

DECOMPOSED GOSLING FEET PROVIDE EVIDENCE OF INSECTICIDE EXPOSURE

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Abstract. Canada goose goslings were exposed to turf sprayed with D·Z·N[®] diazinon 50W application (2.24 kg a.i./ha). The control plot was subjected to a water application. One foot from each bird was placed outdoors for 7 d to decompose and the other foot was kept frozen. Diazinon residues were analyzed on both feet. Results showed that diazinon was detected from undecomposed and decomposed feet of the birds. Diazinon residues were below the level of detection (<0.01 ppm, a.i.) on the feet from the control goslings. Decomposed feet may be used for determining insecticide exposure when the traditional matrices are not available.

Keywords: birds, decomposition, diazinon, feet, insecticides, residues

1. Introduction

Avian mortalities from organophosphorus insecticide exposure are investigated by federal and state wildlife personnel in the United States. The forensic investigation involves not only the recovery of the dead birds from the field but also requires laboratory confirmation of the cause of death. A carcass submitted for laboratory evaluation is subjected to pathological and anatomical examinations (Stroud and Adrian, 1996), and if poisoning is suspected, biochemical and chemical analyses are conducted. For organophosphorus insecticide poisoning, the brain is analyzed for cholinesterase activity levels to identify the mechanism of death, and chemical residue analysis is performed to identify the insecticide responsible for the death. The gastrointestinal tract or its contents are the conventional matrices used for residue analysis.

The determination of the cause of death is contingent on the quality of the samples collected, which in turn is affected by how soon and how thoroughly an investigation occurs after the onset of a mortality event. The vastness of the areas subjected to pesticide applications as well as private property restrictions prevent regular patrolling by wildlife personnel, and therefore forces them to rely on public



reportings. The reportings are limited by the public's ignorance that the wildlife mortality should be reported and to whom it should be reported, fear of prosecution, camaraderie, procrastination, and apathy. When a mortality incident is reported to the appropriate authorities, an immediate investigation may not be possible because of the distance, terrain, weather, private property restrictions, limited resources, and other on-going investigations. Delays in the discovery, the reporting, and the investigation of a mortality incident increase the interval between the time of mortality and carcass collection, which in turn, increase the chances of compromising the quality of the evidence through scavenging and decomposition (Vyas, 1999; Vyas *et al.*, 2003). Consequently, when a carcass is recovered during a field investigation, the biochemical and chemical matrices which are used to ascertain the cause of death from insecticide poisoning may not be in analyzable condition. The loss of these matrices introduces uncertainty in determining the cause of death and reduces the carcasses to circumstantial evidence of poisoning. Due to the constraints on resources, field investigators may submit to the laboratory, only those carcasses which retain the conventional matrices in a condition suitable for analysis.

Our objective was to determine if insecticide residues could be detected from decomposed feet, thereby providing a matrix for determining insecticide exposure.

2. Materials and Methods

2.1. BIRDS

Canada goose (*Branta canadensis*) eggs were incubated and the goslings were hatched at the U.S. Geological Survey's Patuxent Wildlife Research Center in Laurel, MD, U.S.A. Birds were held in rooms (1.8 × 3.0 m) with a concrete floor lined with plastic sheeting (0.12 mm thickness). Rooms were equipped with a waterer, a feeder, and a heat lamp. Goslings had *ad libitum* access to commercial gamebird diet and were allowed to graze in outdoor plots (1.5 × 2.4 m and 3 × 4.6 m, respectively) enclosed with chicken wire. Meadow fescue (*Festuca elatior*), orchard grass (*Hordeum spp.*), and Kentucky blue grass (*Poa pratensis*) were the most abundant grass species in the plots (M. Perry, pers. comm).

Birds (13–14 d old) were placed in cardboard animal transport boxes (45.7 × 61.0 × 40.6 cm; Horizon Omni NESTS[®]) lined with pine needles and shipped via commercial airlines to the U.S. Department of Agriculture's Moore Air Base in Edinburg, Texas for pesticide exposure.

The animal care procedures used in this study were approved by the Patuxent Wildlife Research Center's Animal Care and Use Committee.

2.2. INSECTICIDE EXPOSURE SITE

Goslings were transported to the Moore Air Base to take advantage of an ongoing study on the effects of diazinon to goslings. Birds were randomly assigned to the outdoor study pens (0.15 and 0.16 ha) which were built on Bermuda grass (*Cynodon dactylon*) turf using chicken wire fencing and PVC and wooden posts. Electric fencing wires surrounded the pens to protect the goslings from predators. Each pen was equipped with a shelter (1.8 × 2.4 × 1.8 m) constructed from PVC piping and tarp. Each shelter was supplied with four waterers which were replenished with fresh water twice a day. The shelters were moved within each pen every 2 d to prevent fecal build up. Commercial feed was provided to the goslings for 1 d after arrival and then was removed to accustom the birds to graze on the Bermuda grass. The grass was maintained approximately 9 cm high.

Pen construction and maintenance, and lawn irrigation and mowing was executed by the facilities maintenance staff at Moore Air Base.

2.3. INSECTICIDE APPLICATION AND GOSLING EXPOSURE

Birds were acclimated to the study pens for 10 d before testing. The sprayer tank and hoses were flushed with two rinses of a commercial sprayer tank cleaner prior to its use in our study. On the evening before the pesticide application, all shelters and waterers were removed from the pens and all birds were caught, locked in cardboard boxes, and placed in a nearby building for the night. Plastic sheeting (0.12 mm thickness) was draped over the fences separating the plots and tied down to block pesticide drift. One pen was treated with D·Z·N[®] diazinon 50W (50% diazinon a.i., *O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate) at 2.24 kg a.i./ha using a tractor-mounted boom sprayer. The diazinon application rate used was 50% of the maximum application rate allowable on the D·Z·N[®] diazinon 50W label. The control pen received an appropriate water application. Pesticide application was made by a certified applicator.

Within 2 hr of the completion of the diazinon application, the shelters and waterers were returned and the goslings were released into their respective study pens. The diazinon-exposed birds died within 24 hr of exposure and their feet were collected as soon as the carcasses were observed. Birds in the control group were euthanized with CO₂.

2.4. SAMPLE COLLECTION

Feet were collected from six goslings from the diazinon-treated plot and four goslings from the control plot. The tarsometatarsi were severed from the carcasses using scissors and both feet from each bird were stored together in chemically cleaned jars. All samples were frozen (-23 °C) immediately and later shipped on dry ice to the Patuxent Wildlife Research Center. Samples were frozen (-23 °C) upon receipt

at the Patuxent Wildlife Research Center until either the decomposition trial or residue analysis.

2.5. FOOT DECOMPOSITION

One foot from each bird was removed from the freezer and placed in an outdoor pen (2.4 × 6.1 × 1.8 m) for decomposition. These pens were used for housing animals and have never been subjected to pesticide applications. The vegetation in the pen was mowed to approximately 5 cm high prior to starting the study to reduce shade on the feet. The feet were placed on the bare ground among the vegetation to maximize contact with the soil. The tarsometatarsi of each bird was tied with string to a vinyl stake wire flag to prevent scavengers from carrying away the foot and to mark the location of each foot. The feet were collected after 7 d, placed in individually marked cryovials, and frozen for residue analysis. Some grass and soil from the pen was inadvertently included in the sample while collecting the decomposed foot pieces. Soil and grass blades which were adhered to the samples were not removed and were included in the chemical extraction procedure. The second foot from each bird remained in the freezer until analysis.

2.6. RESIDUE ANALYSES

Each foot (below the distal end of the tarsometatarsus) was cut into approximately 0.6 cm pieces using scissors to facilitate chemical extraction. Samples were extracted three times with 1:1 acetone:dichloromethane, filtered, and adjusted to a 50 mL volume for analysis by gas chromatography (Belisle and Swineford, 1988). The quantitative analysis was performed using a Hewlett-Packard 5890 gas chromatograph equipped with a J&W Megabore 14% cyanopropylphenyl-86% methyl silicone capillary column and flame photometric detector. Quality assurance was conducted following guidelines presented in the U.S. Fish and Wildlife Service's Patuxent Analytical Control Facility Manual (unpublished). During residue analysis, the foot samples were interspersed among other samples containing diazinon, and the results of the appropriate quality assurance analyses are provided. Procedural blanks were analyzed to assure that no analyte is added during the processing of the sample. The blank was an empty tube to which the solvents used in the analysis were added. The blank was processed similarly as the samples. Blank data are usually less than the limit of detection, but, are acceptable up to three times the limit of detection. Spiked samples were analyzed to provide a measure of the accuracy of the methods used for analysis. Two subsamples were taken from a sample. One of the subsamples was processed as a sample and a known quantity of diazinon was added to the other subsample. Spike recoveries for diazinon are acceptable in the 80% to 120% range. Nine matrix spike samples ranging 2.9–6.7 g were spiked with 5.36 µg of diazinon and 12 control blanks were analyzed. For 10% of the samples with residues reported above the limit of detection, the

TABLE I
Diazinon residue levels on undecomposed and 7 d decomposed feet

Application rate (kg a.i./ha)	Bird	Diazinon residue (ppm, a.i.)	
		Undecomposed foot	Decomposed foot
Control	C1	<0.01 ^a	<0.01
	C2	<0.01	<0.01
	C3	<0.01	<0.01
	C4	<0.01	<0.01
2.24	D1	57	1
	D2	25	0.89
	D3	8.8	0.08
	D4	0.36	0.19
	D5	25	2.5
	D6	0.2	3.1

^a Residue level below level of detection.

presence of the pesticide was confirmed on a Hewlett-Packard 5890 gas chromatograph/5970 MSD mass spectrometer (GCMS) equipped with a 50 m cross-linked methyl silicone gum column with 0.2 mm i.d. and 0.32 micron film thickness. The GCMS was linked to a 59970 ChemStation computer data system.

2.7. WEATHER

Weather data during the foot decomposition period (27 June–5 July 2000) were obtained from the U.S. Department of Agriculture's Beltsville Agricultural Research Center's Weather Station #3, in Beltsville, Maryland, U.S.A. The weather station was located approximately 3.0 km from the foot decomposition site.

3. Results and Discussion

Diazinon levels from the undecomposed and the decomposed feet are presented in Table I. Photographs documenting the decomposed feet following chemical extraction are displayed in Figures 1 and 2. Table I and the figures are presented by individual sample to provide wildlife investigators a reference on the degree of decomposition which yielded diazinon residues. Photographs were taken after the chemical extraction procedure. Diazinon was detected from all 7 d old decomposed feet which were exposed to the insecticide. The quality assurance analyses

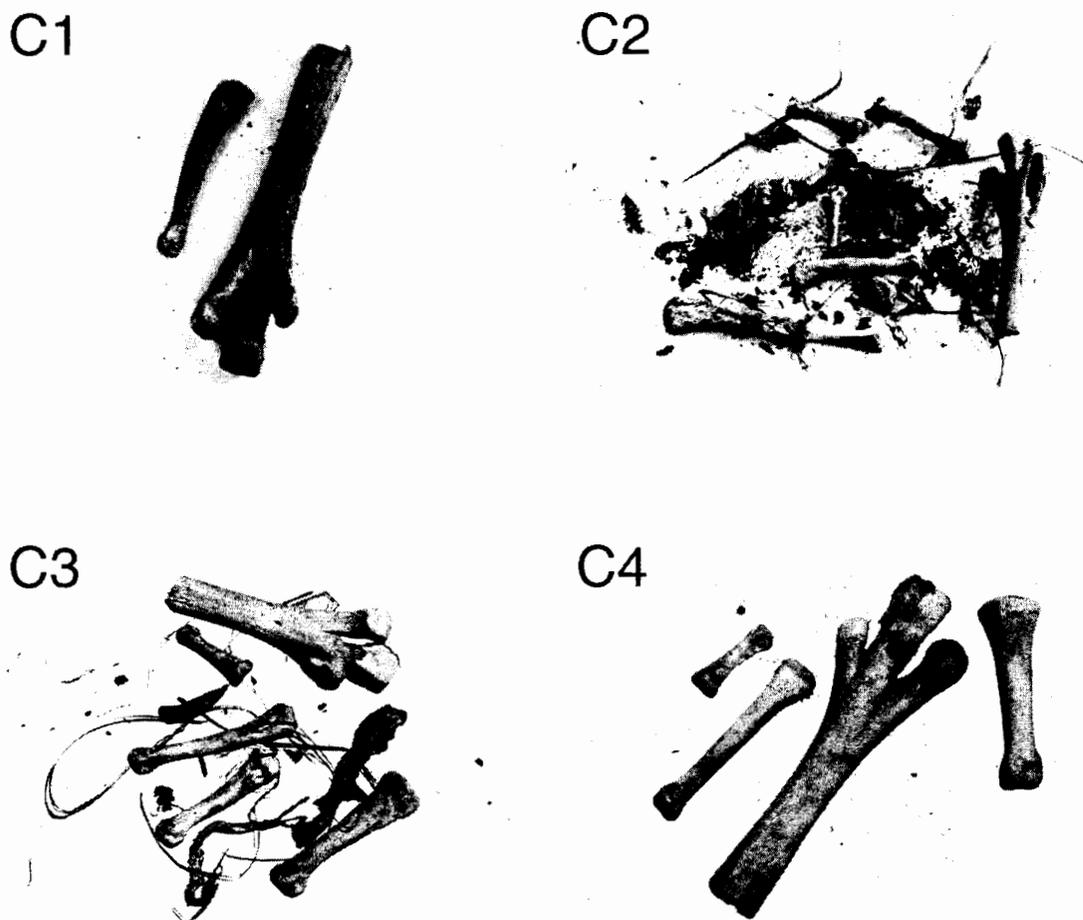


Figure 1. Control birds – 7 d decomposed feet.

revealed that the average spike recovery was 96% and all control blank samples contained $<0.1 \mu\text{g}$ of diazinon. Table II provides the weather conditions during the decomposition period.

Wildlife forensics laboratories may analyze pesticide residues on feet if they are available and as necessary (Frank *et al.*, 1991; Stroud and Adrian, 1996). However, feet in a condition similar to those in Figures 1 and 2 may not be submitted to the laboratory by the field investigators because they may not expect insecticide residues at such an advanced stage of decomposition. We focused on the feet as an indicator of pesticide exposure because:

- (1) For most exposure scenarios, feet are likely to come into contact with insecticides (e.g. walking on treated lawn and soil, perching on contaminated branches, grasping contaminated prey, and wading in contaminated water; Fowle, 1972; Hooper *et al.*, 1989; Driver *et al.*, 1991; Frank *et al.*, 1991; Hunt *et al.*, 1991, 1995; Henderson *et al.*, 1994; Clark, 1997).
- (2) Dermal absorption of pesticides through the feet can be a significant route of exposure for birds. Fowle (1972) provided several songbird (Order Pas-

D1



D2



D3



D4



D5



D6



Figure 2. Diazinon-exposed birds – 7 d decomposed feet.

TABLE II
Weather conditions during the decomposition period

Day of study	Maximum temperature (°C)	Minimum temperature (°C)	Maximum humidity (%)	Minimum humidity (%)	24 hr mean solar radiation (w/m ²)	Mean wind (m/sec)	Total rain (mm)
0	30.3	21.6	103.5	60.0	173.6	1.3	18
1	24.2	20.9	104.1	83.3	87.0	0.3	2
2	28.3	17.8	103.5	58.5	201.6	0.6	9
3	27.0	13.5	104.3	43.3	299.8	0.8	0
4	29.0	14.4	103.3	36.5	328.9	0.6	0
5	30.6	13.6	103.7	43.4	299.0	0.7	0
6	32.2	17.6	102.6	44.4	261.0	0.8	9
7	30.7	21.4	103.8	66.7	216.5	0.8	0
8	29.7	17.6	103.7	48.8	251.6	0.7	0

seriformes) species with either perches or a floor treated with phosphamidon (dimethyl hydrogen phosphate ester with 2-chloro-*N, N*-diethyl-2-hydroxy-crotonomide). Signs of toxicity and mortality were observed in birds within 30 min of exposure. Further, Fowle (1972) treated the feet of several songbird species with phosphamidon using a micro-syringe and observed mortality as soon as 15 min after exposure. House sparrows (*Passer domesticus*) using perches treated with fenthion (*O, O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl]phosphorothioate) for 30 sec–16.5 min demonstrated signs of toxicity as early as 16.5 min after introduction to the perch. Residue analyses of their plumage and skin, internal carcass, and feet revealed the highest fenthion concentrations in the feet (Hunt *et al.*, 1991).

- (3) The possible persistence of insecticides in the feet of live birds indicates the potential for detecting residues from decomposed feet. Henderson *et al.* (1994) exposed rock doves (*Columba livia*) to diazinon, methidathion (*O, O*-dimethyl hydrogen phosphorodithioate, *S*-ester with 4-(mercaptomethyl)-2-methoxy- Δ^2 -1,3,4-thiadiazolin-5-one), and ethyl parathion (*O, O*-diethyl *O*-(*p*-nitrophenyl) phosphorothioate) either dermally via the feet or orally by gavage. Birds dermally exposed to diazinon and ethyl parathion exhibited a slower recovery (6 wk) of plasma cholinesterase activity than the birds exposed orally (3–5 d). The authors suggested that the prolonged effects on the cholinesterase activity of dermally exposed birds occurred because the pesticides could be stored under the scales of the feet and were slowly released into the blood stream.

Diazinon was selected as a representative organophosphorus insecticide because of the large number of avian mortality incidents associated with its applications (Zinkl *et al.*, 1978; Stone and Knoch, 1982; Stone and Gradoni, 1985; Frank *et al.*, 1991; Kendall *et al.*, 1992, 1993). The diazinon detected from our samples represents the chemical on and in the feet. The residue levels from the feet do not necessarily imply a lethal dermal exposure but evidence the insecticide to which the bird was exposed, and report the minimum insecticide concentration that was initially on the foot (Stroud and Adrian, 1996). However, depending on the insecticide and the findings from the field investigation, detection of certain insecticides from the feet may provide evidence to the cause of death.

While all of our diazinon-exposed birds had measurable residues on their feet, the lack of residues on decomposed feet from the field does not imply that the bird was not exposed to an insecticide. Foot decomposition rates depend on the foot type and size, temperature, rainfall, humidity, and invertebrate density and diversity (Payne, 1965; Tullis and Goff, 1987). The success of detecting an insecticide or its metabolites on feet depends on the method of insecticide application, the chemical concentration on the matrix with which the feet were in contact, the amount of contact with uncontaminated surfaces which may wipe the insecticide off the feet, the chemical absorption rate into the blood, and the insecticide. The half-life of the insecticide is affected by the weather, pH, and the presence of appropriate microbes. In addition, the success of detecting insecticide residues depends on the lag time between the mortality and when the feet were collected, and the behavior of the avian species found. Diazinon degrades by hydrolysis, photolysis, and microbial metabolism. The half-lives of diazinon range between 12 hr to 6 mo depending on if the insecticide is in contact with the soil, water, or vegetation, and on the environmental conditions. In general, the persistence of diazinon in the environment increases under conditions of low moisture, low temperature, high alkalinity, and the lack of suitable microbial degraders (Eisler, 2000). The environmental conditions during our study were suitable for rapid decomposition of the foot tissue and the degradation of diazinon.

Field investigators encounter a spectrum of evidence ranging from fresh carcasses in ideal condition for laboratory analysis to scavenged and decomposed carcasses which may be of little use in determining the cause of death (Vyas *et al.*, 2003). White *et al.* (1990) studied the survival of free-living northern bobwhites (*Colinus virginianus*) in cropland subjected to insecticide applications. Despite radio-telemetry monitoring to locate the carcasses, the researchers were not able to determine the cause of mortality for any of the birds because scavenging rendered the carcasses unsuitable for necropsy and pesticide analysis. During field trials with the avicide 4-aminopyridine, Woronecki *et al.* (1979) could not determine the cause of death for 24 of 26 carcasses due to their deteriorated condition. Complete decomposition of a bald eagle (*Haliaeetus leucocephalus*) carcass (except bones, feathers, beak, and feet) was determined to have occurred within 3 d (D. Patterson, pers. comm.). The above examples show how pesticide poisoning can remain un-

proven when the conventionally used matrices of measurement were not available for confirming the cause of death. Our findings provide a tool for determining insecticide exposure when the traditional matrices are not available for analysis. Carcasses previously not submitted for residue analysis because of their advanced stage of decomposition may now be salvaged for their feet. Insecticide analysis of decomposed feet can raise the certainty of the cause of death depending on the complementary information collected during field investigations and the history of wildlife mortalities from a particular insecticide use.

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