From the Field: Carbofuran detected on weathered raptor carcass feet

Nimish B. Vyas, James W. Spann, Craig S. Hulse, Wayne Bauer, and Sabra Olson,

Abstract The cause of death for raptors poisoned at illegal carbofuran-laced predator baits is often not confirmed because the carcass matrices that are conventionally analyzed are not available due to decomposition and scavenging. However, many such carcasses retain intact feet that may have come into contact with carbofuran. Eastern screech owls (*Otus asio*) were exposed to carbofuran via simulated predator baits. Detection of carbofuran from owl feet weathered for 28 days demonstrated the temporal reliability of using feet during a forensic investigation. Raptor carcasses previously not submitted for residue analysis because of a lack of the conventional matrices may now be salvaged for their feet.

Key words carbofuran, forensic science, insecticide, raptors, toxicology, wildlife law enforcement

The deliberate illegal use of insecticides on baits for controlling predators is investigated by federal and state wildlife law enforcement personnel in the United States. Predator baits can include large animal carcasses (e.g., deer [*Odocoileus* sp.], cows, lambs, sheep), small animal carcasses (e.g., chickens, prairie dogs [*Cynomys* sp.], rabbits [*Sylvilagus* sp.]), animal parts, meat patties, fish, eggs, and commercial pet food that are laced with high concentrations of liquid, granular, or powder formulations of highly toxic insecticides that are not registered in the United States for predator control. The insecticides may be topically applied on the surface of the bait, injected or placed into slits cut in the bait, or soaked into the bait. The baits are placed by some ranchers, poultry and gamebird farmers, and hunting establishments to control predatory mammals and birds. The scavenging and feeding behaviors of these animals subject them to high risk of intentional and accidentat poisonings at the bait sites (Vyas et al. 2003*a*).

When illegal baitings are investigated, the suspects may be prosecuted under the Federal Insecticide, Fungicide, and Rodenticide Act for using "any registered pesticide in a manner inconsistent with its labeling"(United States Code,Title 7, Section 136j, 1972). If bird-of-prey carcasses are found in the vicinity of the bait site, the suspects also may be charged with violations of the Migratory Bird Treaty Act (United States Code, Title 16, Section 703, 1918), the Bald and Golden Eagle Protection Act (United States Code,Title 16, Section 668a, 1940), the Endangered Species Act (United States Code,Title 16, Section 1538, 1973), and various state laws. The forensic investigation to establish a cause-effect relationship between an insecticide-laced bait and a raptor mortality involves not only the recovery of the dead bird from the field but also the 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 and carbamate insecticide poisonings, the brain cholinesterase activity is measured to determine the mechanism of death and chemical residue analysis of the gastrointestinal tract or its contents is performed to identify the insecticide responsible for the death (Hill and Fleming 1982). However, scavenging and decomposition may render these conventional matrices unsuitable for analysis (Vyas 1999, Vyas et al. 2003*a*). Our observations have shown that many such carcasses retain intact feet. Therefore, our objective was to measure the persistence of the carbamate insecticide carbofuran (2, 3-dihydro-2, 2-dimethyl-7-benzof uranyl methyl carbamate) on weathered raptor feet to test their long-term reliability as a matrix for determining insecticide exposure to raptors killed by illegal carbofuran-laced baits.

Methods

We selected eastern screech owls (*Otus asio*) from the Patuxent Wildlife Research Center's colony as representative raptors. We used 2 road-killed white-tailed deer (*Odocoileus virginianus*) to recreate an illegal insecticide-laced carcass bait. We carved out pieces (approximately 12.7 × 12.7 cm and 5 cm thick) from the hind quarter and the back strap (along with the hide) from the deer and placed the pieces on the bottom of plastic milk jugs (4.5 L) that served as the exposure pens. The bottle-neck part of each jug (top 7.6 cm) was removed prior to fitting the meat into the jug. We poured 20 ml of a commonly used liquid formulation of carbofuran, Furadan 4F® (FMC Crop Agricultural Products Group, Philadelphia, Penn.) (40% carbofuran), evenly on each deer piece and allowed the insecticide to dry for approximately 18 hours prior to exposure to birds. We placed 9 owls individually on the deer hide to simulate a raptor perched on a large insecticide-laced carcass while feeding. We placed 1 owl on an uncontaminated deer piece; this deer served as a control. We covered jug openings with hardware cloth. We exposed owls to Furadan 4F® for approximately 40 minutes. We then placed birds in individual carrying boxes and transported them 1.5 km to be euthanized by CO_2. The tarsometatarsi were severed from the carcasses using scissors, and each foot was individually placed in a cryovial and frozen at -23°C.

One foot from each bird was removed from the freezer and placed outdoors during summer for weathering. We placed feet in a window-screening wire cage (30 × 30 × 15 cm), which was placed on a wooden pallet inside a chicken-wire pen (32.8 × 16.4 × 19.7 m) to ensure that the feet would be available for residue analysis at the end of the 4-week weathering period. We collected 3 carbofuran-exposed feet after 7, 14, and 28 days of weathering and collected the control foot on day 28. The weathered feet were individually placed in new cryovials and frozen until residue analysis. The second foot from each bird was not weathered and remained frozen until residue analysis.

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 3 times with 1:1 acetone:dichloromethane, filtered, and adjusted to a 50-ml volume for analysis by gas chromatography (Belisle and Swineford 1988). We performed quantitative analysis using a Hewlett-Packard 5890 gas chromatograph equipped with a J&W Megabore 5% phenyl-95% methyl silicone capillary column and a nitrogen phosphorus detector (Hewlett-Packard, Avondale, Penn.). For 10% of the samples, the presence of the carbofuran 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 (Hewlett-Packard, Avondale, Penn.). The GCMS was linked to a 59970 ChemStation computer data system. The detection limit was 0.1 ug/g. Quality assurance was conducted following guidelines established by the United States Fish and Wildlife Service's Patuxent Analytical Control Facility in Laurel, Maryland, USA. We spiked 1 matrix spike sample consisting of control owl feet with 2.1 µg of carbofuran. One method blank and 1 control blank were also analyzed.

We obtained weather data during the foot-weathering period from the United States Department of Agriculture's Beltsville Agricultural Research Center's Weather Station #3 in Beltsville, Maryland, USA. The station was located approximately 3.0 km from the site where the feet were weathered.

Results and discussion

We detected carbofuran from feet that were weathered up to 28 days (Table 1). Residue levels on the control feet were below the level of detec-

Table 1. Carbofuran residues (ppm) on eastern screech owl (*Otus asio*) feet that were weathered outdoors during summer at the USGS Patuxent Wildlife Research Center, Laurel, Maryland, USA.

Treatment	Days weathered	*n*	$\bar{x} \pm$ SD	Range
Control	0[a]	1	<0.1[b]	na
	28	1	<0.1	na
Carbofuran	0[a]	9	10.3 ± 10.5	1.0–32.0
	7	3	14.3 ± 16.5	1.8–33.0
	14	3	1.9 ± 2.6	0.3–4.9
	28	3	12.5 ± 16.0	3.5–31.0

[a] Feet not weathered.
[b] Below level of detection.

tion (0.1 ppm). Quality assurance analysis revealed that the spike recovery was 100% for carbofuran. The 2 blanks contained <0.1µg of carbofuran.

Wildlife forensics laboratories may analyze insecticide residues on feet if they are available and as necessary (Frank et al. 1991, Stroud and Adrian 1996). However, weathered feet may not be submitted to the laboratory by the field investigators and may not be analyzed at the laboratory if the remainder of the carcass appears severely decomposed because insecticide residues may not be expected on these feet. Little is known about the persistence of insecticides on feet of avian carcasses and their reliability as matrices for identifying insecticide exposure to birds. We detected diazinon (*O,O*-diethyl *O*-[2-isopropyl-6-methyl-4-pyrimidinyl] phosphorothioate) and chlorpyrifos (*O,O*-diethyl-*O*-[3,5,6-trichloro-2-pyridyl] phosphorothioate) (applied on lawns) on feet of brown-headed cowbirds (*Molothrus ater*) that were weathered for 1 month (Vyas et al. 2003*b*). Furthermore, decomposed feet of Canada goose (*Branta canadensis*) goslings that were exposed to a diazinon application on lawns provided measurable residues for at least 7 days (Vyas et al. 2004).

We applied 20 ml of carbofuran to our deer pieces to simulate a topical carbofuran application on a large animal carcass bait. Since there is no recommended application rate or method, we based our application on photographs of carbofuran-laced large-carcass baits that depicted carcass hair encrusted with dried, white-colored carbofuran. Based on the viscosity of Furadan 4F®, our application volume allowed complete coverage of the surface of the deer piece such that when dry it would appear similar to the photographs. On large baits, topical applications of carbofuran may involve spreading it over the carcass surface or creating hotspots on the surface. The perpetrators use whatever is at hand to apply the carbofuran and may pour carbofuran on the carcass directly from its container jug. Depending on the size of the carcass and the hotspots, it is estimated that the perpetrators may use 236–738 ml of carbofuran during bait lacing (R. Prieksat, Senior Resident Agent, Division of Law Enforcement, United States Fish and Wildlife Service personal communication; M. Webb, Senior Resident Agent, Division of Law Enforcement, United States Fish and Wildlife Service personal communication). Our application rate, based on the size of our deer piece, is comparable to rates used for lacing.

The owls' feet were in contact with carbofuran for approximately 40 minutes. Large mammals have thick skins, and some raptors may spend >1 hour perching on the carcass while trying to make an opening for feeding (D. Ellis, United States Geological Survey, retired, personal communication). Furthermore, a raptor's feet may be in contact with a contaminated bait for 30–40 minutes while carrying the contaminated bait to a roost tree and then feeding on it (M. Webb, Senior Resident Agent, Division of Law Enforcement, United States Fish and Wildlife Service personal communication). Observations indicate that a golden eagle (*Aquila chrysaetos*) may take 30–60 minutes to consume a black-tailed jackrabbit (*Lepus californicus*; D. Ellis, United States Geological Survey, personal communication).

The wide ranges for carbofuran residue levels from our owls' feet subjected to the same treatments, and the lower carbofuran residues on the feet weathered for 14 days than those weathered for 0, 7, and 28 days, may be artifacts of small sample size. However, the variability in our results show that lack of residues on weathered feet from the field does not imply that the bird was not exposed to an insecticide. The success of detecting an insecticide or its metabolites on feet depends on the method of insecticide application on the bait (e.g., topical application or injection into the bait), chemical concentration on the matrix at the time of contact with feet, exposure time of the feet to the insecticide, amount of contact with uncontaminated surfaces that may wipe the insecticide off the feet, chemical absorption rate into the blood and bait tissue, environmental conditions during weathering, lag time between the raptor mortality and when the feet were collected, and the insecticide's half-life (Allen et al. 1996, Elliot et al. 1996). In general, the persistence of carbofuran in the environment increases under conditions of low moisture, low temperature, low pH, and the lack of suitable microbial degraders (see Eisler 2000). During our weathering period, the maximum and minimum temperatures were 30.2°C ± 2.5°C and 13.3°C ± 3.2°C, respectively, and the maximum and minimum humidities were 101.0% ± 1.0% and 46.5% ± 12.3%, respectively. Precipitation was 4 mm on day 1, 28 mm on day 5, 32 mm on day 6, 38 mm on day 7, 15 mm on day 8, 24 mm on day 12, 1 mm on day 17, 15 mm on day 18, 3 mm on day 21, 2 mm on day 26, and 2 mm on day 27.

Insecticide levels from feet represent chemicals on and in the feet. In general, the residue levels from the feet do not necessarily imply a lethal dermal exposure but are evidence about 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'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.

Carbofuran is used for lacing baits in the United States because of the traditional knowledge within the farming and ranching communities of its high acute toxicity to birds and mammals and because of its availability as an agricultural insecticide (Vyas et al. 2003*a*). State and federal wildlife personnel responding to reports of bird kills regard every incident as a legal case and therefore have focused their efforts on recovering carcasses with analyzable conventional matrices. The loss of the conventional matrices through decomposition and scavenging introduces uncertainty in determining the cause of death and reduces the carcasses to circumstantial evidence of poisoning. Furthermore, due to constraints on resources, field investigators may submit to the laboratory only those carcasses that retain the conventional matrices in a condition that is suitable for analysis. This can result in an underestimation of wildlife losses from insecticide-laced baits, which in turn can influence the prosecution and sentencing of the perpetrators (Vyas et al. 2003*a*).

Our results demonstrate the temporal reliability of carbofuran residues on weathered feet and thereby identify feet as a tool for determining carbofuran exposure after a raptor has been long dead. Carcasses previously not submitted for residue analysis because of lack of the conventional matrices may now be salvaged for their feet.

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Authors' address: Nimish B. Vyas, United States Geological Survey Patuxent Wildlife Research Center, Beltsville Lab, BARC-East Bldg 308, 10300 Baltimore Avenue, Beltsville, MD 20705, USA; e-mail for Nimish: Nimish_Vyas@usgs.gov. James W. Spann is retired.

Nimish B. Vyas is a research biologist whose work focuses on identifying and mitigating human impacts on wildlife. ***James W. Spann*** conducted wildlife research for 45 years, and his work encompassed research on contaminants, wetlands, and population ecology. ***Craig S. Hulse*** has served as a chemist for 29 years with the United States Fish and Wildlife Service and the United States Geological Survey. He is also a visiting scientist with the Environmental Quality Laboratory of the United States Department of Agriculture. ***Sabra Olson*** served as an animal caretaker on the reported study. Currently she is a mother, horse trainer, and an artist in Montana. ***Wayne Bauer*** is an animal caretaker and has been in charge of several captive mammal and avian colonies at the Patuxent Wildlife Research Center. Previously, he was on the husbandry staff of the National Aquarium in Baltimore, Maryland, where he cared for the Neotropical bird and amphibian collections.

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