AMERICAN WIGEON MORTALITY ASSOCIATED WITH TURF APPLICATION OF DIAZINON AG500

Authors: Kendall, R. J., Brewer, L. W., Hitchcock, R. R., and Mayer, J. R.

Source: Journal of Wildlife Diseases, 28(2) : 263-267

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-28.2.263

Journal of Wildlife Diseases, 28(2), 1992, pp. 263-267

AMERICAN WIGEON MORTALITY ASSOCIATED WITH TURF APPLICATION OF DIAZINON AG500

R. J. Kendall,[1,4] L. W. Brewer,[1] R. R. Hitchcock,[2] and J. R. Mayer[3]

[1] The Institute of Wildlife and Environmental Toxicology and Department of Environmental Toxicology, Clemson University, Pendleton, South Carolina 29670, USA
[2] Northwest Communtiy College, Powell, Wyoming 82435, USA
[3] Huxley College of Environmental Studies, Western Washington University, Bellingham, Washington 98225, USA
[4] Author to whom all correspondence should be addressed

ABSTRACT: Nine fairways of a golf course located in Bellingham, Washington were treated with diazinon AG500 at a target application rate of 2.2 kg active ingredient (AI) per ha. The chemical application with a "boomless" sprayer resulted in a variable distribution of diazinon residues on the turf (associated with a deep thatch layer) that ranged from 1.0 to 6.2 kg AI/ha. The diazinon-treated turf was irrigated with 1.3 cm of water immediately following application. The post-irrigation diazinon residue levels ranged from 100 to 333 ppm ($\bar{x}$ = 209; SD = 88; n = 8). These residue levels were higher than expected based on results of turf studies in other regions of the United States. Eighty-five American wigeon (*Anas americana*) died after grazing on one treated fairway on the day of application following irrigation. The brains of all 85 wigeon were analyzed for acetylcholinesterase (AChE) activity. Wigeon that died on the study area (n = 85) showed 44% to 87% depression of AChE ($\bar{x}$ = 76%; SD = 7.1%) when compared to control wigeon (n = 3; AChE Activity = 1.86) AChE levels. Upper GI tract contents of 15 of the 85 dead wigeon contained 0.96 to 18.1 ppm diazinon. Extensive carcass searches revealed no other avian mortality attributable to diazinon toxicity on the treated study area. Although initial post-irrigation diazinon residues in grass samples were higher than expected, diazinon levels in grass samples on day seven post-application had declined to an average of 29 ppm. American wigeon appear to be vulnerable to exposure to diazinon. Use restrictions based on migration times of wigeon may be effective in reducing potential exposure to diazinon or to other pesticides used on turf grasses that may be toxic to grazing waterfowl.

Key words: American wigeon, turf, mortality, diazinon, *Anas americana*, residues, toxicology.

INTRODUCTION

The organophosphate (OP) pesticide diazinon (phosphorothioic acid O,O-diethyl O-[6-methyl-2-(1-methyl ethyl)-4-pyrimidinyl ester]) is widely used on a variety of agricultural crops and turf. Diazinon was commonly used on sod farms and golf courses throughout the United States for turf insect control; however, applications on these areas were discontinued (Thomas, 1988) based largely on potential hazards to waterfowl (Stone and Gradoni, 1985). Although the greatest risk of waterfowl mortality with use of diazinon appears to be on turf grasses, there have been no experimental field studies published evaluating the effects of a known application level of diazinon on birds (Stone, 1979).

Canada geese (*Branta canadensis*) and American wigeon (*Anas americana*) are two species of waterfowl which forage on turf grasses and are particularly susceptible to diazinon poisoning (Zinkl et al., 1978). Our overall research effort considers effects on both species. The data presented here principally concern mortality to a flock of wigeon observed in a screening study (Fite et al., 1988) to assess the hazard of diazinon to wigeon when applied as an emulsible concentrate to golf course turf at the proposed use rate of 2.2 kg active ingredient (AI)/ha (2 lbs AI/a). Wigeon were chosen for study because they frequently graze on golf courses in the Pacific Northwest and their feeding behavior on turf grasses suggested their exposure potential is high to pesticides applied to turf.

The project was designed to assess acute toxicity response of wigeon and other wildlife to diazinon treated golf course turf and to monitor chemical residues in grass, puddles, and other avian food resources such as turf insects. The results of our research with Canada geese will be reported elsewhere.

MATERIALS AND METHODS

Diazinon AG500 (supplied by CIBA-GEIGY Corporation, Greensboro, North Carolina 27409, USA) was applied to nine fairways of the Sudden Valley Golf Course (Bellingham, Washington, USA; 48°43'N, 122°19'W) on 15 October 1986, between 0800 and 1100 hr. The target application rate was 2.2 kg AI/ha. This was one-half the maximum recommended rate on the product label at the time of study initiation. The mixed solution application rate was 199 l/ha (130 gal/a), which was applied in an 18.3 m (60 ft) swath from gang nozzles.

Eight chemical sample pans with liners of absorbent paper were placed on each of two randomly chosen grass sampling fairways (#3 and #7) to quantify the actual diazinon application. Each sample pan was collected after application and the absorbent paper removed. The paper was then placed in sealable plastic bags, put on ice and transported to the laboratory for analysis (amount of diazinon per unit of surface area). A central sampling point was randomly selected on each fairway and pans were placed 1 m from this point. Rates of diazinon application on spray day were estimated from the mean chemical concentrations found on the papers in the sample-pans. Additionally, the concentration (g/l) of diazinon in the 757 l applicator tank was sampled each time this tank was refilled to verify the diazinon concentration.

Immediately after the pre-irrigation grass sampling was completed, the treated fairways were irrigated with 1.3 cm of water from the golf course sprinkler system. The level of irrigation was measured by placing standard rain gauges on the ground on the treated fairways as they were irrigated.

Predetermined carcass search routes were traveled daily seven days before and 14 days after chemical application. The search routes covered all nine fairways and included edges of roughs and along waterways. Any carcass found was identified to species, labelled, and assigned a unique identification number. The collection location was marked on a map of the study area and identified with this number. Carcasses were stored frozen until chemical residue analysis was performed. Bird carcasses found fresh were necropsied and the brains removed for AChE analysis (Ellman et al., 1961).

In preparation of tissue concentrate for the AChE analysis 1 ml 7.4 pH Trizma buffer was used for every 120 mg neural tissue. For homogenization, a Virtis "23" homogenizer (The Virtis Co., Gardiner, New York 12525, USA) equipped with both a vertical and horizontal blade at the end of the shaft was used. The homogenate was decanted into a labeled centrifuge tube (VWR Scientific, Inc., St. Louis, Missouri 63195-6373, USA) and centrifuged in a Sorvall RC5 Superspeed refrigerated centrifuge (Dynatech Laboratories, Chantilly, Virginia 22021, USA), set at 12,500 rpm for 20 min. The homogenate was refrigerated at 0 to 5 C. AChE activity was expressed as micromoles of substrate (ACTI) hydrolyzed per min per g of tissue. The enzyme activity (A/min) was converted based on the equation derived from Ellman et al. (1961). All AChE activity readings were taken on a Perkin-Elmer Lambda 3B Spectrophotometer (Perkin Elmer Corporation, Philadelphia, Pennsylvania 19170, USA).

All diazinon residue analyses were performed on a Perkin-Elmer Sigma 300 gas chromatograph (GC) (Perkin Elmer Corporation).

Upper GI tract contents and grass samples were hexane extracted and spiked with an internal standard (i.e., Dursban) in the analytical procedure. Typical chromatographic conditions (isothermal) included 10 ft OV-210 column (Supelco, Inc., Bellefonte, Pennsylvania 16823, USA) 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 63195, USA)

Grass samples were collected from two fairways of the golf course on the following days: 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, grass samples were collected, immediately after diazinon application and again after irrigation.

Grass samples were collected from randomly chosen sampling points. Grass was collected from 0.5 m^2 quadrants that were located around each sampling point. Quadrants were placed 1 m from the sampling points. Samples from opposite 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, sealable plastic bags, put on blue ice, and transported back to the

TABLE 1. Estimates of diazinon application rates (kg/ha) based on pan samples collected from Sudden Valley Golf Course in Bellingham, Washington, USA. The target application rate was 2.2 kg/ha of diazinon AG500.

Sample number	Rates
Fairway 3	
EM001	1.93
EM002	1.51
EM003	3.17
EM004	2.98
EM005	3.19
EM006	2.45
EM007	5.77
EM008	5.29
Fairway Mean (SD)	3.29 (1.33)
Fairway 7	
EM009	1.95
EM010	1.05
EM011	2.76
EM012	3.92
EM013	3.45
EM014	1.21
EM015	2.33
EM016	2.81
Fairway Mean (SD)	2.44 (0.95)
Total Mean (SD)	2.89 (1.24)

laboratory. All composite grass samples were analyzed for diazinon residues.

RESULTS AND DISCUSSION

A commercial pesticide applicator (Haines Tree and Spray Service, Bellingham, Washington 98225, USA) applied diazinon AG500 (mixed in water) to the golf course using two tank-trucks. Truck number 1 carried 1,968 l of solution and truck two carried 1,514 l of solution. Both vehicles were equipped with Boom-Jet "boomless" nozzles. Each truck sprayed a 18 m swath. Truck 1 traveled at an estimated 2.8 km/hr and covered 1.6 ha (4 a)/tank. Truck 2 traveled an estimated 2.0 km/hr and covered an estimated 1.2 ha (3 a)/tank. Each tank contained 5.7 l (6 qt) of AG500 per 1,514 l (400 gal) of water. Two truck samples resulted in diazinon concentrations of 2.0 g/l and 2.3 g/l. Output of truck 2 was 492 l/ha (130 gal/a). Based on tank output, the total estimated area of coverage was 12.5 ha (31 a) with a total output of 15,364 l (4,130 gal) of solution. Using a scaled map that was overlaid with an acreage-dot-grid, the area of diazinon application was estimated to be 13.2 ha (32.6 a).

Sample-pan analysis (Table 1) indicated a mean application rate of 2.9 kg AI/ha (SD = 1.24; $n = 16$). The range of diazinon concentration on the sample-pans was 1.0 to 5.7 kg AI/ha. This unexpected variation in application rate seemed to have two sources. First, some areas of spray overlap occurred (this was observed on one sample-pan) and second, it appeared that the gang-nozzles used produced an uneven spray such that there were differing amounts of solution being applied within the swath.

At approximately 1730 hr on the day of application, we observed a flock of wigeon near the pond on fairway 3; they grazed for 30 to 40 min and later returned to the edge of an adjacent pond. A few flushed as we approached them at 1830 hr, but most were dead. Seventy-eight wigeon carcasses were collected immediately between 1830 and 1900 hr. Six more dead wigeon were found at approximately 0030 hr, and one was found at 0630 hr on Day 1 post-treatment. None of these wigeon carcasses were collected during the regularly scheduled carcass searches. They were collected immediately upon observation of death so that analytical procedures might proceed as soon as possible. Wigeon carcasses were transported to the laboratory and necropsied by a veterinarian. No physical abnormalities or diseases were noted. The brains and upper GI tracts (esophagus, proventriculus and ventriculus contents) were excised and prepared for chemical analysis. The remainder of the carcasses were frozen. The brains of all wigeon collected were analyzed for AChE activity. Reported values of brain AChE activity were expressed as micromoles of substrate (ACTI) hydrolyzed per min per g of tissue. AChE activity was compared to that of wild wigeon ($n = 3$) obtained from a state owned wildlife managment area near Ferndale, Washington (USA). Wigeon that died on study area 1 showed 44% to 87% depression of AChE activity when compared to the control wigeon. The mean depression was 76% (SD = 7.1%). The severe brain AChE depression in wigeon found dead is consistent with brain AChE depression correlated with death in birds exposed to anticholinesterase compounds reported by other investigators (Hill and Fleming, 1982; Grue et al., 1983). GI tract contents of 15 wigeon randomly chosen from those that died contained 0.96 ppm to 18.1 ppm diazinon ($\bar{x}$ = 7.96 ppm; SD = 7.44).

Though there was extensive use of the turf by songbirds, particularly American robins (*Turdus migratorius*), European starlings (*Sturnus vulgaris*), red-winged blackbirds (*Agelaius phoeniceus*), and American crows (*Corvus brachyrhynchos*), we found no evidence of treatment related mortality or behavioral impairment. Ten other carcasses or animal remains were found on the area during the study. Two of these were found after the diazinon application. These were the remains of a depredated red-winged blackbird found on Day 16 and a shrew (*Sorex* spp.) found on Day 9 post-treatment. The shrew was skinned and the carcass was homogenized with hexane. Diazinon residues were then determined by GC analysis. Insufficient body tissue remained of the blackbird for residue analysis. Diazinon levels were <0.012 ppm (the lower detection level) in the body of the shrew.

The diazinon content of grass samples collected prior to and following treatment are listed in Table 2. Pre-irrigation grass samples residues ranged from 183 ppm to 363 ppm with a mean of 281 ppm. Residues in grass samples collected immediately after irrigation ranged from 100 ppm to 333 ppm with a mean of 209 ppm. Diazinon residues in grass had declined to an average of 29 ppm by Day 7.

Residue values in grass were higher than expected for this application rate when compared to those reported in diazinon turf studies undertaken in other parts of the United States (R. Balcomb, pers. comm.). We suspect that physical conditions of golf course turf, common in the Pacific Northwest, may be influencing the persistence and behavior of diazinon residues. Thatch underlying grass is reported to have a "sponge effect," that can hold

TABLE 2. Diazinon content (ppm) of grass samples with mean and standard deviation for each chronological sampling group collected at Sudden Valley Golf Course in Bellingham, Washington, USA.

Day[a]	Diazinon content	Mean
−1	0.037	
−1	ND[d]	
0 prewash[b]	327.00	
0 prewash	286.00	
0 prewash	267.00	
0 prewash	320.00	
0 prewash	183.00	
0 prewash	284.00	
0 prewash	363.00	
0 prewash	214.00	280.50 (55.55)
0 postwash[c]	213.00	
0 postwash	100.00	
0 postwash	333.00	
0 postwash	328.00	
0 postwash	103.00	
0 postwash	122.00	
0 postwash	251.00	
0 postwash	224.00	209.25 (88.20)
3	102.00	
3	27.90	
3	124.00	
3	90.90	
3	24.40	
3	139.00	
3	104.00	
3	95.10	88.41 (38.83)
7	45.80	
7	34.00	
7	25.70	
7	13.00	
7	24.30	
7	28.80	
7	18.70	
7	43.20	29.19 (10.63)

[a] Day 0, 10-15-86 or the date of diazinon application.
[b] Prewash is before water applied after diazinon application.
[c] Postwash is water applied after diazinon application.
[d] ND, Not detected, <0.012 ppm detection limit for 10 g grass samples.

water near the surface after rain or irrigation (Beard, 1975). In other research in Northwest Washington on the persistence of diazinon residues in turf, we observed that 1.3 cm of irrigation will reduce residue levels to approximately 33% of the initial pre-irrigation levels. However, those levels increased again to approximately 60% of pre-irrigation levels within 18 hr of completion of irrigation. The exact mechanism by which this occurs is unknown; however, it may involve wicking or uptake by the grass. Diazinon residues decreased rapidly with average levels declining to 29 ppm by Day 7.

There was variability in the pre-irrigation residue levels in grass samples from treated fairways in this study. This variability was probably the result of irregularities in the spray swath of the "boomless" gang nozzles as well as some spray overlap. The boomless nozzles are not typically used on golf courses, but we used them in this study to allow rapid treatment of the designated study areas. The variation in residues and spray overlapping may have produced residue "hot spots" that contributed to the severity of the mortality observed. We observed that these irregularities are reduced considerably by using standard boom spraying systems which are generally used on such turf areas.

Though these applications may not be representative of typical use, we believe the results suggest that wigeon because of their grazing habits may be particularly vulnerable to diazinon and, possibly, other organophosphorous insecticides used on turf. It is only on the west coast that wigeon are known to use turf areas for feeding and only at certain times of the year (R. R. Hitchcock, unpubl. data). Generally wigeon are outside the U.S. on their breeding grounds when turf insecticides are normally used. Therefore, if diazinon and other insecticides are used on areas where wigeon feed in the Pacific Northwest we recommend that use occur only after wigeon have migrated out of the area. Preferred turf feeding areas should be avoided for insecticide application when actively being used by waterfowl.

ACKNOWLEDGMENTS

We appreciate the close cooperation and support that we had from the Washington State Department of Wildlife. In particular, we acknowledge the help of Jack Smith, Bob Everitt, and Mike Davison. We also appreciate support from the U.S. Fish and Wildlife Service, Portland, Oregon. This research project was supported primarily by a grant from CIBA-GEIGY Corporation, Greensboro, North Carolina.

LITERATURE CITED

BEARD, J. B. 1975. Turfgrass: Science and culture. Prentice-Hall, Inc., Englewood Cliffs, New Jersey, 658 pp.

ELLMAN, G. L., K. D. COURTNEY, V. ANDRES, AND R. M. FEATHERSTONE. 1961. A new and rapid calorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology 7: 88–95.

FITE, E. C., L. W. TURNER, N. J. COOK, AND C. STUNKARD. 1988. Guidance document for terrestrial field studies. EPA/600/09-88/109. National Technical Information Service Publication No: PB89 124/580, Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, D.C., 67 pp.

GRUE, C. E., W. J. FLEMING, D. G. BUSBY, AND E. F. HILL. 1983. Assessing hazards of organophosphate pesticides to wildlife. Transactions of North American Wildlife and Natural Resources Conference 48: 200–220.

HILL, E. F., AND W. J. FLEMING. 1982. Anticholinesterase poisoning of birds: Field monitoring and diagnosis of acute poisoning. Environmental Toxicology and Chemistry 1: 27–38.

STONE, W. B. 1979. Poisoning of wild birds by organophosphate and carbamate pesticides. New York Fish and Game Journal 26: 37–47.

———, AND P. B. GRADONI. 1985. Wildlife mortality related to use of the pesticide diazinon. Northeastern Environmental Science 4: 30–38.

THOMAS, L. 1988. Diazinon; CIBA-GEIGY Corporation, et al, Petitioners: Final Decision. Federal Register 53: 11119–11131.

ZINKL, J. G., J. RATHERT, AND R. R. HUDSON. 1978. Diazinon poisoning in wild Canada geese. The Journal of Wildlife Management 42: 406–408.

Received for publication 1 February 1991.