight for each goose. The geese were not consuming the EPC treatment diet by the beginning of Day 4; hence, as in Trial 1, the birds were put back on the maintenance diet to begin the post-exposure period which, in the case of Trial 2, lasted 10 days.

Geese were randomly assigned to one of the five groups. If some geese had died during a trial, the percent mortality of control geese and treatment geese was to be compared with a Chi-square contingency test; however, no geese died during Trial 1 or Trial 2. Food consumption by geese was compared between treatment groups using a Multiple Analysis of Variance (MANOVA). The weights of geese were compared between treatment groups and pre- and post-treatment using a MANOVA. The same procedure was used to test for differences in packed blood cell volumes, pre- and post-treatment. Necropsy results were qualitatively described by treatment.

On August 16, 2000 (just prior to the first feeding trial), a sample of each of the two pellet types was placed and sealed in labeled zip-lock bags for later chemical analysis by the Analytical Chemistry Unit of NWRC. The samples were stored in a dark, dry laboratory cabinet at room temperature for two days until submitted to the Analytical Chemistry Unit of NWRC on August 18, 2000. Bait analysis (% active ingredient) was performed by the Analytical Chemistry Unit of NWRC using Analytical Method 71A.

RESULTS AND DISCUSSION

Bait Consumption

The pellets used for the four diphacinone treatment groups consisted of the two whole-pellet types and the two chopped-pellet types.

During Trial 1, a problem with free-ranging raccoons occurred that affected the results of one of the treatment groups. The raccoons were consuming J. T. Eaton chopped pellets in three or four of the 10 pens in that treatment group. Three raccoons were immediately live-trapped and euthanized and the problem did not recur. As a result, only the bait consumption data from four treatment groups [maintenance diet (control); HACCO pelleted bait (HP); J. T. Eaton wax pelleted bait (EP); and HACCO pelleted bait, chopped pellets (HPC)] were analyzed for Trial 1.

There was a significant difference (P < 0.001) in food consumption between maintenance and treatment diets, with much more maintenance diet being consumed than any of the diphacinone diets (Table 1). On average, about 62.4 g (13.7%) of the offered maintenance diet was missing and presumed to be consumed each day. On average, less than 13 g (<3%) of any of the diphacinone diets were missing from the pens each day. This small amount indicates that very little consumption occurred with any of

Table 1. Average daily food consumption, expressed as amount (g) and as a percentage (%) of amount offered each day to Canada geese by treatment and by trial, Fort Collins, Colorado, 2000.

Treatment	Mean Grams Consumed (% of amount offered)	Standard Error of the Mean	
TRIAL 1			
Maintenance diet (control)	62.4 (13.7)	6.0	
J. T. Eaton pellet, whole (EP)	11.2 (2.5)	6.0	
HACCO pellet, whole (HP)	13.0 (2.9)	6.0	
HACCO pellet, chopped (HPC)	4.2 (0.9)	6.0	
TRIAL 2			
Maintenance diet (control)	147.0 (32.2)	11.0	
J. T. Eaton pellet, chopped (EPC)	14.1 (3.2)	11.0	

Table 2. Average Canada goose weight (kg) and percentage packed blood cell volume (%PBCV) by treatment, pre- and post-trial periods, Fort Collins, Colorado, 2000.

Treatment	Weight in Kg (S.E.)		%PBCV (S.E.)	
	Start	End	Start	End
Maintenance diet (control)	3.9 (0.2)	3.8 (0.2)	47.3 (1.3)	50.3 (1.3)
J. T. Eaton pellet,	3.7 (0.2)	3.6	50.0	50.9
whole (EP)		(0.2)	(1.3)	(1.3)
J. T. Eaton pellet,	3.9 (0.2)	3.8	48.0	48.3
chopped (EPC)		(0.2)	(1.4)	(1.4)
HACCO pellet,	3.6 (0.2)	4.0	46.0	49.6
whole (HP)		(0.2)	(1.4)	(1.4)
HACCO pellet,	4.0 (0.2)	4.0	47.3	49.4
chopped (HPC)		(0.2)	(1.3)	(1.3)

the treated diets; small errors in moisture adjustments and spillage could account for much of the missing treated bait. As a result, the diets were deemed unacceptable to the geese, and the geese were returned to the maintenance diet at the beginning of Trial Day 4.

During Trial 1, some rainfall (about 6.6 mm) occurred between the placement and collection of the three days' treatment diets. Consequently, the contents of each food bowl were put in separate, labeled plastic bags; all bags were placed in a drying oven (about 52°C) until the weights stabilized. The J. T. Eaton pellets contain paraffin, so this approach was not entirely successful with those treatments as the liquid paraffin formed a thick layer over the bag's contents that would not allow evaporation of water. The weights were adjusted, nonetheless, by using a weight from moisture check bowls for that treatment. The contents of the moisture check bowls were processed in the same manner as described above for wet treatment foods.

Trial 2 was conducted due to the raccoon problem that affected one treatment group (EPC) in Trial 1. In the second trial, there was a significant difference (P < 0.0001) in food consumption with much more maintenance diet

being consumed than the J. T. Eaton chopped-pellet diet (Table 1). On average, about 147 g (32.2%) of the offered maintenance diet was missing and presumed to be consumed each day. On average, about 14.1 g (3.2%) of the diphacinone diet was missing from the pens each day.

Again, this small amount indicates that very little consumption of the treated diet occurred and, indeed, the small errors in moisture adjustments and spillage could account for most of the small amounts of missing treated bait. As a result, the treatment diet was deemed unacceptable to the geese, and the geese were put back on the maintenance diet at the beginning of Trial Day 4.

During Trial 2, some rainfall (about 15.2 mm) occurred between the placement and collection of the 2nd day's treatment diets. Consequently, the contents of each food bowl were poured through a 2 mm wire mesh funnel to remove water. The solid contents in the mesh funnel were placed into separate, labeled plastic bags; all bags were allowed to air-dry in the laboratory (about 22°C) until the weights stabilized. Again, the weights were adjusted by comparing them to the respective moisture check bowl contents which were handled in the same manner as described above for the wet treatment foods. A summary of the food consumption by goose in Trial 2 is presented in Table 1.

Mortality

No geese died during either trial. This includes the pretrial quarantine period, the three-day periods when the treatment diets were offered, and the post-exposure periods (14 days in Trial 1; 10 days in Trial 2) when all geese were put back on the maintenance diet. One goose (JD #13; in the EPC group of Trial 1) that had consistently lost weight during Trial 1 died (on September 6, 2000) after the trial's post-exposure period and before Trial 2 was begun on September 18, 2000. That bird, which would have been used in Trial 2, was replaced with a bird from the group pen. Another goose (JD#974; in the HP group of Trial 1) escaped (on or about August 26, 2000) and was not recaptured during the post-trial maintenance diet period of Trial 1, hence the total number of birds for that treatment group (HP) was 9 not 10 like the other treatment groups.

Weights and Packed Cell Volumes

There were no differences (P = 0.78) in geese body weights between treatments (Table 2). There were no differences (P = 0.25) among weights of geese before or after the trials (Table 2). Geese generally weighed between 3.6 and 4.0 kg. Most geese either maintained their weight or lost a small amount of weight.

There was no difference (P=0.55) in percentage packed blood cell volume (%PBCV) change (pre- and post-trial) between treatments (Table 2). There was, however, a notable difference (P=0.002) between the %PBCV of the geese overall before and after the feeding trials, independent of treatment (Table 2). Geese generally had packed blood cell volumes between 46.0% and 50.9% and most geese had somewhat higher %PBCV at the end of the study, indicating good health. Campbell (1988) noted that most caged birds have %PBCVs of 35-55%; values lower than 35% may indicate anemia, while values

above 55% may indicate dehydration.

Necropsy

Only a subset (30 of 50) of the geese used in the study were necropsied because the geese consumed only a very small amount of the treatment diet. Had the geese consumed much more of the treatment diet, or had any substantial differences been noted between treatment and control birds as a result of the necropsy by the veterinary pathologist the remainder of the frozen carcasses would have been necropsied as well.

The veterinary pathologist found no notable differences between the treatment groups and no obvious lesions compatible with anticoagulants in any of the birds. Some birds in each group showed mild bruising of the pectoral muscles and edema of the legs; this is consistent with the frequent contact with the chain-link fencing of the pens by many of the wild-caught birds when the pens were approached by people or when a goose acted aggressively towards a goose in a neighboring pen.

Bait Assay

Three sub-samples of each of the two bait pellet types were analyzed for diphacinone concentration by the NWRC Analytical Chemistry Unit on September 5, 2000, about two weeks after the beginning of the first trial. The percent active ingredient of diphacinone in the three sub-samples of HACCO bait pellets averaged 0.00272% with a standard deviation of 0.000036%. The percent active ingredient of diphacinone in the three sub-samples of J. T. Eaton wax bait pellets averaged 0.00389% with a standard deviation of 0.000035%. For both types, the concentrations determined by the NWRC analytical method were far below the stated label concentration of 0.005%.

CONCLUSIONS

The 0.005% diphacinone rodenticide pellets used in this study have been proposed for use in field applications to control introduced rodents on conservation lands in the state of Hawaii. Based on these trials with captive, wild Canada geese, it appears that neither whole nor chopped (to simulate broken or weathered pellets) pellets pose a significant risk to the Hawaiian goose, a species considerably smaller than the Canada goose. The pellets (whole or chopped) were not acceptable to the Canada geese used in this study despite the geese having only a small amount of green grass sod as an alternative food. While it would be desirable to conduct this trial with Hawaiian geese, their endangered status precluded that option.

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