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# Mortality of Passerines Adjacent to a North Carolina Corn Field Treated with Granular Carbofuran

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ABSTRACT: Red-winged blackbirds (*Agelaius phoeniceus*) were collected during an epizootic in southeastern North Carolina (USA). Activity of brain cholinesterase (ChE) was inhibited by 14 to 48% in three of five specimens, and returned to normal levels after incubation. Gastrointestinal tracts were analyzed for 30 anti-ChE agents. Carbofuran, the only compound detected, was present in all specimens at levels from 5.44 to 72.7 μg/g wet weight. Application of granular carbofuran in an adjacent corn field, results of necropsy examinations, and chemical analyses are consistent with a diagnosis of carbofuran poisoning in these specimens.

*Key words:* Carbofuran, Furadan®, anticholinesterase poisoning, North Carolina, passerines, red-winged blackbirds, *Agelaius phoeniceus*.

Carbofuran (2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) is a widely used insecticide-nematicide with a history of avian mortality after applications apparently in compliance with product labelling and after illegal use (Balcomb, 1983; Balcomb et al., 1984; Flickinger et al., 1986; Stansley, 1993). A review of granular carbofuran's risks to birds resulted in an agreement between the manufacturer and the U.S. Environmental Protection Agency (1991) to eliminate most permitted uses over a 4-yr period. The agreement included a prohibition on use of granular carbofuran in selected areas, including coastal North Carolina (USA), beginning in late 1991. Arguments have been made to ease restrictions established by that agreement due, in part, to a lack of recently reported wildlife mortalities. Our objective is to document a recent mortality incident in southeastern North Carolina.

In March 1992, the U.S. Fish and Wildlife Service (USFWS) was notified of a bird epizootic in Shallotte Point, Brunswick County, North Carolina (33°58′N, 78°23′W). The reporting individual estimated 40 to 50 dead and disabled birds on 26 March 1992 in her yard, which was adjacent to an approximately 7-ha no-till corn-farming operation.

Wildlife law enforcement officers inspected the site on 27 and 28 March and interviewed the person who planted crops on the adjacent farm. Planting was conducted with two tractors on 25 March. The first vehicle carried corn and Furadan® 15G (FMC Corporation, Philadelphia, Pennsylvania, USA) (active ingredient [a.i.] carbofuran) applied at a rate of 6.7 kg product/ha. A second vehicle applied Gramoxone® (ICI Americas Agricultural Products, Wilmington, Delaware, USA) (a.i. paraquat [1,1′-Dimethyl-4,4′-bipyridinium ion]), Bicep® (Ciba-Geigy, Greensboro, North Carolina) (a mix of atrazine [2-chloro-4-ethylamino-6-isopropylamino-s-triazine] and metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide]), and liquid nitrogen. Two officers walked a portion of the farm field and its perimeter. What appeared to be pesticide granules were found in a small depression in the field where planting machinery did not contact the soil. Investigators concluded that products were applied properly and did not constitute an intentional misuse. Brunswick County is within the area where granular carbofuran use was prohibited by the U.S. Environmental Protection Agency's 1991 agreement with the manufacturer; however, geographic restrictions did not apply to granular carbofuran already in the hands of users at the time of the agreement.

During the investigation, 23 dead birds including red-winged blackbirds (*Agelaius phoeniceus*), eastern bluebirds (*Sialia sialis*), American robins (*Turdus migratorius*), and an unidentified finch were found. Fifteen red-winged blackbirds were wrapped in aluminum foil and frozen for analyses.

Seven red-winged blackbirds were sent to the National Wildlife Health Center (NWHC) in Madison, Wisconsin (USA); the five of these which appeared freshest were necropsied on 2 April 1992. All necropsied birds were juvenile females. Perihepatic hemorrhage was seen in specimens 1 and 2; there was no other evidence of infectious disease or trauma. All specimens had moderate to abundant subcutaneous, pericardial, peritoneal, and perirenal fat. Contents of the proventriculi included a black insect in specimen 3 and seed in specimen 4. The gizzards of all specimens contained what appeared to be small seeds and sand.

*Citrobacter freundii, Serratia foniticola,* and *Klebsiella pneumoniae* were isolated from the liver using eosin methylene blue agars (Difco Laboratories, Detroit, Michigan, USA) and API 20E system products (Analytab Products, Division of Sherwood Medical, Plainview, New York, USA); however, no lesions were associated with these isolates and they were determined to be postmortem contamination. No salmonellae were isolated from intestine using selenite broth, xylose lysine desoxycholate, and Brilliant Green agars (Difco Laboratories). Attempts to isolate viruses from lung and trachea, using cell culture methods of Docherty and Slota (1988), were unsuccessful. Sections of heart, lung, trachea, liver, intestine, kidney, adrenal gland, ovary, and spleen were sampled at necropsy and placed in 10% buffered formalin. Paraffin-embedded sections of these tissues were cut at 5 μm, stained with hematoxylin and eosin, and examined microscopically. Focal acute hepatic hemorrhage was confirmed by microscopic examination of livers from specimens 1 and 2 but the remainder of the tissues were unremarkable for the degree of autolysis present. The acute, focal hepatic hemorrhage was consistent with trauma, possibly impact with the ground as a terminal event.

TABLE 1. Brain cholinesterase (ChE) activity (μmoles/min/g), percent ChE-inhibition, and brain ChE activity after 18 hr, 37 C incubation of sample from dead red-winged blackbirds.

| Sample number | Brain ChE activity[a] | Percent inhibition | ChE activity after incubation[a] |
|---|---|---|---|
| 1 | 12.7 (2) ± 0.7 | 48 | 24.6 (2) ± 0.7 |
| 2 | 21.1 (2) ± 0.5 | 14 | 25.4 (2) ± 0.0 |
| 3 | 21.1 (2) ± 0.7 | 14 | 27.7 (2) ± 0.0 |
| 4 | 22.8 (2) ± 1.4 | —[b] | — |
| 5 | 22.3 (2) ± 0.4 | — | — |

[a] Mean (number of replicates) ± standard deviation.

[b] In these samples, brain ChE activity was above the diagnostic threshold, defined as two standard deviations below the mean ChE activity in five reference red-winged blackbirds [24.5 ± 1.1]. Because ChE activity was not inhibited in these samples, they were not incubated to assess recovery.

Brains of the five birds were analyzed for cholinesterase (ChE) activity. Assays were performed in duplicate at the NWHC by the methods of Ellman et al. (1961) as modified by Hill and Fleming (1982). Cholinesterase inhibition was reported if mean ChE activity was below the diagnostic threshold. The diagnostic threshold was defined as two standard deviations below the mean NWHC reference value for five adult red-winged blackbirds of 24.5 ± 1.1 (mean ± standard deviation) micromoles acetylthiocholine hydrolyzed per minute per gram of wet weight brain tissue (μmoles/min/g).

Brain ChE activities of three specimens were below the diagnostic threshold. Inhibition of brain ChE in these ranged from 14 to 48%. Two specimens had no ChE inhibition. Brain ChE activity in inhibited specimens reactivated to levels above the diagnostic threshold after 18 hr incubation at 37 C (Table 1).

Brain ChE depression approached the >50% level considered diagnostic of death by anti-ChE agents (Hill and Fleming, 1982). We therefore analyzed ingesta for anti-ChE agents, particularly carbamates

because of the reversible ChE-inhibition. Because of the limited amount of ingesta, portions of the upper gastrointestinal tract were placed in acid-washed glass jars and frozen for residue analyses. Samples 2 through 5 consisted of the esophagus, proventriculus, gizzard, and their contents from specimens 2 through 5. Two subsamples, consisting of the esophagus and its contents and gizzard contents (subsample 1-1) and the gizzard, proventriculus, and intestine with its contents (subsample 1–2) were taken from specimen 1.

Samples were shipped to the USFWS Patuxent Analytical Control Facility (PACF) in Laurel, Maryland (USA), for analysis of 24 organophosphorus compounds and six carbamates. Active ingredients of pesticides commonly used in the area, such as acephate, chlorpyrifos, diazinon, ethoprop, malathion, methyl parathion, parathion, terbufos, carbaryl, carbofuran, and methomyl, were included in the analytical panel. Analyses were by gas chromatography under PACF standard operating procedures adapted from methods previously described (Belisle and Swineford, 1988).

The lower limit of detection was 0.5 μg/g wet weight for organophosphorus compounds and 1.0 μg/g for all carbamates except methomyl, which had a detection limit of 5.0 μg/g. A procedural blank indicated no background contamination of analytical equipment or reagents. Spike recoveries and results of duplicate analyses were within acceptable ranges for method precision and accuracy.

Carbofuran, the only analyte detected, was detected in all samples from 5.44 to 72.7 μg/g wet weight; its presence was confirmed by mass spectrometry (Table 2). The lethal potentials of these concentrations were evaluated by comparison with published toxicity data. The concentration of carbofuran in a gut sample multiplied by the weight of the gut sample yielded the total extracted carbofuran. This quantity was divided by the body weight of the original specimen to approximate a pesticide dose in the gut. Smith (1987) gives a median lethal dose producing 50% mortality in a test population ($LD_{50}$) from carbofuran for red-winged blackbirds of 0.42 mg/kg body weight. The approximate doses calculated from total extracted carbofuran in our samples were 73 to 980% of this $LD_{50}$ (Table 2). Although the amount of carbofuran extracted from the gut is only a portion of that ingested, the detection and confirmation of a pesticide approaching or exceeding the $LD_{50}$ of the compound for the species is regarded as firm evidence of poisoning (Grieg-Smith, 1991). As little as one granule of a 10% carbofuran formulation has been demonstrated as lethal to passerines in laboratory tests (Balcomb et al., 1984).

TABLE 2. Concentrations (μg/g wet weight) of carbofuran in gastrointestinal tract of dead red-winged blackbirds. The lethal potential is suggested from comparison of the total extracted carbofuran in each sample, relative to body weight of the specimen, with published $LD_{50}$.[a]

| Sample number | Carbofuran in gut (μg/g) | Total carbofuran in gut (μg) | Body weight (g) | Percent of $LD_{50}$[a] |
|---|---|---|---|---|
| 1–1[b] | 33.7 | 50.6 | 45.6 | 260 |
| 1–2[b] | <1 | — | — | — |
| 2 | 7.14 | 12.9 | 41.7 | 73 |
| 3 | 5.44 | 16.3 | 44.1 | 88 |
| 4 | 7.85 | 14.1 | 39.8 | 85 |
| 5 | 72.7 | 167 | 40.7 | 980 |

[a] $LD_{50}$ of 0.42 mg/kg body weight from Smith (1987).
[b] For specimen 1, subsamples were 1–1 (esophagus and its contents and gizzard contents) and 1–2 (gizzard, proventriculus, and intestine with its contents).

In addition to residue confirmation, inhibition of brain ChE by >50% often is considered diagnostic of death by anti-ChE agents (Hill and Fleming, 1982). In this case, brain ChE was depressed between 0 and 48% and the highest concentration of carbofuran was in a bird with no ChE depression. Cholinesterase depression in brains of birds dying from carbamate compounds, such as carbofuran, is known to be reversible by postmortem reactivation (Martin et al., 1981) and is quite variable under laboratory and field condi-

tions (Hill, 1989). This factor is important in diagnosing potential poisoning cases because the time between death and specimen collection often is not precisely known and ChE levels may have returned to normal. In cases of suspected poisoning where ChE levels are not depressed in all or some animals, it is important to consider the case history and necropsy findings. It may be worthwhile to do residue analyses, especially when only a few animals died or were available for examination.

The case history, results of necropsy examinations, and chemical analyses are consistent with a diagnosis of carbofuran poisoning in all analyzed birds. Any modification of the granular carbofuran crop restrictions and geographic prohibitions should address continuing avian mortalities from ingestion of this product.

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