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DEVELOPMENT OF NICARBAZIN AS A REPRODUCTIVE INHIBITOR FOR RESIDENT CANADA GEESE

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Abstract: Expanding populations of resident Canada geese that remain in suburban and urban areas year-round often result in increased conflicts with humans. Non-lethal and humane means are needed for managing the size of Canada goose flocks residing near or on airports, golf courses, industrial parks, government sites, and city parks. A side effect of nicarbazin, a veterinary drug used to control coccidiosis in chickens, is decreased egg production and hatching. Exploiting this side effect, studies of nicarbazin for reducing the hatchability of eggs from Canada geese were conducted. An initial study in Coturnix quail verified reduction in hatchability in a species other than chickens. Because plasma nicarbazin was not routinely measured, a study in chickens was conducted to determine the relationship between plasma and egg nicarbazin. A comparative study in chickens, mallards, and Canada geese showed that nicarbazin absorption was lowest in geese. Studies in both penned and wild Canada geese showed that reduction in hatchability was possible but neither study used bait suitable for general field application. Bait development led to the OvoControl-G® (Innolytics LLC) bait, which resulted in reduction in hatchability of 51% at treated sites compared to control sites in the field. Previous studies showed that nicarbazin is practically non-toxic and is environmentally friendly; timing and management of baiting will minimize non-target hazards. OvoControl-G® 2500 ppm nicarbazin bait is recommended for incorporation into a comprehensive management plan as a reproductive inhibitor for use in controlling resident Canada goose flock sizes.

Key words: avian contraception, avian population control, Canada geese, hatch control, nicarbazin, OvoControl-G®, resident geese, wildlife contraception, wildlife population control

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INTRODUCTION

Resident Canada goose (*Branta canadensis*) populations are rapidly increasing across the United States, causing increasingly frequent conflicts with humans. Although many communities want reductions in resident goose populations, lethal control alone is often not considered acceptable. Reproductive control methods have been well accepted by the public as a

tool for managing resident Canada goose populations. However, current methods require locating individual goose nests to allow coating the eggs with oil (egg oiling) (Cummings et al. 1997), shaking eggs (egg addling), or puncturing eggs to prevent them from hatching. Because Canada geese often nest in areas difficult to access and at low densities, techniques targeting individual nests are time consuming and costly, sometimes with poor success in terms of the number of nests treated when conducted by volunteers from the community. Development of contraceptive bait that could be fed to resident Canada geese at central locations in nesting areas would allow treatment of many nests at one time.

NICARBAZIN

Nicarbazin has been registered with the Food and Drug Administration (FDA) since 1955 to treat and control coccidiosis in broiler (meat) chickens, a disease caused by intestinal protozoa and primarily manifested as weight loss. Accepted worldwide as a safe and effective product, nicarbazin is a 1:1 equimolar crystalline complex of two compounds, 4,4'-dinitrocarbanilide (DNC, CAS #330-95-0) and 2-hydroxy-6,6-dimethylpyrimidine (HDP, CAS # 108-79-2) (Figure 1). Nicarbazin is hydrated through exposure to aqueous environment (e.g., gastrointestinal fluid, rain, etc.) for 3-5 h, with slightly acidic pH providing faster hydration (J. Hurley, NWRC, personal communication). Upon hydration the parent complex dissociates into DNC and HDP, releasing crystals of DNC $\leq$ 1 µm in size for active transport of DNC from the gastrointestinal tract into the bloodstream (Rogers et al. 1983). However, DNC crystals are hydrophobic and quickly form aggregates up to 20 µm in size, which are too large for absorption (Rogers et al. 1983). Thus, hydration of nicarbazin must occur in the intestines for absorption of DNC and reproductive inhibition.

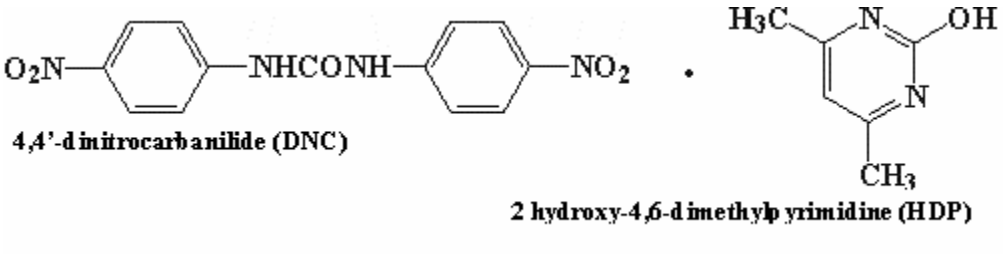

Figure 1. Structure of Nicarbazin: Nicarbazin is a 1:1 equimolar crystalline complex of two compounds, 4,4'-dinitrocarbanilide (DNC) and 2-hydroxy-6,6-dimethylpyrimidine (HDP).

NICARBAZIN AS AN AVIAN REPRODUCTIVE INHIBITOR

Accidental feeding of nicarbazin to laying or breeding hens results in reductions in both egg laying and egg hatchability. Nicarbazin (25-100 ppm) added to the diet of chickens (*Gallus domesticus*) reduced hatchability of eggs from 6 to 10 days after treatment began (Jones et al. 1990). Hatchability of eggs produced by hens treated with 100 ppm nicarbazin was reduced to <1 %. During treatment, egg yolk DNC levels increased in proportion to the amount of nicarbazin fed, with 100 ppm nicarbazin resulting in yolk DNC levels averaging 15.67 µg/g. Other studies of nicarbazin in chickens showed that plasma DNC levels are not constant until day 6 (Furusawa 2001) and that treatment for 8 to 10 days is required to reach maximum egg DNC levels (Jones et al. 1990).

Whether nicarbazin had similar reproductive effects in other avian species was unclear, so the National Wildlife

Research Center (NWRC) conducted a pilot study with Coturnix quail (*Coturnix japonica*). Quail were treated with 125 ppm nicarbazin (estimated dose of 36 mg/kg) in commercial chicken feed (Koffolk, Inc.) for 25 d. Maximal reduction of hatchability to 0% and maximal yolk mottling were seen in week 4.

The relationship between plasma DNC levels, egg DNC levels, and hatchability had not been established, so a study in commercial laying chickens was conducted to determine these relationships (Johnston et al. 2001). Methods to assay avian plasma and egg DNC levels were developed by NWRC Analytical Chemistry (Primus et al. 2001). White Leghorn hens were fed 0 ppm, 25 ppm (1.68 mg/kg), 50 ppm (3.36 mg/kg), 75 ppm (5.04 mg/kg), and 100 ppm (6.72 mg/kg) nicarbazin in commercial chicken feed (Koffolk, Inc.) for 14 d. Peak plasma DNC levels were 0 µg/ml, 1.5 µg/ml, 3.0 µg/ml, 7.0 µg/ml, and 8.0 µg/ml, respectively. Peak egg DNC levels were 0 µg/g, 3.5 µg/g, 8.0 µg/g, 13 µg/g, and 14 µg/g, respectively. Treatment with 50, 75, and 100 ppm nicarbazin reduced hatchability to 12%, 17%, and 8%, respectively. This study showed that plasma DNC levels were approximately 50% of egg DNC levels (Johnston et al. 2001).

NICARBAZIN FOR CANADA GEESE

Based on the findings in chickens and quail, a study was performed to assess the absorption of nicarbazin in Canada geese as compared to chickens and mallards. All 3 species were gavaged for 8 days with gelatin capsules containing 8.4 mg/kg body weight nicarbazin, the approximate dose in chickens free feeding on 125 ppm commercial chicken feed. The highest plasma DNC levels were observed at 6-8 days in the 8 day treatment period and were 2.9 µg/ml, 2.4 µg /ml, and 1.5 µg/ml in chickens, mallards, and Canada geese, respectively (Figure 2). Plasma DNC levels fell to 0 µg/ml by 2 day post-treatment in ducks, 4 day post-treatment in geese, and 6 day post-treatment in chickens. This study showed that application of nicarbazin to reduce egg hatchability in Canada geese would require use of a higher dose than that in chickens due to reduced drug absorption in the goose. It was estimated that plasma DNC levels of 2.5-3.0 µg/ml and egg DNC levels of 5.0-8.0 µg/g would be required to achieve a 50% reduction in the percent hatchability of eggs laid by treated versus control birds.

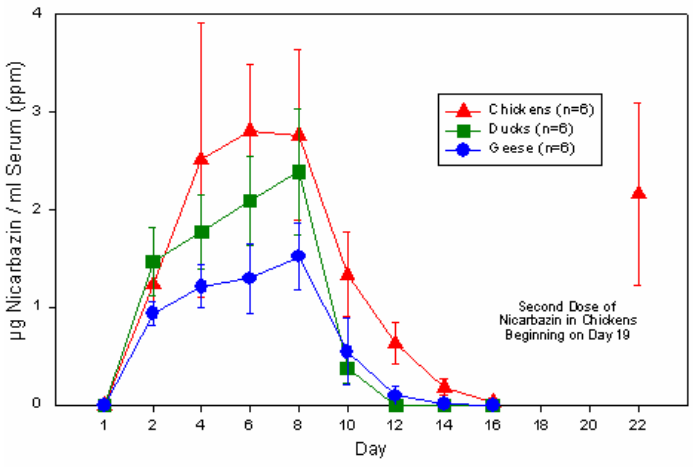

Figure 2. Comparative Absorption of Nicarbazin in 3 Species: Plasma DNC levels in chickens, mallards, and Canada geese gavaged with 8.4 mg/kg nicarbazin. Error bars represent standard error of the mean.

Penned Canada geese pairs from a domestic flock in Minnesota were treated with 0 ppm, 125 ppm, 250 ppm, and 500 ppm nicarbazin in commercial pellets manufactured locally by Cargill Feeds (VerCauteren 2005). Treatment of Canada geese with 500 ppm nicarbazin achieved an average daily intake of 91 g bait and resulted in a 51% reduction in hatchability of eggs. However, the commercial pellet bait used was unpalatable to wild Canada geese in the field (Yoder 2005). A target consumption rate of 91 g bait per goose per day in the field is unrealistic and higher nicarbazin concentrations in the bait are required to achieve similar reductions in egg hatchability in the field.

A field study in Colorado was conducted to assess the efficacy of nicarbazin for reducing Canada goose egg hatchability in the field. Canada geese were treated with 1350 ppm nicarbazin topically coated on cracked corn with 1.5% corn oil and 1.5% milk powder (Yoder 2005). Treatment over two consecutive breeding seasons resulted in 41% reduction in percent hatchability in year one and 47% reduction in hatchability in the second year. However, concerns over the stability of topical nicarbazin on bait in the field and about non-target bait consumption of the cracked corn bait continued to plague researchers.

NICARBAZIN BAIT DEVELOPMENT

Overall, studies showed that nicarbazin was unpalatable to Canada geese at the concentrations required to achieve effective plasma DNC levels. Nicarbazin bait similar to commercially available poultry pellet feeds was not palatable to wild Canada geese either in pens or in the field without extensive pre-treatment acclimation efforts (VerCauteren et al. 2000, Curtis et al. 2001, Clark 2005). Concentrations of nicarbazin up to 1350 ppm over-coated onto cracked corn with 1.5% corn oil and 1.5% milk powder had acceptable palatability (Yoder 2005), but concerns regarding bait stability and non-target bait consumption made cracked corn unacceptable for general field application. Nicarbazin was effective in reducing hatchability of eggs laid by treated birds, but it remained difficult to achieve ingestion of the appropriate dose by free-feeding.

Developing bait that Canada geese would consistently eat was the most challenging aspect of the project. Canada geese are extremely neophobic to new food types, and the presence of nicarbazin in bait further reduced palatability (Clark 2005). The ideal nicarbazin bait must be highly palatable to geese, be formulated to maintain its shape and integrity in harsh field conditions (e.g., heat, cold, or precipitation), and be of a design (e.g., size, shape, texture, friability) to minimize consumption by non-target species. Additionally, nicarbazin bait has to be so palatable that Canada geese will continue to consume bait even when the grass “greens” in the spring.

To allow incorporation of nicarbazin into the bait matrix rather than applying it topically, attention shifted to custom extruded baits. Initial testing of extruded baits involved hard extruded corn pellets (Phibro Animal Health Inc.) with a corn-flour base, yellow coloring, and shaped like corn (Bynum et al. 2005a). Although there was some limited acceptance in small field palatability tests in Colorado, bait was essentially rejected during all pen trials with Canada geese. Trials of hard extruded corn pellet bait were characterized by low initial bait consumption followed by rejection of bait (Figure 3). Consumption was so low that several trials had to be stopped early due to loss of body condition in study animals that refused to eat the bait offered.

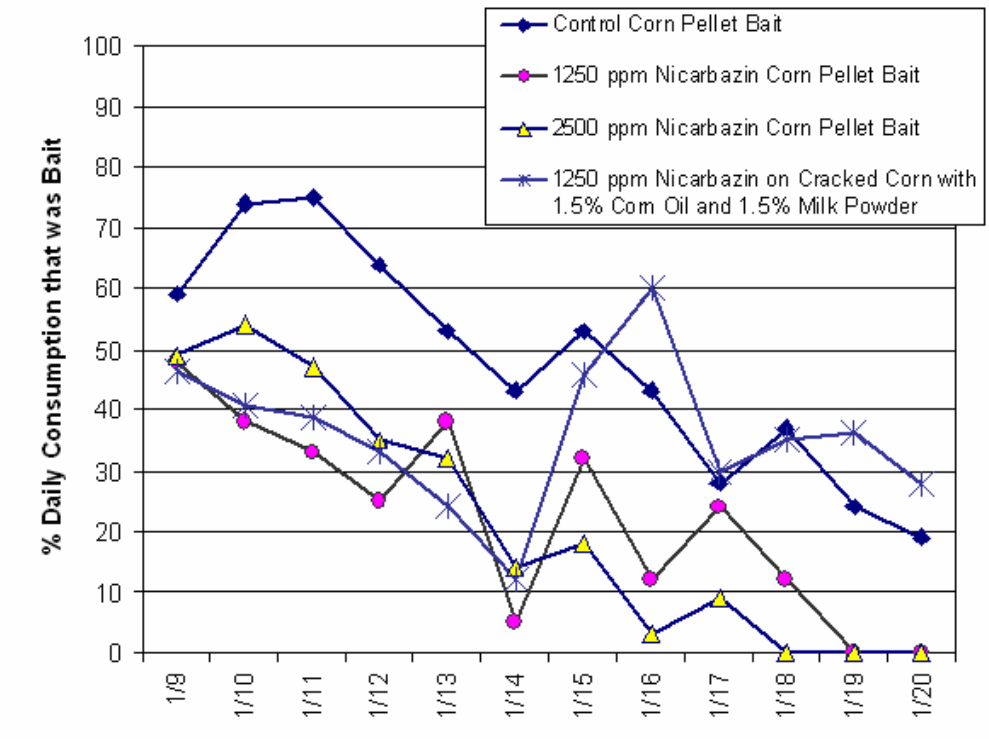

Figure 3. Percent of Daily Food Consumption that was Bait: Percent of daily food consumption that was bait during one trial of hard extruded corn pellets. Note the decreasing trend in the amount of bait consumed.

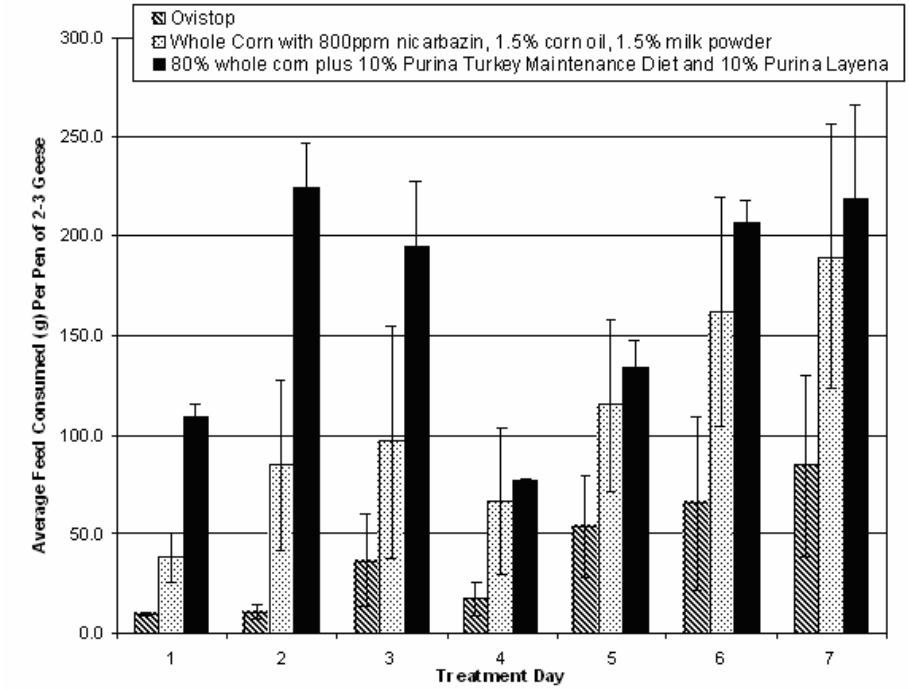

Figure 4. Average Daily Consumption of Ovistop® Bait: Average daily consumption of Ovistop 800 ppm nicarbazin bait was substantially lower than consumption of topically coated 800 ppm nicarbazin on whole corn with 1.5% corn oil and 1.5% milk powder. Error bars represent standard error of the mean.

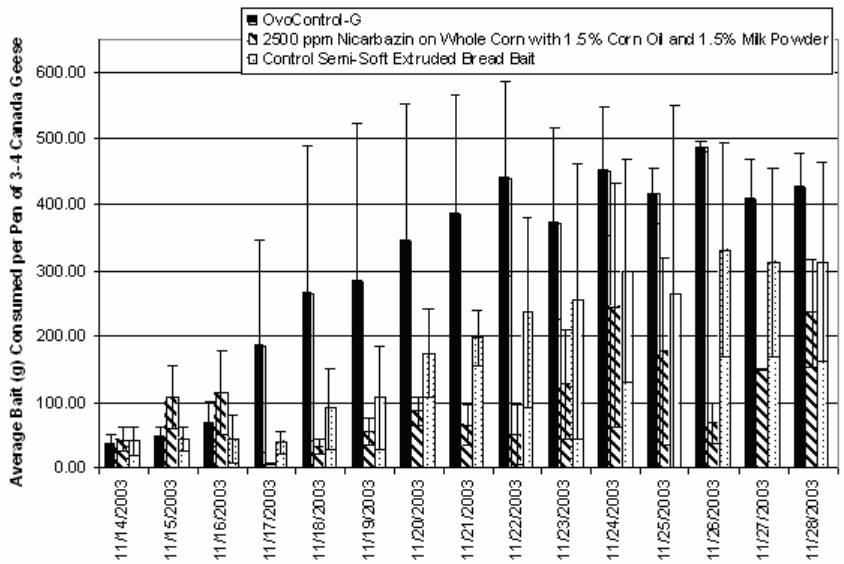

Figure 5. Average Daily Consumption of OvoControl-G® Bait: Average daily consumption of OvoControl-G® 2500 ppm nicarbazin bait was substantially not significantly different from consumption of topically coated 2500 ppm nicarbazin on whole corn with 1.5% corn oil and 1.5% milk powder. Note the increasing trend in the amount of OvoControl-G® bait consumed. Error bars represent standard error of the mean.

Even with the difficulties involved to ensure even coating and stability, OviStop® (Acme Drugs, Italy) was registered in Italy as a nicarbazin-in-wax topically coated whole corn bait for pigeon control. Because it was a current commercial product, Ovistop 800 ppm nicarbazin bait was tested by NWRC for Canada geese (Bynum et al. 2005a). Consumption of Ovistop bait was significantly lower than 800 ppm nicarbazin coated topically onto whole corn with 1.5% corn oil and 1.5% milk powder (Figure 4). Plasma DNC levels resulting from treatment with Ovistop averaged 0.829 $\pm$ 0.139 µg/ml, which is not high enough to affect reproduction in Canada geese. As Ovistop baits with higher nicarbazin concentrations were not available, no further testing of Ovistop for Canada geese was conducted.

Development efforts then focused on a bread-like bait, which would likely appeal to resident Canada geese due to the common practice of recreational feeding of bread to waterfowl across the United States. Changes in bait characteristics included making bait semi-soft instead of hard and using wheat flour instead of corn flour, although yellow and shaped like corn characteristics were retained. Semi-soft nicarbazin bait was developed in collaboration with Innolytics LLC (Long Valley, New Jersey). Control baits for pen and field studies were identical to nicarbazin baits except they did not contain nicarbazin.

Performance of the OvoControl-G® exceeded expectations and was the first bait tested that showed a trend in increasing consumption by Canada geese over time during laboratory studies (Bynum et al. 2005a). Comparing nicarbazin coated topically onto corn with semi-soft extruded bread baits, both 1000 ppm and 2500 ppm nicarbazin concentrations were tested. By focusing efforts on the 2500 ppm nicarbazin concentration (25 mg nicarbazin/ kg bait), target bait consumption per Canada goose could be minimized to 25 g bait per day to provide 62.5 mg nicarbazin. Consumption of 2500 ppm OvoControl-G® was not significantly different from consumption of

corn bait topically treated with 2500 ppm nicarbazin, 1.5% corn oil, and 1.5% milk powder ($p=0.201$, ANOVA) (Figure 5), which had previously been the only bait with high enough palatability in Canada geese to result in acceptable plasma DNC levels. Canada geese treated with OvoControl-G® had plasma DNC levels averaging 7.2 $\pm$ 2.7 µg/ml, which far exceeded the 3 µg/ml target to affect reproduction in Canada geese. Based on the results of pen studies, it was decided that field testing of OvoControl-G® was warranted.

THE OREGON FIELD EFFICACY STUDY

Planned as the primary field efficacy test, this study was undertaken in February 2004 following U.S. Environmental Protection Agency protocol approval. At 10 sites in Oregon, wild Canada geese were treated with approximately 8,000 kg of bait over 56 days, with 5,100 kg of OvoControl-G® consumed between 6 treated sites and 2,900 kg of control bait consumed between 4 control sites (Bynum et al. 2005b). Sixty-nine nests at treated sites and 46 nests at control sites were monitored to determine hatching success of eggs. Calculated as the number of eggs hatching out of the total number of eggs of known fate at the site, overall reduction in percent hatchability was 35.6% ($p=0.062$, General Linear Model). When considering individual nests at sites rather than flocks as a whole, percent hatchability was significantly reduced by 50.7% ($p<0.001$, General Linear Model) at treated versus control sites.

Nicarbazin can completely inhibit egg production in chickens (Sherwood et al. 1956). However, the number of Canada geese that consumed enough nicarbazin to suppress egg production could not be determined under field conditions in this study. Due to the high estimates of individual bait consumption by Canada geese, it was likely that reproductive success at treated sites was reduced considerably more than accounted for by determination of percent hatchability alone. Data on numbers of eggs laid at each site in previous breeding seasons was not available to use to estimate the reduction in egg laying. Confounding factors across years (e.g., predation rates, weather, etc.) would be difficult to account for even if such data was available.

Within a biological system, sources of variability are infinite and cannot always be accounted for. Comparison of percent hatchability between treated and control flocks overall revealed marginal significance ($p=0.062$), based on the assumption that all females in the flock with nests consumed bait at equal rates. Although comparison between flocks is the most statistically correct due to the lack of independence between nests, comparison of the percent hatchability of nests within flocks may be more useful in understanding the effect of treatment. Comparing percent hatchability of nests within flocks significantly lowered percent hatchability per nest at treated versus control flocks ($p<0.001$). Data from this study indicate that 2500 ppm nicarbazin bait (OvoControl-G®) is effective in reducing hatchability of eggs laid by resident Canada geese and should be considered for incorporation into Canada goose management programs.

Substantial effort was made to monitor non-target species at bait stations and consuming bait during the Oregon OvoControl-G® study (Bynum et al. 2005b). Bait stations at each of 10 sites were monitored by video camera every third day, resulting in approximately 252 observation hours per site, totaling 2,520 hours for the entire study (10 sites). Video tapes were viewed and recorded observations included species, time spent at bait station, time spent feeding on bait, and

the time of day visitation occurred. The most prevalent non-target avian species were American Crows, ravens, and mallards and the most prevalent mammals were ground squirrels of the S*ciurius* genus. There was some domestic dog and human interaction with the bait, and there was at least one human that consumed bait regardless of the signs posted to not handle the bait pans or feed and media coverage on the study. More frequently, humans inspected the bait and bait pans, and/or used the bait to hand feed Canada geese at the site.

NICARBAZIN TOXICITY AND ENVIRONMENTAL FATE

Regarding safety, nicarbazin is considered practically non-toxic and the concentration of nicarbazin in the proposed bait formulation is not expected to have a toxic effect on non-target species or on the environment. Because nicarbazin bait will be offered in a bait station, the most likely route for nicarbazin to reach soil, water and plants digestion and excretion by a goose. A secondary source would be from degrading baits leaching or depositing nicarbazin into the soil. Once consumed by an animal, dissolved in water, or deposited on the soil surface, nicarbazin separates into its components, DNC and HDP. When not in complex with HDP, DNC aggregates to form particles too large for absorption in the intestines, thereby having no effect on the animal consuming the DNC (Rogers et al. 1983).

Toxicity studies in mammals and birds given short and long term doses of nicarbazin showed minimal effects. For example, a rat would have to consume over 2.2 lbs of OvoControl-G® in a single feeding to reach the LD_{50} (Ott et al. 1956, Roberts et al. 1998). Extrapolated from chicken toxicity data, a crow would have to consume approximately 1.4 lbs of OvoControl-G® each day for 84 days before it would reach the LD_{50} (Ott et al. 1956, Roberts et al. 1998). The sheer volume of OvoControl-G® nicarbazin bait that would have to be consumed by non-target birds and mammals precludes them from being affected by exposure to OvoControl-G®. Adverse effects noted in animals have been observed only after daily long-term treatment ($\geq$ 1 year) (Ott et al. 1956, Roberts et al. 1998), which was not possible with baiting limited to 56 days.

Urban and suburban predators and scavengers could consume geese that had consumed nicarbazin bait. In a worst-case scenario, a 6.6 lb (3 kg) goose consuming 50 g of OvoControl-G® per day has a maximum whole body nicarbazin residue of about 41.7 mg/kg. This assumes that none of the nicarbazin is metabolized or excreted prior to predation, which is practically impossible. A mammalian predator or scavenger (coyote, raccoon, etc.) would need to eat over 40 geese (265 lbs) in a single day to reach the acute LD_{50} (>5000 mg/kg) for dogs weighing about 25 lbs (Ott et al. 1956, Roberts et al. 1998), or over 13 geese (81 lbs) per day for 163 days to approach the chronic LD_{50} (>1600 mg/kg/day for 163 days) (Ott et al. 1956, Roberts et al. 1998).

Litter from chickens treated with nicarbazin, which includes DNC and HDP, as well as nicarbazin in feed, is routinely used as a fertilizer for agricultural fields. Application of litter to agricultural fields at 10 times the normal rate resulted in no significant differences in nitrate, nitrite, ammonium, soil bacteria, actinomycetes, or fungi in the soil compared to soil treated with chicken litter from untreated chickens (MacDonald 2003). Another study of the effects of chicken litter from treated or control chickens on total and methane gas production during anaerobic digestion in the soil showed no differences. Litter from

nicarbazin treated chickens was anaerobically digested similar to litter from untreated chickens (MacDonald 2003). Likewise, nicarbazin had no detectable effect on the growth of oat, corn, tomato, lettuce, bean, turnip, pea, and sunflower plants.

The main concern about nicarbazin field application is bait consumption by non-target avian species. Nicarbazin bait must be consumed daily for at least 3-4 days to achieve blood levels that affect the hatchability of forming eggs. Plasma DNC levels are reduced by half within one day after bait consumption stops and nicarbazin is undetectable in the plasma by 4-6 days after consumption of nicarbazin bait has stopped (Figure 2). By 2 days after bait consumption has stopped, no effects on the forming egg are seen. As baiting would end far in advance of the breeding season of non-target birds, any effects of nicarbazin consumption would be gone before breeding began. Also, baiting practices can help minimize non-target avian exposure by ensuring that only enough bait to treat the target Canada goose flock is offered and removing any uneaten bait from the treatment areas. Between the timing of treatment and minimizing exposure to bait, non-target hazards of nicarbazin to avian species can be controlled.

MANAGEMENT IMPLICATIONS

The field study in Oregon demonstrated that 2500 ppm nicarbazin semi-soft bread bait (OvoControl-G) effectively reduced the hatchability of eggs under field conditions. Toxicity, non-target safety, and environmental safety studies showed that nicarbazin is a safe compound for field application. Nicarbazin reproductive inhibition can become an effective addition to integrated management plans for controlling resident Canada goose populations.

Egg-oiling is a popular method of reproductive control for resident Canada geese. However, the egg-oiling method requires personnel to locate individual nests to apply treatment. An advantage of nicarbazin is that bait can be applied in a few areas strategically located at breeding sites to allow treatment of several breeding pairs without having to locate the nests. Nicarbazin treatment also prevents human disturbance at the nest, which may result in nest abandonment and re-nesting at an alternative, untreated location.

Nicarbazin bait (2500 ppm OvoControl-G®) is recommended for incorporation into a comprehensive management plan as another tool to control resident Canada goose flock sizes. As a long-lived species (average of 9-12 years) with a long breeding life, reproductive control alone is unlikely to result in significant population decreases over the short-term that would reduce the conflicts between humans and resident Canada geese. However, it is suggested that lethal control through round-up and culling to bring the population down to a manageable level be used first, after which reproductive control could be used to help maintain population numbers at a manageable flock size.

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