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Abstract: The nutria (*Myocastor coypus*) is a large semi-aquatic rodent, declared as one of the 100 worst invasive species in the world by the IUCN Invasive Species Specialist Group in 2000. Through USDA Small Business Innovative Research Phase I (Mach 2004) and Phase II (Mach 2006) funding, Genesis is developing a new nutria rodenticide as an alternative to the currently-registered zinc phosphide bait. Our course of research and development is described herein: live-trapping, laboratory non-toxic choice studies, laboratory toxicity bioassays, and field toxicity studies. Two active ingredients and two bait types proved to be successful, however, only cholecalciferol in each of two bait types will be tested in future field studies.

Key Words: cholecalciferol, coypu, invasive species, *Myocastor coypus*, nutria, rodenticide.

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INTRODUCTION

The nutria or coypu is a large (over 6 kg), semi-aquatic rodent with a voracious appetite and high reproductive potential. It is a rodent native to South America that was introduced to the United States (US) in fur farms; however, they have become established in the wild in numerous states. The marsh habitat of Louisiana proved to be ideal and populations exploded, reaching an estimated 20 million animals in less than 20 years (Evans 1970).

Numerous studies of the wetland environments of Louisiana have documented the deleterious effects of nutria grazing. Nutria herbivory has been documented on species of bald cypress (*Taxodium distichum*) (Conner and Toliver 1987), *Sagittaria latifolia* and *S. platyphylla* (Grace and Ford 1996, Evers et al. 1998), *Spartina patens* and *S. alterniflora* (Taylor et al. 1997, Ford and Grace 1998), and many other species of marsh vegetation (Fuller et al. 1985, Foote and Johnson 1992, Taylor and Grace 1995).

Trapping of nutria for their pelts formed the backbone of the Louisiana trapping industry from the 1960s until the early 1980s when price for fur fell dramatically. Since then, the annual trapping harvest has dwindled to 29,544 in the 2000-2001 season. Reports of damage to wetland habitats emerged in the late 1980s. In November 2002, an incentive-payment program for the nutria control was initiated through funding by a 20-year program, Coastal Wetland Planning, Protection, and Restoration Act (CWPPRA) with the intent of curbing the amount of marsh herbivory. The program has been successful in that an approximate mean of 300,000 nutria tails per year have been turned in for payment. Adjustment has been made in the incentive payment, currently $5 per tail, to entice additional control and retain a high level of trapping pressure.

Through USDA Small Business Innovative Research (SBIR) grants, we are developing a unique buoyant rodenticide to be applied aerially or by hand spot-baiting for nutria control. A unique toxicant and bait form will provide an alternative to current control methods.

PROJECT METHODS AND RESULTS

Prior to the initiation of any of the studies, several nutria were live-captured by personnel of Genesis Laboratories, Inc. and Louisiana Department of Wildlife and Fisheries, Fur and Refuge Division, New Iberia, Louisiana. All nutria were captured at Salvador Wildlife Management Area, an area managed by the State and located near Paradis, Louisiana, southwest of New Orleans. They were captured by hand or by large fishing net while driving an airboat alongside the swimming or running nutria. As they were captured, they were placed in portable wire mesh cages. The nutria were transported to the Department of Wildlife and Fisheries office in New Iberia or to Genesis Laboratories, Inc., in Wellington, Colorado, for holding and laboratory research and development. IACUC approved protocol numbers N04015 and N07004 were reviewed and followed for appropriate animal care.

Nutria were held individually in stainless steel cages with a floor area of 576 in^2 (24 x 24 x 15 in)

or in small groups in larger stock tanks with a floor area of approximately 1,200 in^2. Nutria were offered vegetables *ad libitum* as food and a water source. Bedding was changed at least once each week.

The project progressed with the following studies: choice bioassays, toxicant efficacy, and secondary toxicity. Following is a short description of the methods and the results of each study.

Choice Bioassays

Native vegetation was intended to be the challenge diet that we would use for all choice tests, but it quickly became evident that the nutria could consume large amounts and transportation acquisition was very difficult. If nutria are eating normally, they can consume as much as 25% of their body weight each day (Christen 1978). We began using raw russet potato as the "challenge diet."

Due to the large amount of food that the nutria could consume, we used limited exposure periods during the work hours of each study day. For example, for a 2-day test, we would offer the challenge diet (potato) and the test substance for approximately 4-6 hours during each study day. We were limiting the exposure period of the test substance to assure that the nutria would not consume all of one food item and then begin eating the less preferred item. Allowing consumption of an entire diet item would give false results on the performance of the test diets. To achieve a valid data set, spilled diets were retrieved from the bedding of each cage. Test substance acceptance (%) was calculated with the following formula:

We began testing different formulas, vegetables, fruits, commercially available pet feeds, modified floating fish baits, and many other food-grade formulations. Many of the formulations did not float and were dismissed from additional testing.

Finally, two types of formulas were chosen as possibilities, a vegetable bait (celery or carrot) to be covered with the toxicant, and a gum-based formula in to which the toxicant could be formulated.

Toxicant Efficacy

We have evaluated the use of four different toxicants that are currently registered for other rodent species and uses throughout the world. We evaluated bromethalin, diphacinone, cholecalciferol, and sodium monofluoroacetate. Not all of these toxicants are currently registered in the US, but their potential for this particular use was justifiable for research purposes. Zinc phosphide was not considered as it is already registered and used as a topical toxicant on vegetables for nutria control.

Nine nutria were tested with each of the following active ingredients: cholecalciferol, bromethalin, diphacinone, and sodium monofluoroacetate (Table 1). These data provided initial toxicity of these compounds to nutria and provided justification to remove some of the compounds from the list of acceptable toxicants.

After this test, we did not choose to re-examine bromethalin because of the very poor efficacy (33%) achieved by a 275 ppm bromethalin bait, a concentration 2.75 times higher than that of any other commercial rodenticide, even though bait acceptance appeared sufficient. Although diphacinone tested well with 100% mortality, we were unwillingly to possibly sacrifice efficacy due to nutria consuming

$$\frac{\text{Total Test Substance Consumption (g)}}{\text{Total Test Substance Consumption (g) + Total Challenge Diet Consumption (g)}} \times 100\%$$

Table 1. Active ingredient efficacy trials conducted in December, 2004.

Nutria Per Test	Challenge Diet (Sweet Potato)	Test Diet (Toxicant-covered Sweet Potato)		Test Diet Acceptance (%)	Mortality and Duration to Last Death
	Mean Consumption (g)	Toxicant and Concentration	Mean Consumption (g)		
9	914	Cholecalciferol (825 ppm)	475	34	100% 8 days
9	94	Sodium Monofluoro-acetate (550 ppm)	173	65	100% 1 day
9	1025	Bromethalin (275 ppm)	827	45	33% 2 days
9	1455	Diphacinone (55 ppm)	1132	44	100% 8 days

vegetation high in vitamin K. Vitamin K is the antidote to diphacinone, and is common in green leafy plants.

Our remaining options were sodium monofluoroacetate and cholecalciferol. Additional active ingredient testing with sodium monofluoroacetate (SM) and cholecalciferol was conducted. Using celery as the carrier, we added 750 ppm cholecalciferol and 500 ppm SM to groups of six animals. We achieved 100% efficacy (i.e., mortality) in both tests. We decided to test how low we could go with the concentration of the SM on celery and still produce mortality. We formulated a 100 ppm SM bait and offered it in a choice test. We achieved mortality in 3.5 hours (184 grams bait consumed). Again, we dropped the concentration and formulated a 10 ppm SM bait and offered it in a choice test. Mortality was achieved in 6 hours (208 grams bait consumed). As a final step, we offered 50 grams of the 10 ppm bait to the last nutria in a no-choice test. Mortality was achieved between 6 (first symptoms) and 23 hours (found dead). Testing with 750 ppm cholecalciferol baits are consistently effective at controlling nutria.

Although the testing with SM appeared to be effective at very low levels, it is highly scrutinized as a vertebrate pest management tool. It was removed from registration as a food bait toxicant in the US in the 1970s. Therefore, it was not considered for further testing, even though our research has shown it to be very effective as a nutria toxicant.

Cholecalciferol was our final choice for a toxicant for nutria control.

Secondary Toxicity

Two species were evaluated for secondary toxicity of cholecalciferol. The red-eared slider (*Pseudemys scripta*) was used as a surrogate scavenger to the many species of reptiles that are found in the marshes, and the domestic ferret (*Mustela putorius furo*) was used as a surrogate mammalian predator for the mustelid species found in the marshes.

To prepare the dose for each respective species, five males and five female Norway rats (*Rattus nowegicus*, Wistar strain) were offered 750 ppm bait in a no-choice feeding regime until dead (Table 2). This type of feeding regime would simulate a "worst case scenario" as if an animal solely chose to consume the bait. After the rats had died, they were placed into individually labeled plastic bags and stored in a freezer to minimize any further degradation of the carcass and active ingredient in the carcass. After the rat skin and feet were removed, the carcass was ground in a meat grinder to a medium consistency and then re-ground with the same setting to help mix the carcass. The carcass was then re-ground again with a fine setting and further mixed by hand post-grinding to maintain a homogeneous mix.

Red-eared Slider. The turtles were acquired from a local pet store and were transported to Genesis Labs in a cardboard box. Sex and age were unknown, but carapace diameter suggests that they were of adult age.

Table 2. No-choice consumption of the cholecalciferol bait by Norway rats.

Rat ID	Sex	Total Bait Consumption (g)	Total mg/kg Body Weight Consumed	Days until Death
1		66.7	97.0	3
2		48.5	81.6	4
3	Male	8.1	18.7	9
4		45.0	71.5	4
5		47.3	79.2	4
26		60.9	187.0	3
27		79.7	230.3	5
28	Female	70.5	196.9	5
29		71.5	180.4	4
30		35.6	109.4	3

To deliver the test substance to the turtles, the exit hole of 10 ml syringe was drilled out to allow larger particles to travel through the syringe. A Soverign Feeding Tube and Urethral Catheter (size: 16" 14 Fr.) was cut to 6" with a bevel and attached to the exterior diameter of the syringe neck. The syringe and tube was preloaded with the meat and tared on a balance. Then, an additional amount of meat was added to the syringe and weighed as a pre-dose weight. The turtle was held by a technician and the turtle mouth was kept open with a flat screwdriver, held vertically in the turtle's mouth. The mouth was not pried open, but kept open after the turtle opened its mouth to bite the screwdriver. The tube was slowly placed into the turtle's mouth by rotating the tube and syringe to minimize application resistance of the tube. The syringe plunger was depressed until the test material was observed in the throat or mouth. After dosing the turtle, the syringe was weighed to determine dose. The turtles were held in a plastic tub for 3 hours to assure consumption of the meat and lack of regurgitation.

Upon consultation with Gary Witmer of the USDA National Wildlife Research Center, alligator (*Alligator mississippiensis*) secondary toxicity studies with zinc phosphide suggested that intoxication in reptiles may be postponed instead of the expected quick onset of symptoms. Due to this possibility, our post-treatment observation period was extended.

Table 3 identifies the dosage given to each turtle during the two doses. Female rat #28 (Table 2) was used to dose all four turtles (70.5 g bait consumption, 196.5 mg active ingredient per kg body weight). To produce a worst case scenario, we dosed the turtles on days 0 and 6 with the assumption that a turtle might feed every 6 days within a 12-day maximum exposure period during a field baiting.

During the 20-day post-test observation, no signs were observed that indicated any ill-health of the turtles. This suggests that this species of turtle, and possibly other reptiles, may not be affected by secondary exposures to cholecalciferol from consuming poisoned nutria.

Domestic Ferret. The ferrets were acquired from a reputable supplier and were transported to Genesis Labs in wood framed/hardware cloth cages. They were held in plastic-coated wire cages with a floor area of 576 in^2 (24 x 24 x 16 in). Ferrets were offered Purina ferret chow *ad libitum*. Bedding pans with wood shavings were placed below each cage and changed at least once each week.

Table 4 identifies the total dosage consumed by each of the two ferrets during the five-day exposure period. The male ferret consumed portions of rat 1, 2, 4, 26, and 29. The female ferret consumed portions of rat 3, 5, 27, 28, and 30 (Table 2).

As an additional measure of intoxication or sub-lethal toxicity, a blood draw was conducted 5 days after the initial exposure for analysis of albumin and calcium levels. Both of the two treatment ferrets and an extra control animal were tested. The control animal was tested to measure normal blood levels. The blood draw results were normal for the control and the treated female, but were elevated with the treated male ferret (Table 5).

Albumin is also measured to identify a concurrent effect of intoxication because albumin is a carrier for calcium. For ferrets, the normal range of calcium

Table 3. Total dose of rodent-poisoned Norway rat carcass gavaged to the turtles.

Turtle ID	Turtle Body Weight (g)	Theoretical Maximum mg a.i./kg body weight (Dec 15, 2005)	Theoretical Maximum mg a.i./kg body weight (Dec 21, 2005)	Total Theoretical Maximum mg a.i./kg body weight
1	290	2.31	3.94	6.25
2	268	3.23	2.50	5.73
3	331	2.74	3.51	6.25
4	329	1.92	2.15	4.07

Table 4. Ferret consumption of the poisoned Norway rat carcass.

Ferret ID (sex)	Ferret Body Weight (g)	Total Carcass Consumption (g)	Total A.I. Consumed (mg a.i. / kg body weight)[a]
1 (m)	1,420	905.5	322.2
6 (f)	814	567.4	258.3

[a] The theoretical consumption is based upon equal distribution of the toxicant in the body and no degradation.

Table 5. Ferret blood sample analysis results.

Ferret ID	Albumin Level (g/dL)	Calcium Level (mg/dL)
Control	2.7	10.4
Treated Male #1	4.1▲	18.9▲
Treated Female #6	2.8	11.5

▲ = The hematology instrument indicated elevated levels compared to normal levels.

levels is 8.0 to 11.8 mg/dL. A typical level of calcium that may initiate clinical physical symptoms is ~14 mg/dL. The effects are reversible. Although the male levels indicate an effect, long-term observation (30 days) of the ferrets did not indicate any visible physical effects at any time. During the 25-day post-test observation, no physical signs were observed that indicated any ill-health of the ferrets. This suggests that ferrets may not be affected by secondary exposures to cholecalciferol in nutria carcasses.

DISCUSSION

Based on this series of research studies, we conclude that 750 ppm cholecalciferol (vitamin D_3) bait is an ideal formulation to test further for nutria control.

Although vitamins are essential to survival, large amounts of vitamin D_3 can be toxic. Its mode of action involves the mobilization of calcium from the bones into the circulatory system causing hypercalcemia, a highly toxic condition that is not easily reversed. Marshall (1984) reports field studies in which 90 to 98% efficacy was achieved with Norway and roof rats (*R. rattus*) and house mice (*Mus musculus*) in 31 different locations of the US, low toxicological risk to birds (chronic oral LC_{50} quail [*Coturnix coturnix*] 2,000 ppm; mallard [*Anas platyrhyncos*] 4,000 ppm; LD_{50} mallard >2,000 mg/kg), virtually insoluble in water, and low toxicity to fish. A secondary toxicity study conducted with beagles (*Canis familiaris*) resulted in no symptoms and/or death after consuming poisoned rats for 14 consecutive days (Marshall 1984).

The next step in testing is to field test the baits. At this time, pilot field testing will be conducted to test the vegetable-based formula and the gum-based formula. Each bait offers certain positive characteristics that need to be evaluated in a field test. After the pilot field test is conducted on each bait formula, a final formula will be chosen and used in a larger, definitive field test. Other ecotoxicology tests are expected to be conducted as well.

CONCLUSION

Genesis Laboratories, Inc. has initiated research necessary to develop a floating, efficacious nutria toxicant bait. To date, cholecalciferol is the preferred toxicant, and two candidate formulations are to be tested in a pilot field study. The most successful formulation will be further developed. If another nutria toxicant can be registered, resource managers will have another tool with which to manage the invasive nutria.

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