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RESPONSE OF CANADA GEESE TO A TURF APPLICATION OF DIAZINON AG500

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ABSTRACT: We investigated the effects of a turf application of the insecticide diazinon AG500 on Canada geese (*Branta canadensis*) on a golf course in coastal Washington (USA). On both 19 and 26 March 1987, 1 ha of turf on a golf course located in Birch Bay, Washington was treated with diazinon AG500 at a target application rate of 2.2 kg active ingredient per hectare (AI/ha). Treated areas were then irrigated with 6 mm water. Grass and water samples were collected from three different sites one day before and 1, 3, 7 and 14 days after each application. Diazinon residues ≥20 ppm were found in golf course grasses for one week after each application. Diazinon residues in study area ponds and creeks were ≥17 ppb. Samples from two irrigation puddles one day post-application had 1.00 and 0.20 ppm of diazinon, respectively. Numbers of geese present declined following diazinon application; however, no goose mortality was observed. Geese spent 422 and 538 min feeding on the treated areas after the first and second diazinon applications, respectively. One goose feeding in treated areas demonstrated signs of poisoning (lethargy, ataxia) for several hours. Two other geese feeding in the treated areas may have been slightly intoxicated. During carcass searches, three American wigeon (*Anas americana*) carcasses were found. Based on brain cholinesterase (ChE) levels and gastrointestinal (GI) tract residues of diazinon present, we concluded that these wigeon died from diazinon poisoning. Numerous songbirds (Passeriformes) also fed on the treated turf but no apparent response to the insecticide was observed.

Key words: *Branta canadensis*, diazinon, avian toxicology, organophosphate, insecticide.

INTRODUCTION

Diazinon (Phosphorothioic acid O,O-diethyl O-[6-methyl-2-(1-methylethyl)-4-pyrimidinyl] ester) is an organophosphorus insecticide that was commonly applied to golf courses and turf farms throughout the United States. This use was recently disapproved by the U.S. Environmental Protection Agency, largely because of reports of mortality to waterfowl (Stone, 1979; Thomas, 1988). Prior to the federal ban, the manufacturer CIBA-GEIGY Corporation, proposed a reduction in the use rate to a maximum of 2.2 kg AI/ha to improve waterfowl safety (R. Balcomb, pers. comm.). We describe a field study of exposure and effects on Canada geese under the conditions of the proposed lower use rate. A previous study describing effects on American wigeon (*Anas americana*) at this application rate has been reported (Kendall et al., 1992).

METHODS

Study area

This study was conducted on Sea Links Golf Course in Birch Bay, Washington (USA) (48°55′N, 122°40′W) from 3 March to 9 April 1987. The fairways and tees were vegetated with ryegrass (*Elymus* spp.) and greens were vegetated with bentgrass (*Agrostis* spp.); these grasses typically are found on golf courses in the Northwest. Fairways had scattered Douglas fir (*Pseudotsuga menziesii*) and western red cedar (*Thuja plicata*). A freshwater marsh ran along the southern border of the study area and there were four ponds on the study area ranging in size from approximately 0.25 to 0.50 ha.

Chemical application

The Washington State Department of Wildlife (WDW) and the U.S. Fish and Wildlife Service (USFWS) issued a permit for research personnel to spray diazinon AG500 on an area of the golf course of high goose utilization, not to exceed 1 ha. The study area was monitored from 1 October to 18 December 1986 and 3 to 19 March 1987, to determine which areas were most consistently utilized by Canada geese and other wildlife. During this period, observations were made from 07:00 to 13:00 each day. On 19 March and again on 26 March diazinon AG500 was sprayed on those areas of the golf course most heavily utilized by Canada geese. Application rate was 2.2 kg active ingredient (AI)/ha and a total of approximately 1 ha was treated on each application. Diazinon was applied utilizing a gasoline-powered utility cart equipped with a tank and boom with fan nozzles that were spaced at 305 mm. After application the diazinon was washed into the soil with 6 mm of water from the golf course sprinkler system. Borders of areas sprayed were marked with red flags to allow observers to determine when geese were in the treated areas. Following standard practice at this course, an unsprayed strip 1 to 3 m wide was left around edges of two ponds adjacent to treated areas. These two ponds were 0.25 ha and 0.5 ha in area, respectively.

Bird observations

Since mortality in American wigeon was evident in an earlier study (Kendall et al., 1992) and our research permit emphasized assessment of exposure of Canada geese, wigeon were hazed from the study area before diazinon was applied. The hazing activities did not appear to disturb Canada geese. The geese remained on the course but did not use the same areas as wigeon for feeding and loafing. From 19 to 22 March and 26 to 29 March, respectively, the study area was monitored 24 hrs per day for four days after each application of diazinon. From 23 to 25 March and from 30 March to 9 April, personnel were on the golf course from before dawn until after dark.

From 13 March through 1 April, an hourly census of Canada geese was conducted on the study area from 07:00 to 13:00. The maximum number of geese counted was determined daily. Mean maximum daily goose counts on treated areas for six days pre-treatment, six days post-treatment (application no. 1), and six days post-treatment (application no. 2) were compared with a single-factor ANOVA (Sokal and Rohlf, 1981).

Observations of goose feeding behavior were conducted during all daylight hours from 12:00 the day of diazinon application to 12:00 four days later. When Canada geese were observed on treated areas their activity was recorded at 1 min intervals. For example, three geese observed feeding in the treated area at the instant sampling occurred, represented 3-goose-feeding-min. The numbers of goose-feeding-minutes were summed over each 4-day post-application interval to provide an index of oral exposure to diazinon during the study.

Carcass searching

Thorough carcass searches on the entire study area were conducted at approximately 06:30 and at 18:00. Carcass searches also were conducted opportunistically through the day. Team members collected all observed carcasses, labeled them, and placed them in a cooler on ice. Carcasses were transported back to the laboratory for necropsy. Brain cholinesterase (ChE) levels were determined in all bird carcasses found following the methods of Ellman et al. (1961). Diazinon levels in the gastrointestinal (GI) tract of birds found dead were analyzed using gas chromatography as described by Kendall et al. (1992).

To test the carcass search efficiency of the two teams, 15 carcasses of northern bobwhite (*Colinus virginianus*) were placed on the study area from 09:00 to 10:00 and again at 18:00 on 1 April. A map of the study area was divided into 44 numbered sections of approximately equal size. Fifteen random numbers were selected for each carcass search efficiency test. Carcasses were placed in the sections of the study area designated by the randomly chosen numbers. Carcasses placed on the study area at 18:00 remained on the course overnight. Carcass searchers were not informed on which days tests would be run.

At the end of each carcass search test, bobwhite still remaining on the study area were collected by investigators. Any carcasses not found were assumed lost to scavengers. Results from both carcass search efficiency tests were pooled. The search efficiency rate was calculated by dividing the total number of carcasses placed on the study area into the total number of carcasses recovered by searchers. An estimate of the actual number of wild bird carcasses that occurred on the golf course during this study was calculated by the following formula:

$$N = \frac{(n/x)}{(1 - y)}$$

where N = An estimate of the total number of wild bird carcasses occurring on the study area; n = Number of wild bird carcasses found during carcass searching; x = Search efficiency rate; and y = Scavenging rate (number of quail carcasses carried away divided by the number of quail carcasses placed in the study area).

On 22 March, we used a Cessna 172 aircraft to conduct an aerial survey for goose carcasses around the golf course. A search was made of nearby Birch Bay and the fresh water marsh adjacent to the golf course. Flight altitude was approximately 122 m and six circuits were flown over each area to provide thorough coverage of the sites. At this search height, geese were readily observed on the golf course and could be counted.

The behavior of Canada geese and other avian species was closely monitored by observers throughout this study. A written description, video recordings, or photographs were made of any abnormal behavior demonstrated by avian species on the study area. Observations were made with binoculars from distances that did not appear to interfere with normal avian behaviors. All avian and mammalian use of the study area was recorded.

Environmental chemistry

Grass samples were collected from three fairways of the golf course one day before chemical application (Day −1), on the day of chemical application (Day 0), and on Days 1, 3, and 7 post-application. On Day 0, two grass samples were collected, one immediately after diazinon application, and the other after irrigation. This same grass sampling regime was used for both diazinon applications. Grass samples also were collected on Day 14 after the second chemical application.

Grass samples were collected from randomly chosen sampling points on each of the three experimental fairways. Grass was collected from 0.5 m^2 quadrats which were located in the four cardinal directions around each sampling point. Quadrats were placed 1 m from the sampling points. Samples from opposite cardinal directions (NS, EW) were combined and mixed to create two composite samples for each sampling point. Grasses were sheared close to the ground, placed in labeled plastic bags, put on ice, and transported back to the laboratory.

All diazinon residue analyses were performed on a Perkin-Elmer Sigma 300 gas chromatograph (GC; Perkin Elmer Corporation, Philadelphia, Pennsylvania, USA). Grass samples were hexane-extracted in the analytical procedure. Typical chromatographic conditions (isothermal) included 25 mm OV-210 column (Supelco, Inc., Bellefonte, Pennsylvania) with nitrogen carrier gas (34 ml/min; 60 psi). The detector was operated in flame photometric mode set for phosphorous analysis. The injector temperature was operated at 215 C, detector temperature 215 C, and the oven temperature 190 C. All injections were performed manually using a 100 ml Hamilton syringe (VWR Scientific, Inc., St. Louis, Missouri, USA).

Two sample-pans containing absorbent paper were placed on the fairways before each application of diazinon on each of the three fairways from which grass samples were collected. A central sampling point was randomly selected on each fairway and pans were placed 1 m from this point in varying cardinal directions. Amounts of diazinon applied on both spray days were estimated from the chemical concentrations found on papers in the sample-pans and from the total amount of diazinon applied to the measured treatment area. Additionally, the concentration (g/l) of diazinon in the 757 l applicator tank was sampled each time this tank was refilled. A t-test was used to compare sample-pan concentrations of diazinon (Sokal and Rohlf, 1981).

RESULTS

Based on pan samples, the rate of diazinon application was 71 to 79% of that intended (2.2 kg AI/ha) for the two applications: 1) $\bar{x}$ (±SD) = 1.69 (±0.11) kg AI/ha, CV = 6.5; and 2) $\bar{x}$ (±SD) = 1.57 (±10.8) kg Al/ha, CV = 10.8. Although the variability increased for the second application, the mean sample-pan concentrations were not significantly different between periods (t_{n-1} = 2.57, $P > 0.05$). Concentrations of diazinon in tank samples were more variable even though care was taken in mixing the solutions.

There were 422 and 538 goose-feeding-min observed within the treated areas during the two 4-day post-application periods, respectively. The goose feeding estimates were conservative because it was often difficult to determine if geese were feeding within or just outside the treated areas. When locations of geese were in doubt, feeding data were not recorded.

No Canada goose carcasses were found during ground or aerial searches and no significant difference in the number of Canada geese using the study area before and after the application of diazinon were detected by the ANOVA test (Table 1). During this study carcass searchers recovered three American wigeon: one hen and one drake on 23 March and another hen on 24 March. No carcasses were found after the second diazinon application. Brain ChE inhibition in these three birds was 71%, 64%, and 62%, respectively, of the mean for control birds (n = 3) of this species obtained in a previous study (Kendall et al., 1992). Gastrointestinal tract contents

TABLE 1. Mean maximum numbers of Canada geese counted on the Sea Links Golf Course, Birch Bay, Washington, from 07:00 to 13:00, 13 March to 1 April 1987. One ha of the course was treated with diazinon AG500 at 2.2 kg A.I./ha on 19 March and again on 26 March. Mean maximum daily goose counts were calculated for six days pre-treatment (13 to 18 March), six days post-treatment period no. 1 (20 to 25 March), and for six days post-treatment period no. 2 (27 March to 1 April).

	Sample size	Mean[a] maximum number	Lower 95% confidence limits	Upper 95% confidence limits
Pre-treatment	6	24.50	20.66	28.34
Post-treatment 1	6	23.60	17.13	30.07
Post-treatment 2	6	17.33	14.14	20.52

[a] Means do not differ statistically (single-factor ANOVA, $P < 0.05$). However, 95% confidence limits do not overlap between pre-treatment counts and post-treatment no. 2 counts.

TABLE 2. Mean (±SD) concentration of diazinon AG-500 found in grass samples taken from three fairways on the Sea Links Golf Course, Birch Bay, Washington. Diazinon AG500 was applied to the golf course (2.2 kg active ingredient/ha) on 19 March 1987.

Number of samples	Day[a]	Diazinon concentration $\bar{x} \pm$ SD
First application (19 March)		
6	−1	NA
6	O-P	114.2 ± 13.8
6	O-W	43.1 ± 7.7
6	1	32.6 ± 10.5
6	3	24.9 ± 4.4
6	7	14.5 ± 1.4
Second application (26 March)		
6	O-P	158.3 ± 31.1
6	O-W	24.8 ± 15.0
6	1	37.2 ± 16.7
6	3	23.9 ± 8.2
6	7	26.5 ± 13.7
6	14	5.3 ± 2.2

[a] Day −1 is one day pre-application 1; day O-P represents the day of application immediately after spraying and before irrigation; day O-W represents the day of application immediately after irrigation with 6 mm of water; and day 1, 3, 7, and 14 are the number of days post-application after irrigation (O-W).

contained 2.14, 0.18, and 0.13 ppm of diazinon, respectively.

Carcass searchers found 27 (90%) of 30 northern bobwhites placed on the study area in the efficiency test. No northern bobwhites were taken by scavengers.

Numerous avian species, including American robins (*Turdus migratorius*), gulls (*Larus* spp.), European starlings (*Sturnus vulgaris*), red-winged blackbirds (*Agelaius phoeniceus*), and American crows (*Corvus brachyrhynchos*), often were seen feeding on areas treated with diazinon. In spite of the frequent feeding activity on treated areas, the carcass of only one bird was found; an American robin was recovered at 07:00 on 26 March. This robin had been preyed upon and too few remains were found for residue analysis or ChE assay. On 26 March numerous red-winged blackbirds concentrated their feeding efforts on the treated areas just after application and irrigation of diazinon. Presumably these birds were feeding on intoxicated invertebrates as a result of the diazinon application. No mortality or adverse behavioral effects were observed in these blackbirds.

With the exception of Day 7 post-application 1, and Day 14 post-application 2, grass samples with >20 ppm of diazinon were found consistently in treated areas (Table 2). However, mortality was not observed in geese using and feeding on diazinon treated fairways; but at least one incident of apparent behavioral toxicity was noted.

The maximum concentrations of diazinon in water samples occurred on both application days (Table 3). The maximum concentration of diazinon recorded in the ponds or creek on the study site was 17 ppb. However, the two puddle samples collected on 20 March contained 1.00 and 0.20 ppm of diazinon.

DISCUSSION

The first application tank sample [$\bar{x}$ (±SD) = 2.30 (±1.11); CV = 48.3] showed more variability than the second application tank samples [$\bar{x}$ (±SD) = 2.64 (±0.49); CV = 18.61]. Based on a two-tailed t-test (t_{n-1} = 4.30, $P > 0.05$), the tank concen-

TABLE 3. Concentration of diazinon AG500 found in water samples from four sites[a] on the Sea Links Golf course, Birch Bay, Washington. Diazinon AG500 was applied to the golf course (2.2 kg A.I./ha) on 19 March and 26 March 1987.

Sample #	Location	Day[b]	Diazinon concentration (ppb)
SLB 045	A	−1	<0.24
SLB 046	B	−1	<0.24
SLB 047	C	−1	<0.24
SLB 048	Creek	−1	<0.24
SLB 049	A	O-W	1.85
SLB 050	B	O-W	<0.24
SLB 051	C	O-W	17.0
SLB 052	Creek	O-W	4.94
SLB 053	A	1	2.32
SLB 054	B	1	1.74
SLB 055	C	1	2.33
SLB 056	Puddle	1	1,000
SLB 057	Creek	1	2.14
SLB 066	Puddle	1	200
SLB 069	B	3	<.024
SLB 071	C	3	2.0
SLB 073	Creek	3	1.09
SLB 074	Creek	3	1.38
SLB 075	A	3	1.26
SLB 077	A	7	<0.24
SLB 078	B	7	<0.24
SLB 064	C	7	0.58
SLB 079	Creek	7	0.84
	Second application (26 March)		
SLB 098	A	O-W	10.8
SLB 099	B	O-W	5.06
SLB 100	C	O-W	5.64
SLB 101	Creek	O-W	5.20
SLB 105	A	1	1.72
SLB 106	B	1	2.30
SLB 107	C	1	2.26
SLB 108	Creek	1	6.90
SLB 109	A	3	2.86
SLB 110	B	3	1.48
SLB 111	C	3	4.30
SLB 112	Creek	3	4.16
SLB 113	A	7	1.08
SLB 114	B	7	<0.24
SLB 115	C	7	2.47
SLB 116	Creek	7	1.57
SLB 141	A	14	0.74
SLB 143	C	14	0.38
SLB 144	Creek	14	0.88

[a] Water samples were taken from ponds (A, B, C) on the golf course and from a creek just northwest of the course.

[b] Day −1 is 1 day pre-application 1; day O-W represents the day of application immediately after wash-in; day 1, 3, 7, and 14 are the number of days post-application.

trations were not significantly different between applications. It is interesting to note that while the tank samples were more variable for the first application period, the sample-pan concentrations were less variable for this period. Additionally, while the mean concentration was higher in tank samples for the second application, the mean sample-pan concentrations were lower for the second application.

The mean daily goose counts during post-treatment period 2 were 29% lower than the pre-treatment period, and the count variability during post-treatment period 1 (CV = 48.3) was much greater than during the pre-treatment period 2 (CV = 18.6). Additionally, the 95% confidence limits (Sokal and Rohlf, 1981) of the post-treatment 2 counts did not overlap the pre-treatment limits (Table 1). The small sample size and variability of the data may have reduced the sensitivity of the ANOVA. Based on these data, there may have been a true difference between pre- and post-application goose counts. However, since we did not find any goose carcasses during the thorough searches, we do not believe the differences in goose counts were the result of goose mortality. Even though we saw diazinon residues of ≥20 ppm, we did not observe direct mortality in foraging geese. Sublethal effects of ingesting contaminated grass may have influenced the movement and feeding behavior of the geese. The differences in pre- and post-treatment counts also may have been the result of natural differences in goose behavior related to the chronology of their wintering ecology. Such a relationship could have been identified if we had a reference population feeding elsewhere on untreated turf.

On 19 March, the day of the first diazinon application, 15 geese were observed grazing on a treated green at approximately 14:00. Two of these geese were seen drinking from a puddle on the treated area. At 15:24, a goose that may have been one of those observed drinking staggered from a nearby pond onto an adjacent fairway and fell forward on its breast. It got up, staggered a few more steps and settled down again. This goose remained alone and stationary until approached by investigators at 20:40. When approached, the bird got up and walked a few feet while flapping its wings. Investigators concluded that the goose was recovering and by morning the bird had apparently rejoined the flock. On March 30, four days after the second application of diazinon, one of four geese observed grazing on a treated tee displayed wing droop. Both wings of this goose were held close to the ground for about 6 min. Four min later, posture was normal. This goose was the last of four to leave the area and swim away in the adjacent pond. These events occurred from 13:39 to 13:54. At 16:58, a goose was spotted near a pond approximately 137 m off a treated fairway. This goose was sitting alone approximately 1 m from the edge of the pond. Its left wing was almost fully extended and was resting upon the ground. The bird remained in this position until 18:01 when it staggered to the water's edge, took a few drinks, and then assumed a normal sitting position. At 18:55, an investigator walked to within 27 m of the goose which responded by entering the water, swimming midway across the pond and then flying off the golf course. Its flight appeared normal.

The GI tract residues confirm that these birds died from diazinon exposure. The drake was found a few meters from a treated area and the two hens were approximately 64 m and 32 m, respectively, from the nearest area treated with diazinon. All three birds were either partially eaten or had puncture wounds which indicated they had been bitten by mammalian predators or scavengers. Thus it is unknown if the hens moved to the location where they were found or were carried there by predators or scavengers. On necropsy, there were blood stains outside the puncture wounds on the breast of the wigeon drake, which indicates it may have been alive when the wounds were inflicted. Thus the drake may have been debilitated by diazinon poisoning and then killed by a mammalian predator.

The lack of scavenging of any of the northern bobwhite carcasses seemed inconsistent with the American wigeon carcasses being scavenged. However, if the intoxicated wigeon were moving, they would attract the attention of a predator. We estimated 3.33 as the total number of wild bird carcasses occurring on the study area during the investigation. We estimated that ≤ 4 wigeon died from diazinon exposure on the study area.

The higher concentrations of diazinon in puddles may have contributed to chemical exposure to wildlife; for example, geese in this study were observed drinking occasionally from puddles. However, based on published LD_{50} values (Hudson et al., 1984) geese would have to drink a large quantity of puddled water to reach acutely toxic dosages. Since a dose-response curve for Canada geese was not found in the literature a precise assessment of the potential for mortality related to drinking puddled water is not possible.

In conclusion, two applications of diazinon (2.2 kg AI/ha), seven days apart, to turf on a golf course in western Washington, resulted in no observed mortality to Canada geese which grazed on that course; but some evidence of behavioral disturbances were observed. Small amounts of food containing as little as 20 ppm of diazinon have been reported to be potentially lethal to Canada geese (Zinkl et al., 1978). Evidence of American wigeon mortality resulting from diazinon was apparent and confirmed the risk to this species as reported earlier by Kendall et al. (1992). The 2.2 kg AI/ha application rate studied would reduce mortality in Canada geese grazing on golf courses, but would fail to protect American wigeon feeding on turf.

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