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Deaths of Canada Geese Following Spraying of Turf with Diazinon

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Diazinon has been registered for the control of insect pests on turf since the early 70s in Canada. Its use increased in 1986 when the use of chlordane was suspended. One of the main insect pests against which diazinon is currently used is the black turf grass ataenious or black fairway beetle (Ataenious spretulus). This insect is a newly introduced beetle with a seven year life cycle and appears to have become a widespread problem of golf courses by 1987. The insect feeds on the turf thatch and is controllable if treatment is applied while it is in its first or second instar and the insecticide is washed into the thatch. Treatment with diazinon or chlorpyrifos has been recommended on the basis of the U.S. experience with this pest. No insecticide is currently registered for this specific pest in Canada.

Since the introduction of diazinon, sporadic mortality of waterfowl feeding on treated turf or on orchard grass has come to light in Canada. This paper is a report of incidents which took place in Ontario between 1986 and 1988 with an in-depth study of a case in 1987.

MATERIALS AND METHODS

Field staff of the Ontario Ministry of Natural Resources or the Ministry of the Environment collected dead and dying Canada Geese (Branta canadensis) from the golf courses and other turfed sites where deaths had occurred and delivered them to the Wildlife Diseases Section of the Department of Pathology at the University of Guelph. Field investigators collected pertinent information on the facts surrounding the deaths. Carcasses were examined for causes of death and various specimens and tissues collected for pesticide analyses by the Agricultural Laboratory Services Branch of the Ontario Ministry of Agriculture and Food. Routine histology and bacterial culture were carried out on a range of appropriate tissues from the dead geese.

A one to 10 gram sample of tissue was homogenized and extracted by blending with acetonitrile and water (2 + 1) according to the procedure of Braun and Lobb (1976). A measured portion of the extract was concentrated and residues were determined by gas chromatography on a 1.8 m x 2 mm id column packed with 5% OV-1 on 100-200 mesh Gas-Chrom Q with flame photometric detection (P mode,

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525 nm filter) at an estimated temperature of 170 degrees C. Recoveries were determined in each sampling period by fortification of pureed tissues just before extraction and then carrying through the procedure as described. Recoveries of diazinon were 85-95% and the limit of detection was 0.1 mg/kg.

When possible, brains were frozen and shipped on dry ice to the National Wildlife Research Centre of the Canadian Wildlife Service for measurement of cholinesterase (ChE) levels. Analysis was by the Ellman (1961) method as modified by Hill and Fleming (1982). This assay measures total cholinesterase, which in avian brain tissue is thought to be mostly acetylcholinesterase (Hill and Fleming 1982). Brain samples were run in duplicates and the average value accepted if the two runs were within 10% of each other.

RESULTS AND DISCUSSION

Records of five incidents of Canada Goose poisonings, following the spraying of turf with diazinon, appear in Table 1. The 1986 case was from a treated condominium lawn, the others were from golf courses.

Table 1. Cases of poisoning of Canada Geese on turfed sites in Ontario 1986-88.

Year	Kill Location	Goose Deaths	Internal Reference (Nos)
1986 (June)	Burlington	13	A
1987 (June)	Burlington	16	B
1987 (July)	Markham	10	C
1988 (Aug.)	Burlington	3	D
1988 (Sept.)	Toronto	15	E
Total		57	

Case A

The condominium lawn was treated on June 25 or 26 by a licensed lawn care company. The birds were killed between June 28 and July 1. The treated property is along Lake Ontario and because the birds were found in the water, it is thought that more birds could have died but not been retrieved.

Analyses of the stomach content of one bird revealed 5.8 mg/kg of diazinon. A lung sample from the same bird contained 0.29 mg/kg. Clinical symptoms included submucosal dilation of the proventriculus, and congested and oedematous lungs. No other significant abnormalities were present to explain mortaility.

Five brains were sent for ChE measurements. Two Canada Goose brains were obtained to serve as concurrent controls. The two control brains gave activity levels of 13.3 and 13.4 U/g of tissue. (One unit [U] is 1 micro-mole of substrate hydrolysed per minute). Values for the 5 brains of birds killed on the lawn ranged from 4.36 to 5.73 U/g or an inhibition level ranging from 57% to 77% of control. A single sample was reactivated with the oxime 2-PAM at 30 degrees C for 60 min. and its activity increased

from 4.36 to 12.6 U/g. An aliquot from the same brain only increased to 5.58 U/g through spontaneous hydrolysis for 60 min. at 30 degrees C in water only. This differential recovery of the sample when subjected to 2-PAM is diagnostic of organophosphorus poisoning (Martin 1981).

Case B

A 500 EC formulation of diazinon was applied on June 25 to all fairways of a golf course at the labeled rate of 9 Kg a.i./ha. The birds were picked up on June 27 by Humane Society officials. Brains of all 16 birds were analysed for ChE levels. They ranged from a low of 2.67 U/g to 6.76 U/g. This represents an inhibition level ranging from 49% to 80% relative to the two control birds reported in case A.

The golf course operator involved reported that up to several hundred geese have been known to forage on this particular course. He also indicated that a kill of a few birds on diazinon-treated turf had also taken place in 1986 but had not been reported.

Case C

In 1987 a full description of a poisoning event was recorded. Between 1300 and 1700 hours on July 7, the golf course was sprayed with 8.6 Kg a.i./ha of a 500 EC formulation. The intent was actually to apply 4 Kg a.i./ha but an error was made in preparing the spray solution. In any case, the resulting application was still within the range allowed on the label. The fairways were treated with a boom-type sprayer driven at a speed of 5-6 km/h and application at a pressure of 280 kilopascals.

The fairway on which the Canada Geese appeared was sprayed at 1300 hrs and immediately irrigated for one hour with 12 to 25 mm of water. Approximately 25-30 Canada Geese were seen grazing the fairways and the first casualties were picked up approximately 3.5 hours after the application. Surviving geese exhibited the following signs: ataxia and neck swaying, flopping to the ground, foaming at the mouth and passing blood in stools.

A turf sample collected around 1800 hrs on July 7 contained 390 mg/kg of diazinon. The diazinon treatment gave a good control of the pest.

The results of analyses of six of the dead geese are presented in Tables 2 and 3. The analytical results and the gross and microscopic findings strongly support the view that the death of geese A to E was caused by diazinon ingestion. Goose F appeared to have died of aspergillosis; Tissue residues were low and brain cholinesterase level normal. Spearman's rank correlations were computed for all variables listed in table 2 for birds A to E. A highly significant ($P<.01$) negative rank correlation was obtained between brain ChE levels and kidney, lung or stomach content residues. A highly significant positive rank correlation was obtained between the following residue levels: kidney-lung, kidney-stomach contents and lung-stomach contents. None of the other rank correlations were significant.

Case D

The greens and tees of this golf course were treated on August 2 with Diazinon 50 EC. Three geese and one gull (species not recorded) were found on the afternoon of the next day in a large pond on the course. The pooled gizzard contents from the three

birds contained 5.9 mg/kg diazinon. The gull was not analysed. The lungs of all birds were mildly congested and markedly oedematous and the vents were stained reddish brown. The birds were in poor condition but there were no other significant findings to explain the kills.

Case E

The golf course in question was treated on September 12 but no other application details were made available. The dead birds were picked up the next day. A single bird was necropsied. Some blood stained the vent and surrounding feathers but the internal organs appeared normal. Upper gastrointestinal tract contents contained 6.7 mg/kg diazinon and the liver 0.029 mg/kg. The brain from this same bird had a ChE activity of 3.26 U/g which is a 75% inhibition level relative to the previously reported controls. Reactivation with 2-PAM raised the activity level to 8.26 U/g and there was no measurable spontaneous hydrolysis indicating once again that an organophosphate insecticide was involved.

All of the kills reviewed here had a positive diagnosis of diazinon intoxication. Brain ChE levels proved useful in arriving at this diagnosis in all cases where samples were analysed and this, despite the obvious variation in the treatment of the carcasses, and the length of time between death and proper storage of the birds. This suggests that brain samples inhibited by diazinon are not overly subject to rapid spontaneous reactivation. The point was further illustrated by the unsuccessful attempts to induce spontaneous reactivation of the two brain homogenate samples in the laboratory.

Positive residue levels were also detected in all samples analysed but these were found to vary greatly, even between birds within a single incident. Diazinon oxon was not detected in any sample. Its limit of detection is estimated to be double that of the parent material. Brain ChE depression and stomach content residue levels correlated best with lung and kidney levels but not with brain, liver or foot residue levels in one incident.

Mortality of geese grazing on diazinon-treated turf is not surprising or unusual. There are extensive reports of such mortality in the U.S. (e.g., Stone and Gradoni 1985) and the use of diazinon on golf courses and sod farms has been withdrawn in that country because of the large number of reported kills. From an examination of the turf residue data from 8 separate studies submitted in the course of the U.S. cancellation hearings on diazinon, it was determined that median levels of diazinon in turf sprayed and then properly irrigated ranged from 45.4 to 256 mg/kg for a single application of 1 Kg a.i./ha of the EC formulation (Mineau 1987). The value of 390 mg/kg obtained from the single grass sample sprayed with approximately 9 Kg a.i./ha is therefore at the low end of possible residue levels following such a heavy application.

Maximum residue levels for grass recovered from the oesophagi of geese killed by diazinon on turf and collected while still fresh have ranged from 55 to 79 mg/kg (Stone and Gradoni 1985). These residue levels could easily result from an application as low as 1 Kg a.i./ha followed by irrigation. Current label rates in Canada permit applications to turf as high as 12 Kg a.i./ha but are more typically between 4 and 6 Kg a.i./ha. It is therefore clear that, where geese and other grazing waterfowl species come in contact with turf recently treated with diazinon, kills are very likely to occur.

Table 2. Residue of diazinon and brain cholinesterase in 6 of 10 Canada Geese found dead or dying on a golf course in July 1987.

	Tissue Diazinon Level (mg/kg)						
Goose	Brain	Stomach content	Liver	Kidney	Lung	Foot	Brain Cholinesterase[1] Activity (U/g)
A	N.S.[2]	3.9	0.12	0.079	0.039	0.073	2.9 +/- 0.1
B	0.016	3.8	0.011	0.047	0.01	0.096	4.8 +/- 0.1
C	0.017	25.0	0.19	0.56	0.64	0.33	1.5 +/- 0.3
D	0.017	25.0	0.23	0.32	0.10	1.10	2.2 +/- 0.2
E	0.045	23.0	0.022	0.15	0.096	0.44	2.4 +/- 0.02
F	0.010	0.067	0.010	0.01	0.029	0.75	13.9 +/- 0.3

[1]Two Canada geese analysed earlier had brain cholinesterase activity values of 13.4 and 13.3 U/g. U/g: 1 umole of substrate hydrolysed per minute per gram of brain.
[2]N.S. - No sample.

Table 3. Clinical pathology and diagnosis in the deaths of 6 of 10 Canada Geese found dead or dying on a golf course in July 1987.

Goose Identity	Clinical Pathology		Bacterial/ Microscopic findings
A	mild pulmonary congestion and oedema, no lesions.	normal	non-specific[1]
B	marked hemorrhagic enteritis, no lesions in other tissues	normal	non specific
C	clear oedema fluid in trachea and nostrils, marked pulmonary congestion	normal	non specific
D	marked pulmonary congestion	normal	non specific
E	marked pulmonary congestion	normal	non specific
F	white necrotic nodules (1-5mm) in lungs and air sacs. Intestine tract empty. Other tissues and organs, lesions and oedematous	dehydrated, emaciated, poor feathering, external trauma	pulmonary[2] aspergillosis

[1]Non specific, no bacteria from internal organs
[2]Moderate to large numbers of Aeromonas hydrophila, Bacteroids spp, Fusobacterium spp and Clostridium sp in lung abcesses

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