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Abstract: In 1989 and 1990, 58 sandhill cranes (*Grus canadensis*) were collected along the central Platte River in Nebraska during a study of mortality caused by powerline impact. Brains were assayed for acetylcholinesterase activity; gut contents were analyzed for residues of 25 organophosphate and 6 carbamate compounds; and livers were analyzed for 20 inorganics (including lead, mercury, and boron) and 22 organochlorine chemicals. Brain acetylcholinesterase activities appeared to be within normal ranges, and no measurable organophosphate or carbamate residues were found in the gut contents of 5 birds with the lowest brain enzyme activities. Heptachlor epoxide, oxychlordane, p,p'-DDE, and hexachlorobenzene were detected in livers. Inorganics were generally below concern levels and were similar to levels found in a previous study of greater sandhill cranes in the Rocky Mountain population. Applications of these data to other investigations of contaminants in sandhill cranes are also discussed.

Key Words: acetylcholinesterase, boron, carbamate, contaminant residues, DDE, *Grus canadensis*, lead, Nebraska, organophosphate, sandhill crane

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More than 80% of the world's sandhill cranes (approximately 500,000 individuals) stage annually along the Platte River of central Nebraska during spring migration to accumulate fat and nutrient reserves before completing the flight to their nesting grounds (U.S. Fish and Wildlife Service 1981). During this concentrated staging time, some of the cranes collide with powerlines adjacent to the river while returning to or leaving their riverine roosts at night or in the morning. To evaluate the magnitude of this problem, the Wyoming Cooperative Fish and Wildlife Research Unit (WCFWRU), University of Wyoming, conducted a study of crane mortality caused by powerline impact (Morkill and Anderson 1991). They collected dead cranes found under the powerlines for morphometric measurements and sex determination.

Because cranes concentrate in the area, and intensive conventional agriculture is the primary land use in the Platte River system, we analyzed crane carcasses found during the mortality study for contaminant residues. Presently the literature contains little information about contaminants in sandhill cranes. Only 3 pertinent studies have been published (Mullins et al. 1979, Wallace et al. 1983, Windingstad et al. 1984). More than 10 years ago, Mullins et al. (1979) studied the Rocky Mountain population of sandhill cranes to determine residue levels for organochlorines, lead, and mercury in eggs and tissues. Since that time, use of organochlorine pesticides has markedly decreased, but use of organophosphate and carbamate pesticides has increased. Wallace et al. (1983) and Windingstad et al. (1984) studied the incidence and effects of lead shot in sandhill cranes. Lead shot has been banned for waterfowl hunting, but reworking of river sediments by streamflow and the sandhills' habit of probing wet meadows for invertebrates may expose them to older lead shot through ingestion.

Nearly 1.8 million kg of organophosphate (OP) and carbamate pesticides are applied annually to corn in Nebraska alone (Johnson and Kamble 1984). In addition, fungicidal treatments of seed corn frequently are based on arsenic or mercury compounds. Corn is by far the major crop grown in the stopover habitat of the crane, and waste corn left from the previous harvest is a major food source.

Because of the correlation between OP and carbamate pesticides and depressed enzyme activity in the brain, we also determined the acetylcholinesterase activity of the brain tissue and attempted to correlate it with OP and carbamate levels in the gut contents of cranes.

Inorganic residues in livers from 17 birds, organic compound residues in livers from 8 birds, brain acetylcholinesterase levels in 57 birds, and organophosphate and carbamate levels in the gut contents from 5 birds were obtained.

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STUDY AREA AND METHODS

The study area for this contaminant investigation, detailed by Morkill and Anderson (1991), lies 3.2 km north and 1.6 km south of the Platte River, 60 km eastward from the town of Overton to Gibbon in southcentral Nebraska (Fig. 1). The area included portions of Dawson, Buffalo, and Kearney counties. Habitats of importance to sandhill cranes in the area are cropland (primarily corn), wet

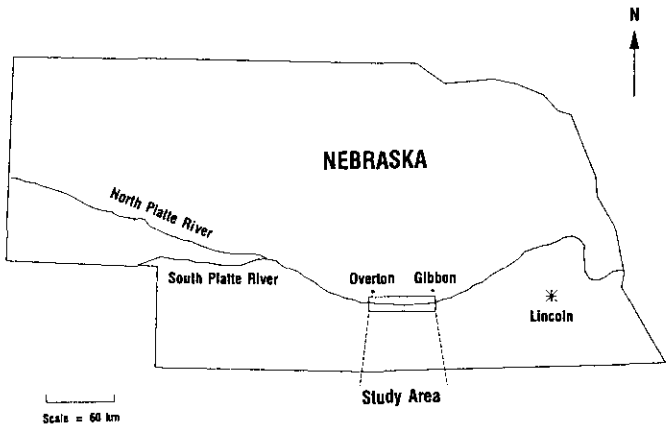

Fig. 1. Study area from which sandhill crane carcasses were collected for contaminants analysis, 1989–90.

meadows adjacent to the Platte River, and unvegetated sandbars and shallow areas along open reaches of the Platte River.

Nine powerline segments were under observation in the study area. During spring of 1989 and 1990, all of the segments were searched daily in order to find carcasses within 24 hours after death. This quick retrieval of carcasses in conjunction with generally cool spring temperatures minimized postmortem decay. Upon notification of retrieved carcasses, we performed an external examination of each bird, which included taking photographs and noting any external lesions or other abnormalities. During necropsy, brain, gut contents, and liver tissues were collected from each bird. The specimens were placed in labeled chemically clean glass jars, put on ice, and frozen (−18 C) within 6 hours. Samples were excised and frozen generally within 36 hours of death.

Whole brains were excised in 1989 but were not analyzed until spring of 1990. Because Hill (1989) found that brain enzyme activity decreased with time in frozen storage, brains taken in 1990 were excised, then halved. One half-brain from each bird taken in 1990 and whole brains from birds collected in 1989 were analyzed in spring of 1990. The remaining half-brains from 1990 samples were analyzed in 1991 to determine if activity did in fact decrease during a year of frozen storage. Brain acetylcholinesterase activity was determined by the University of Illinois College of Veterinary Medicine using the modified Ellman method (Ellman et al. 1961). In total, 57 cranes were analyzed for brain acetylcholinesterase.

Gut contents from the 5 birds having the lowest brain acetylcholinesterase activities were analyzed by the Patuxent Analytical Control Facility (PACF), U.S. Fish and Wildlife Service, Laurel, MD, for OP and carbamate residues. The sample was homogenized, then mixed with acetone and methylene chloride to separate the pesticides from the tissue. The organic extract was filtered and adjusted to volume prior to gas chromatography (GC) by using a flame photometric detector for OP determinations and a nitrogen/phosphorus detector for carbamate determinations. Megabore capillary columns were used for the GC separations.

Liver specimens were analyzed for inorganics at Research Triangle Institute, Research Triangle Park, NC, or Environmental Trace Substances Research Center, Columbia, MO. Tissue for mercury determination was prepared by homogenization and lyophilization with subsequent nitric acid digestion and analysis by cold vapor atomic absorption spectroscopy. Liver samples for selenium, arsenic, and lead determinations were homogenized, lyophilized, and heated with nitric acid before analyses with graphite furnace atomic absorption spectroscopy. Liver specimens for determination of other inorganics were homogenized, lyophilized, heated in nitric acid, and preconcentrated by evaporation in a microwave oven before analyses with inductively coupled plasma emission spectroscopy. All chemical analyses passed intralaboratory quality control/quality assurance (QC/QA) procedures, and interlaboratory QA procedures of PACF.

Liver specimens were also analyzed for organochlorine compounds at Mississippi State Chemical Laboratory, Mississippi State, MS. Samples were ground with acetonitrile and extracted 3 times with hexane, and the organic extract was concentrated under nitrogen. The concentrated extract was cleaned and fractionated through a florisil mini-column before high performance liquid chromatographic analyses of the fractions.

RESULTS AND DISCUSSION

Brain acetylcholinesterase activities in the 57 cranes (Table 1) were within expected normal activity ranges (K. Harlin, pers. commun., Hill 1988). A 2-tailed *t*-test of the half-brains analyzed the year of excision and their correspondent halves analyzed after 1 year of frozen storage showed no significant difference in enzyme activity ($P > 0.05$) due to frozen storage (Table 1).

No detectable residues of organophosphate or carbamate pesticides were found in gut contents of the 5 sandhill cranes with the lowest measured brain acetylcholinesterase activity. The detection limit of the organophosphorus compounds measured (acephate, azinphos-methylyl, chlorpyriphos-dursban, coumaphos, demeton, diazinon, dichlorvos, dicrotophos, dimethoate, disulfoton, dursban, EPN, ethoprop, famphur, fensulfothion, fenthion, malathion, methamidophos, methyl parathion, mevinphos, mono-

Table 1. Brain acetylcholinesterase (AChE) activity (μmoles/g/min) in brains of 57 sandhill cranes from the Platte River, Nebraska, 1989–90.[a]

Mortality dates	Processing year	$\bar{x}$	SD	Range	*n*
13 Mar–5 Apr 1989	1990	20.7	1.43	16.84–23.51	23
12 Mar–24 Apr 1990	1990	23.1[b]	3.24	14.26–27.84	34
12 Mar–24 Apr 1990	1991	21.8[b]	2.91	15.13–27.39	34

[a] Analyses were generally completed within 2 weeks of receipt by the College of Veterinary Medicine, University of Illinois.
[b] No significant difference ($P > 0.05$) between AChE activities in halves of the same brains processed shortly after mortality and halves processed 1 year later.

crotophos, parathion, phorate, terbufos, trichlorfon) was 0.05 μg/g wet weight (WW). The detection limit for carbamates (aldicarb, carbaryl, carbofuran, methiocarb, and oxamyl) was 1 μg/g WW, except for methomyl, which had a detection limit of 3.8 μg/g WW.

Most inorganic residues in liver were uniformly below present levels of concern to the U.S Fish and Wildlife Service (Tables 2 and 3). However, residues of lead and boron warrant further discussion.

The maximum lead residue of 55 μg/g dry weight (DW) (Table 3) is equal to a wet-weight concentration of 20.4 μg/g. This level is greater than that found in livers of 11 species of birds diagnosed with plumbosis (Kendall and Scanlon 1985). The crane from which the sample was excised was not found by WCFWRU personnel, but rather was an incidental sample from a feeding ecology study conducted in the same area by the Iowa Cooperative Fish and Wildlife Research Unit (Davis 1991). The birds in the feeding ecology study were collected by shooting them with a rifle; it is possible that the bullet contaminated internal fluids of the crane, and that some of this fluid in turn contaminated the liver specimen.

In general, lead residues found in sandhill crane livers in this study compare with those found in sandhill cranes by Mullins et al. (1979). The range of lead residue levels they found in livers was 0.31–43.8 μg/g WW; we found residues from <0.03 μg/g WW (<0.1 μg/g DW) to 20 μg/g WW (55 μg/g DW) (Table 3). Only 2 lead residue values in our study were >0.3 μg/g DW: 2.6 μg/g DW and 55 μg/g DW (Table 3).

The maximum boron concentration detected in liver (2.42 μg/g DW, Table 2) was near a level (3 μg/g DW, Hoffman et al. 1990) found in female mallard ducklings that exhibit delayed growth, reduced growth rate, and

Table 2. Inorganic residues (μg/gram dry weight) quantified by inductively coupled plasma emission spectroscopy for liver samples of 17 sandhill cranes from the Platte River, Nebraska, 1989–90.

Residue	Al	Ba	Be	B	Cd	Cr	Cu	Fe	Mg	Mn	Mo	Ni	Ag	Sr	Sn	Tl	V	Zn
N_d/N_t[a]	2/17	2/17	1/17	8/17	11/17	8/17	17/17	17/17	17/17	17/17	17/17	1/17	0/17	13/17	0/8	0/9	2/17	17/17
Conver.[b]	log10	log10	log10	log10	normal	log10	normal	normal	normal	normal	normal	log10	normal	log10	normal	log10	log10	normal
$\bar{x}$	3.17	0.09	0.04	1.22	0.31	0.42	7.72	1,379	569	8.49	2.13	0.40	1.24	0.17	2.75	4.50	0.14	94.65
SD	2.19	2.03	2.01	1.32	0.21	1.39	1.99	614	88	1.99	0.76	1.58	0.26	2.46	0.00	1.00	1.45	21.30
Max.	14.00	0.59	0.45	2.42	0.80	0.51	11.10	2,850	734	12.20	3.93	1.27	1.50	0.81	2.75	4.50	0.44	125.00
Min.	1.50	0.05	0.03	1.00	0.05	0.14	4.40	631	348	5.40	1.00	0.25	1.00	0.05	2.75	4.50	0.10	53.20

[a] N_d indicates the number of samples with detectable concentrations, N_t the total number of samples analyzed. For statistical analyses samples having concentrations below detection limits were assigned a value equal to one-half the detection limit.
[b] Conversion: log10 indicates that the sample distribution was non-normal and the mean is a geometric mean, and normal indicates that the mean is an arithmetic mean of a normal sample distribution.

behavioral effects (reduced standing and bathing time), and also in mallard ducklings exhibiting similar behavioral changes (increased resting time and reduced bathing time).

Mercury residues in livers from this study (Table 3) were within the range of those found by Mullins et al. 1979. The range of mercury residue levels that those researchers found in livers was 0–1.63 μg/g WW; we found residues from nondetectable concentrations (about 0.005 μg/g WW) to 0.079 μg/g WW (0.28 μg/g dry weight in Table 3).

Seven of the 8 sandhill crane livers analyzed in this study had detectable organochlorine residues (Table 4). In contrast to the findings of Mullins et al. (1979), no residues of p,p'-DDT were found. This is gratifying because DDT has not been used legally for over a decade, and one would expect residues in populations to be declining. The other organochlorines that Mullins et al. (1979) investigated in their study were dieldrin and p,p'-DDE. Dieldrin was not detected in liver samples in the present study. Residues of p,p'-DDE in the present study ranged from nondetectable (<0.01 μg/g WW) to 0.03 μg/g WW. These values do not exceed the range of p,p'-DDE residues found by Mullins et al. (1979) (i.e., 0.007–0.461 μg/g WW), or concentrations of p,p'-DDE (0.07 and 0.08 μg/g WW) found in livers of 2 whooping cranes (Lamont and Reichel 1970).

Contaminant residue information gathered from these powerline-induced mortalities indicates that sandhill cranes staging in southcentral Nebraska during spring migration appear to have relatively low levels of contaminants, and only lead and possibly boron are present in appreciable quantities. However, not every potential environmental contaminant that could affect sandhill cranes was included in tissue analyses, and further analyses may be appropriate depending on probable exposure. These additional analyses of crane tissues will help define the overall contaminant profiles and possible biological effects in the sandhill crane populations migrating through Nebraska.

Applications of data such as those obtained in this study may be used (1) to build a database of residue levels in this sample of sandhill cranes for extrapolation to the Great Plains population, (2) to determine the distribution of residue values in this sample of the population for extrapolation to the entire population, (3) to extrapolate the residue levels in sandhill cranes to the population of endangered whooping cranes which use the same habitats in spring and fall, and (4) to determine correlations of residue levels among tissues so that residue levels in living birds may be estimated without sacrificial sampling.

Table 3. Inorganic residues (μg/gram dry weight) quantified by atomic absorption spectroscopy for liver samples of 17 sandhill cranes from the Platte River, Nebraska, 1989–90.

Residue	As	Pb	Se	Hg
N_d/N_t[a]	1/17	11/17	17/17	14/17
Conversion[b]	normal	log10	normal	log10
$\bar{x}$	0.11	0.20	3.62	0.03
SD	0.06	5.60	0.86	2.61
Max.	0.20	55.00	5.77	0.28
Min.	0.05	0.05	2.00	0.01

[a] N_d indicates the number of samples with detectable concentrations, N_t the total number of samples analyzed. For statistical analyses samples having concentrations below detection limits were assigned a value equal to one-half the detection limit.

[b] Log10 indicates that the sample distribution was non-normal and the mean is a geometric mean, and normal indicates that the mean is an arithmetic mean of a normal sample distribution.

Table 4. Organochlorine residues (μg/gram wet weight) in livers of 8 sandhill cranes from the Platte River, Nebraska, 1989–90.

Liver sample	Moisture (%)	HCB	Oxy-chlordane	Heptachlor epoxide	p,p'-DDE
1A	74.5	ND[a]	ND	ND	0.01
2A	74.5	ND	ND	0.06	0.01
4A	73.5	ND	ND	0.15	0.03
5A	73.5	ND	0.02	0.36	0.01
5B	74.5	ND	ND	0.05	ND
5C	72.5	ND	ND	ND	ND
5D	74.0	ND	ND	0.06	0.01
5E	77.0	0.01	ND	ND	ND

[a] ND = not detected at a detection limit of 0.01 μg/g wet weight. α-BHC, Γ-BHC, ß-BHC, s-BHC, Γ-chlordane, toxaphene, t-nonachlor, total PCB's, α-chlordane, dieldrin, o,p'-DDE, o,p'-DDD, endrin, o,p'-DDT, p,p'-DDD, p,p'-DDT, mirex, and *cis*-nonachlor were also undetected at the same limit of 0.01 μg/g wet weight.

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