CSI: Patuxent - Forensic Research Supporting Wildlife Law Enforcement

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ABSTRACT

Wildlife law enforcement agents depend on forensic analyses to identify the cause of death in order to build their case. The cause of death from organophosphorus and carbamate insecticide poisonings is often not confirmed because the carcass matrices that are conventionally analyzed are not available due to decomposition and scavenging. In such cases additional methods are needed to determine insecticide exposure. Many scavenged and decomposed carcasses retain intact feet that may have come into contact with the insecticides. We tested three scenarios with respect to pesticide, foot type, and exposure to determine the temporal reliability of pesticides on weathered and decomposed feet. Results show that certain insecticides can be detected on feet for at least 28 days. We provide wildlife law enforcement agents a tool for determining pesticide exposure after a bird has been long dead and the conventional matrices are no longer available for analysis. Insecticide analysis of feet can raise the certainty of the cause of death, thereby providing valuable evidence for wildlife law enforcement agents that in turn can influence prosecution and sentencing.

INTRODUCTION

Wildlife law enforcement agents responding to a reporting of an avian mortality regard every incident as a possible legal case. However, finding dead birds is not enough. A forensic evaluation must be conducted to determine the cause of death in order to build a case. If poisoning from organophosphorus or carbamate insecticides is suspected, the brain is analyzed for cholinesterase activity to identify the mechanism of death and chemical residue analysis of the gastrointestinal contents is performed to identify the insecticide responsible for the death. By the time a carcass is recovered during a field investigation, the conventional biochemical and chemical matrices that are used to ascertain the cause of death are often lost due to scavenging or decomposition (Fig 1). The loss of the conventional matrices introduces uncertainty in determining the cause of death and reduces the carcasses to circumstantial evidence of poisoning.

Photo by Don Patterson

Photo by Kinard Boone

Fig 1. Carcasses lacking conventional matrices but with intact feet.

Levels I – IV of the Mortality Pyramid (Fig 2) summarizes how our research fits into the broader picture of how evidence is lost and the variables that limit the determination of the cause of death.

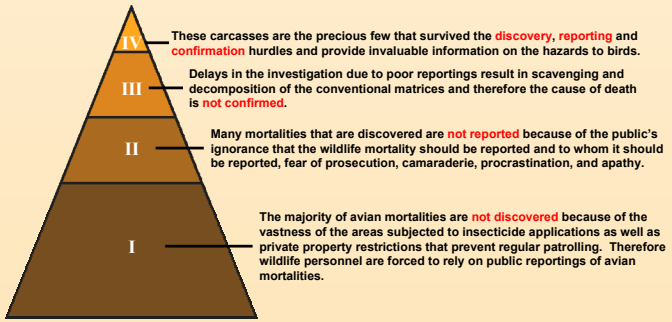

Fig 2. Mortality Pyramid – Schematic of how evidence is lost.

Our objective was to provide wildlife law enforcement agents an additional matrix for determining pesticide exposure when the conventional matrices are not available. We tested the reliability of weathered and decomposed bird feet for confirming pesticide exposure. Feet were selected because they come into contact with pesticides, many decomposed and scavenged carcasses retain intact feet (Fig 1), and dermal absorption of insecticides through the feet can be a significant route of exposure and toxicity for birds. Our research focuses on reducing the evidence lost at Level III of the Mortality Pyramid.

MATERIALS & METHODS

Exposure Scenarios

Birds were exposed to pesticides via walking or perching on a contaminated surface. Three scenarios were tested with respect to pesticide, foot type, and exposure.

Scenario 1. Songbirds on pesticide treated lawns.

Adult brown-headed cowbirds (Molothrus ater) walked on turf that was sprayed at label rates of either Earth Care®, 25% diazinon a.i.(76 L a.i./ha) or Ortho-Klor®, 12.6% chlorpyrifos a.i.(328 L a.i./ha), followed by 9.1 L of water. One bird served as a control and was not exposed to the treated turf.

Scenario 2. Raptors at illegal pesticide-laced predator baits.

Some ranchers, poultry and gamebird farmers, and hunting establishments illegally lace baits with pesticides to control predatory mammals and birds. Predator baits can include large and small animal carcasses, animal parts, meat patties, fish, eggs, and commercial pet food (Fig 3). The scavenging feeding behavior of raptors subject them to intentional and accidental poisonings at the bait sites (Fig 4). Eastern screech owls (*Otus asio*) were perched on pieces of white-tailed deer (*Odocoileus virginianus*) that were coated with 20 ml of a commonly used liquid formulation of carbofuran, Furadan 4F® (40% carbofuran a.i.) to recreate exposure at an insecticide-laced carcass bait. The insecticide was allowed to dry for approximately 18 hours prior to exposure to birds. One owl was placed on an uncontaminated deer piece and served as a control.

Photo by Diane Fries

Fig 3. Actual illegal predator bait laced with carbofuran (white residue on hair) by rancher.

Photo by Diane Fries

Fig 4. Hawk killed at a carbofuran-laced predator bait; carcass lacking conventional matrices but with intact feet.

Scenario 3. Waterfowl on pesticide treated lawns.

Canada goose (*Branta canadensis*) goslings walked on turf following label rate application of D•Z•N® diazinon 50W, 50% diazinon a.i. (2.24 kg a.i./ha). Four birds served as controls and were not exposed to the treated turf.

Weathering/decomposition and residue analysis

At the end of the exposure period, all survivors were euthanized by CO_2. The tarsometatarsi were severed from the carcasses using scissors (Fig 5) and each foot was individually placed in a cryovial and frozen until either the weathering and decomposition trials or residue analysis. One foot from each bird was removed from the freezer and allowed to either weather or to decompose outdoors during the summer at the Patuxent Wildlife Research Center. Cowbird and owl feet were weathered for up to 28 days in a window screening wire cage on top of a wooden palate. Gosling feet were placed on the bare ground for decomposition for 7 days (Fig 6). The weathered and decomposed feet were individually placed in new cryovials and frozen until residue analysis. The second foot from each bird remained frozen until analysis. Prior to residue analysis, each cowbird and owl foot (below the distal end of the tarsometatarsus) was cut into approximately 0.6 cm pieces using scissors for residue analyses (Fig 5).

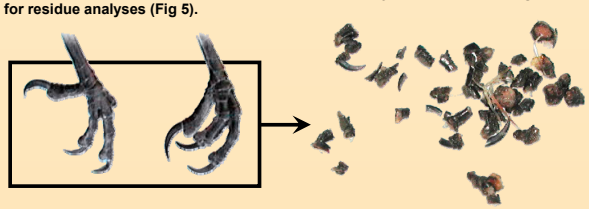

Fig 5. Feet below the distal end of the tarsometatarsus cut into small pieces for residue analysis.

RESULTS

Pesticide residues were found on all weathered and decomposed feet (Tables 1 - 4).

Table 1. Diazinon residues (ppm, a.i.) from unweathered and 28 day weathered feet of cowbirds exposed to turf.

Treatment	Unweathered feet $\bar{x}\pm$ SD (n) [range]	Weathered feet $\bar{x}\pm$ SD (n) [range]
Control	<1.0 [a] (1) [na][b]	<1.0 [a] (1) [na][b]
Diazinon	6.7 ± 2.7 (9) [2.3 – 11.0]	3.0 ± 2.2 (3) [1.6 – 5.6]

[a] Residue level below level of detection.
[b] Not applicable.

Table 2. Chlorpyrifos residues (ppm, a.i.) from unweathered and 28 day weathered feet of cowbirds exposed to turf.

Treatment	Unweathered feet $\bar{x}\pm$ SD (n) [range]	Weathered feet $\bar{x}\pm$ SD (n) [range]
Control	<1.0 [a] (1) [na][b]	<1.0 [a] (1) [na][b]
Chlorpyrifos	8.3 ± 2.9 (9) [6.1 – 14.0]	3.4 ± 1.4 (3) [1.8 – 4.3]

[a] Residue level below level of detection.
[b] Not applicable.

Table 3. Carbofuran residues (ppm, a.i.) from unweathered and 28 day weathered feet of eastern screech owls exposed to pesticide-laced predator bait.

Treatment	Unweathered feet $\bar{x}\pm$ SD (n) [range]	Weathered feet $\bar{x}\pm$ SD (n) [range]
Control	<0.1[a] (1) [na][b]	<0.1[a] (1) [na][b]
Carbofuran	10.3 ± 10.5 (9) [1.0 - 32.0]	12.5 ± 16.0 (3) [3.5 - 31.0]

[a] Residue level below level of detection.
[b] Not applicable.

Fig 6. Seven-day old decomposed gosling foot with diazinon residues.

Table 4. Diazinon residues (ppm, a.i.) from undecomposed and 7 day decomposed feet of goslings exposed to turf.

Treatment	Undecomposed feet $\bar{x}\pm$ SD (n) [range]	Decomposed feet $\bar{x}\pm$ SD (n) [range]
Control	<0.01[a] (4) [na][b]	<0.01 [a] (4) [na][b]
Diazinon	19.4 ± 21.5 (6) [0.2 – 57]	1.3 ± 1.2 (6) [0.08 – 3.1]

[a] Residue level below level of detection.
[b] Not applicable.

DISCUSSION

Our results demonstrate the temporal reliability of certain organophosphorus and carbamate insecticide residues on weathered and decomposed feet. In general, the residue levels from the feet do not necessary imply a lethal dermal exposure but evidence the insecticide to which the bird was exposed. However, depending on the insecticide's toxicity, its history of wildlife mortalities, and the findings during the field investigation, detection of certain insecticides from the feet can provide evidence of the cause of death.

Wildlife law enforcement agents build their cases by focusing their efforts on carcasses with analyzable conventional matrices that can provide evidence for the cause of death. We provide a tool for determining pesticide exposure after a bird has been long dead and the conventional matrices are no longer available for analysis. Our research reduces some of the uncertainty at the Level III of the Mortality Pyramid. Carcasses previously not collected or submitted for analysis because of the lack of the conventional matrices may now be salvaged for their feet. The residues from the feet may improve the estimate of the magnitude of a mortality incident. The magnitude of a kill is a critical element of the prosecution and therefore can affect the sentencing guidelines and result in a more appropriate sentencing of the perpetrators.

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