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Winter Poisoning of Coyotes and Raptors with Furadan-Laced Carcass Baits

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ABSTRACT: Three bald eagles (*Haliaeetus leucocephalus*), a red-tailed hawk (*Buteo jamaicensis*), and two coyotes (*Canis latrans*) found in a field in north-central Kansas (USA) in December 1992 were poisoned by flowable carbofuran (Furadan® 4F) placed on sheep (*Ovis aries*) carcasses to kill coyotes. The carbofuran was placed on the carcasses in October 1992, but the coyotes and raptors apparently were killed in late December. Thus, flowable Furadan® can cause direct and secondary deaths of wildlife under some circumstances for at least 60 days following placement.

Key words: Carbofuran, Furadan®, *Haliaeetus leucocephalus, Buteo jamaicensis, Canis latrans*, winter.

Carbamate and organophosphate insecticides can cause mortality in wildlife when applied as recommended, when used improperly, and when used illegally as a means to control predators. In particular, both accidental and intentional poisoning of wildlife with all forms of the insecticide carbofuran have been well documented (Mineau, 1993). Here we present a case of the illegal use of flowable carbofuran (Furadan® 4F, EPA-279-2876-ZA, FMC Corporation, Agricultural Chemicals Division, Philadelphia, Pennsylvania, USA). The carbofuran was placed on sheep (*Ovis aries*) carcasses, and was intended to kill coyotes (*Canis latrans*), but subsequently also killed birds of prey. We are not aware of other documented cases of intentional poisoning with the flowable form.

On 26 December 1992, the Kansas Department of Wildlife and Parks (KDWP) received a report of a dead bald eagle (*Haliaeetus leucocephalus*) in a field of harvested corn in Republic County in north-central Kansas (USA) (39°48′N, 97°53′W). On 26 and 28 December, KDWP law enforcement officers found 67 sheep carcasses and approximately 2100 live sheep, and the carcasses of two coyotes, one red-tailed hawk (*Buteo jamaicensis*), two immature bald eagles (one male, one female), and one adult female bald eagle in the 106-ha field. Two sheep carcasses that appeared to have been cut in several places, all of one coyote and parts of the other, the red-tailed hawk, and the eagles were collected by L. Thompson and other law enforcement officers. Their primary concern in this case was to document the poisoning of an endangered species (the bald eagles), a migratory bird (the red-tailed hawk), and a possible violation of label directions under the U.S. Federal Insecticide, Fungicide, and Rodenticide Act. Therefore, the raptor carcasses, sheep muscle tissue and wool samples, and the stomach removed from one of the coyotes in the field were sent to the National Fish and Wildlife Forensics Laboratory in Ashland, Oregon (USA) for necropsy and pesticide analyses. Additionally, for a quick response, one sheep carcass, one coyote carcass, and the remaining portions of the field-dissected coyote were delivered to the Veterinary Diagnostic Laboratory at Kansas State University in Manhattan, Kansas.

On 4 January 1993, the carcass of an adult male bald eagle was found approximately 6 km north of the site where the raptors, coyotes, and sheep had been found earlier (at 39°53′N, 97°53′W). That

carcass also was sent to the National Fish and Wildlife Forensics Laboratory for necropsy examination.

The coyotes necropsied at the Veterinary Diagnostic Laboratory at Kansas State University were judged to be in good body condition because of the presence of a small amount of fat in the coronary groove of the heart, in the perirenal area, and in the mesentery. There were no signs of trauma or significant gross lesions. The stomach of the whole coyote contained muscle tissue and wool.

The sheep carcass had white powder on the wool along the back, shoulder, hip, and left front leg. It appeared that the front left leg had been cut in the axillary region and reflected. A white powder was present on the intercostal muscles. The internal organs of the sheep had been removed by scavengers. Samples of the sheep's wool, the stomach contents from the whole coyote, and the liver from the coyote that had been partly field dissected were sent to the Animal Health Diagnostic Laboratory at Michigan State University, E. Lansing, Michigan (USA), for analyses not possible at Kansas State University. The stomach contents and liver were analyzed by gas chromatography-mass spectrometry (GC/MS) using modifications of the procedure described by Schock and Braselton (1982). Five g and 20 g aliquots of stomach contents and liver, respectively, were placed in a blender with 300 ml of non-spectro grade acetonitrile (Burdick and Jackson, Muskegon, Michigan) and 1 ppm diphenylamine (Mallinkrodt, St. Louis, Missouri, USA) as an internal standard, and blended. The blended samples were suction-filtered through a Whatman #4 filter (Whatman LabSales, Hillsboro, Oregon), and the filtrate was transferred to a 1 l separatory funnel (Kimble Science Products, Vineland, New Jersey, USA) containing 10 ml of saturated sodium chloride and 600 ml of deionized water. The aqueous phase was extracted two times with 100 ml each of methylene chloride (Burdick and Jackson) and the methylene chloride fractions were combined. The aqueous mixture was adjusted to pH 9, and reextracted twice with methylene chloride, which was combined with the previous methylene chloride fractions (neutral-basic fraction). The neutral-basic fraction was evaporated to approximately 1 ml, diluted to 4 ml with cyclohexane (Burdick and Jackson) : methylene chloride (85:15), eluted through a gel permeation column to remove lipids, and evaporated to 100 µl. The neutral-basic fraction was injected into a Hewlett-Packard model 5970MSD (Hewlett-Packard Company, Palo Alto, California, USA) gas chromatograph-mass spectrometer equipped with a 15 m × 0.25 mm DB5 bonded phase capillary column (J&W Scientific, Folsom, California, USA). Individual, unknown mass spectra were compared to a computer mass spectral library, and carbofuran was qualitatively detected in both samples. Carbofuran was confirmed by GC/MS analysis of reference carbofuran under the same conditions. Approximately 0.1 g of the sheep wool was extracted with 5 ml methanol (Burdick and Jackson), and 2 µl were analyzed by solids probe insertion into a TSQ70 mass spectrometer (Finnigan MAT, San Jose, California). A 70 eV electron-impact mass spectrum of carbofuran was obtained and matched with that of the reference compound. The finding of carbofuran in all of the samples was considered to be diagnostically significant, so the carbofuran was not quantified.

All of the eagles were in a good state of preservation when found. At death, the immature eagles and the adult female bald eagle had abundant mesenteric and subcutaneous fat. The adult male found on 4 January also was in good body condition. The red-tailed hawk was an immature female with moderate mesenteric and subcutaneous fat deposits. There were no external or internal lesions in any of the birds suggestive of trauma, gunshot, or electrocution. Radiographic examination of the birds resulted in no significant findings.

A subsample of the gastrointestinal tract

contents from each of the raptors was examined by chemical analysis for the presence of carbamates by high pressure liquid chromatography (HPLC) with postcolumn derivatization (EPA Method 531.1, Graves, 1989). Compounds not visible to the UV detector must be derivatized, that is, combined with a visible compound, to be analyzed. The carbamates (N-methylcarbamates or oxime carbamates) were separated by reverse phase chromatography under gradient conditions. The separated carbamates were hydrolyzed at 100 C by sodium hydroxide and then derivatized with opthaladehyde (OPA) and Thiofluor™ (Pickering Laboratories, Mountain View, California, USA) to produce a highly fluorescent isoindole (Dong et al., 1991, 1992). A fluorescent detector then was used to detect the derivatized carbamate.

Ten grams of each sample were extracted with 75 ml of ethyl acetate (Optima grade, Fisher Scientific, Santa Clara, California) with 5% ethanol and 30 g of sodium sulfate (reagent grade, Fisher Scientific) for 30 min. The sample was then centrifuged at 1000 × *G* for 10 min. A 20 ml aliquot was removed, and three drops of 5% decanol (Kodak Corporation, Rochester, New York, USA) in acetone (Optima grade, Fisher Scientific) were added. The extract was evaporated under a stream of nitrogen. The samples then were reconstituted with 970 µl of 5% ethanol in ethyl acetate and 30 µl of BDMC (4-bromo-3,5-dimethylphenyl N-methyl carbamate) internal standard (ChemService, West Chester, Pennsylvania). The samples were analyzed by a Hewlett Packard 1090 HPLC with a Hewlett Packard 1046A programmable fluorescence detector (Hewlett Packard Company, Palo Alto, California) and a Pickering PCX 5000 postcolumn derivatization system (Pickering Laboratories). Ten microliters of the samples were injected onto a Pickering reverse phase C18 analytical column (250 mm × 4.6 mm I.D., 5 µm particle size). Separation of the carbamates was accomplished with a 42-min linear gradient of 75% water and 25% methanol (Optima grade, Fisher Scientific) to 25% water and 75% methanol at a flow rate of 1.0 ml/min at 42 C.

A 10 g subsample of meat from the coyote stomach sent to the National Fish and Wildlife Forensics Laboratory contained meat from sheep, identified by reaction to ovine antisera (Organon Teknika Corporation, West Chester, Pennsylvania) (Ouchterlony, 1949). It also contained 124 µg (12.4 ppm) of carbofuran.

The gastrointestinal tract contents of all of the eagles included meat from sheep, identified by reaction to ovine antisera. The crop of the immature male eagle contained 166 g of freshly ingested meat, which contained 5.4 ppm carbofuran. The gastrointestinal tract contents of the immature female included 70 g of freshly ingested meat containing 4.8 ppm carbofuran. The tract from the mature female included 196 g of freshly ingested meat containing 1.1 ppm carbofuran. Carbofuran was not detected in the gastrointestinal tract from the adult male found on 4 January, but it contained only 7 g of material. The cause of death of that eagle was undetermined.

The gastrointestinal tract of the red-tailed hawk contained the remains of the head and the foot of a European starling (*Sturnus vulgaris*) and the foot of a rabbit (*Sylvilagus floridanus*) totaling 51 g, and had a carbofuran concentration of 27.1 ppm. It contained no meat identified as sheep. We suspect that the hawk's death was a case of secondary poisoning, the hawk having fed on a starling that had been killed or intoxicated by the carbofuran. Secondary poisonings with carbamate and organophosphate pesticides are well documented (Henny et al., 1987).

Diagnosis of carbamate poisoning based only on residue data is reasonable because spontaneous postmortem reactivation of brain acetylcholinesterase (AChE) confounds AChE activity interpretation (Greig-Smith, 1991). In this instance, confirmation of carbofuran in the stomach of the coyote and in the crops of the raptors was considered by the U.S. Fish and Wild-

life Service to be sufficient to pursue the legal case, so brain cholinesterase analyses were not necessary. However, the acute oral median lethal single-exposure dosage (LD_{50}) for carbofuran in wild birds ranges from 0.2 to 7 mg/kg of body mass (Schafer et al., 1983; Hill and Camardese, 1984; Hudson et al., 1984). The oral LD_{50} for technical grade carbofuran ranges from 0.238 to 8.0 ppm for bird species studied to date, but for most species studied it is below 2.0 ppm (Mineau, 1993). Therefore, we believe that the deaths of three of the eagles and the red-tailed hawk were due to ingestion of carbofuran.

As a result of the investigation by the KDWP and the USFWS, two people admitted having placed Furadan® 4F on two of the sheep carcasses in the field to kill problem coyotes. The owner stated that the sheep had been had been turned out into a harvested cornfield in October. Soon afterward they began experiencing losses in the flock, and considered predation by coyotes to be part of the cause. The responsible parties testified that it was at that time, and only at that time, that they baited two of the dead carcasses with Furadan®. Cold, dry weather and snow cover during some of the time between the placement of the Furadan® on the carcasses and the wildlife poisonings likely contributed to its preservation on the carcasses. Based on the fresh condition of their carcasses and the fact that they had not been reported earlier, we believe that the coyotes and the raptors were killed not long before they were noticed in December; this is 2 mo after the carbofuran was applied to the sheep carcasses.

Many factors affect carbofuran degradation, but it was considered by Eisler (1985) to have a relatively short life in the environment. Smith (1987) stated that it is moderately persistent. There are several possible explanations for the persistence of the carbofuran in this case. Its decomposition is closely related to pH, with slow breakdown at pH below 7, and temperature, with slower degradation at lower temperatures (Eisler, 1985). The pH of the tissues on which the Furadan® was applied may have been low enough to slow degradation of the chemical, and ambient temperatures were low. Bacteria believed to degrade carbofuran (Felsot et al., 1981) may not have been on the carcasses or may have been affected by the cold. Most likely, however, is that because carbofuran is degraded mainly through hydrolysis, the dry white residue was stable and the granular form is stable (Mineau, 1993). Based on this case, residue from the flowable Furadan® lasted at least 2 mo and was a continued hazard to wildlife through both direct and secondary poisoning. The legal case was taken to federal court where, in a pre-trial diversion, the responsible parties agreed to pay a $12,000 penalty for their unlawful actions.

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