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MONITORING EXPOSURE OF NESTLING SONGBIRDS TO AGRICULTURAL APPLICATION OF AN ORGANOPHOSPHORUS INSECTICIDE USING CHOLINESTERASE ACTIVITY

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Abstract—In June 1992 we collected 53 blood plasma samples from nestling red-winged blackbirds (*Agelaius phoeniceus*), house sparrows (*Passer domesticus*), and brown-headed cowbirds (*Molothrus ater*) at five study sites in northwestern Minnesota to evaluate the feasibility of using plasma and brain cholinesterase (ChE) activity and reactivation as a means of assessing exposure of nontarget organisms to the operational use of ChE- inhibiting insecticides in an agricultural setting. Three sites were adjacent to sugar beet fields that were likely to be treated with chlorpyrifos as Lorsban® (an organophosphorus [OP] insecticide) for control of sugar beet root maggot (*Tetanops myopaeformis*), and two sites were distant from fields likely to be treated (reference sites). Application of chlorpyrifos in fields surrounding study plots was monitored through contact with landowners and direct observations. Cholinesterase activity levels (total cholinesterase [ChE], acetylcholinesterase [AChE], and butyrylcholinesterase [BChE]) in nestling plasma were measured and tested for reactivation (ChE and AChE) in the presence of 2-PAM, an indication of exposure to an organophosphorus insecticide. In addition, 11 nestlings were euthanatized and in these samples we measured brain ChE activity and reactivation, and we analyzed gastrointestinal tracts and carcass washes for chlorpyrifos residues. Total ChE and BChE activity were lowest in similar-aged nestlings at sites adjacent to treated beet fields (ChE, $t = -2.51$, $d.f. = 21$, $p = 0.033$; BChE, $t = -2.56$, $d.f. = 21$, $p = 0.043$). Nestlings from sites that were near fields where chlorpyrifos was applied were more likely to exhibit plasma AChE reactivation than nestlings from reference sites where OP or carbamate insecticide application was improbable ($\chi^2 = 3.805$, $d.f. = 1$, $p \sim 0.05$). The magnitude of plasma ChE reactivation was highest within 1 to 3 d of insecticide application, although significant reactivation was measured up to 11 d after application of chlorpyrifos. Plasma AChE reactivation in the presence of pyridine-2-aldoxime methochloride (2-PAM) appeared to be a more sensitive indicator of exposure to chlorpyrifos than plasma ChE or BChE activity levels.

Keywords—*Agelaius phoeniceus* Chlorpyrifos Cholinesterase Organophosphorus 2-PAM reactivation

INTRODUCTION

Considerable field and laboratory research has focused on the lethal and sublethal effects of cholinesterase-inhibiting compounds to nontarget vertebrates, especially birds [1–6]. Although a number of avian die-offs have been attributed to exposure to these compounds [7–9], the extent to which migratory birds are exposed to operational applications of these compounds is not well documented. In the United States organophosphorus (OP) and carbamate insecticides are used on a wide variety of crops including cotton, white and wild rice, potato, sunflower, field corn, and winter wheat [10–12; Minnesota Department of Agriculture, unpublished data]. Smith [10] estimated that from 1981 to 1987, 160 million acre-treatments of OPs and carbamates were applied annually to agricultural crops and forests in the United States. Because a large total area is treated annually with these chemicals the potential for exposure of nontarget species to OP and carbamate insecticides is high. However, only a few researchers have focused on methodologies for monitoring the exposure of wildlife to OPs and carbamates [13–18].

There are a large number of OP and carbamate compounds [10] and most inhibit cholinesterase (ChE) enzymes [19]. Direct measurement of OP and carbamate insecticide residues from operational applications is difficult and often impractical because these insecticides are quite labile in biological tissue and a large number of compounds are in use. However, measurement of ChE activity or ChE inhibition in brain tissue or blood plasma of birds can be used as an indicator of exposure to ChE-inhibiting insecticides [1,18,20].

We used ChE activity [20,21] and ChE reactivation [17,18,22] to evaluate the exposure of nestling birds to chlorpyrifos (0,0,-diethyl 0-(3,5,6-trichloro-2-pyrdinyl)phosphorothioate) applied as Lorsban® to sugar beet fields in northwestern Minnesota. We collected blood samples from red-winged blackbird (*Agelaius phoeniceus*), house sparrow (*Passer domesticus*), and brown-headed cowbird (*Molothrus ater*) nestlings from nests near sugar beet fields. Nestlings were selected as subjects for this study because their exposure to insecticides is related to events in the vicinity of nests. Our goal was to evaluate the feasibility of using plasma and brain ChE activity and reactivation as a means of assessing exposure of nontarget organisms to the operational use of ChE-inhibiting insecticides in an agricultural setting.

METHODS

Study site selection

We conducted this study in 1992 in Norman County, in northwestern Minnesota, where there was a high probability of a

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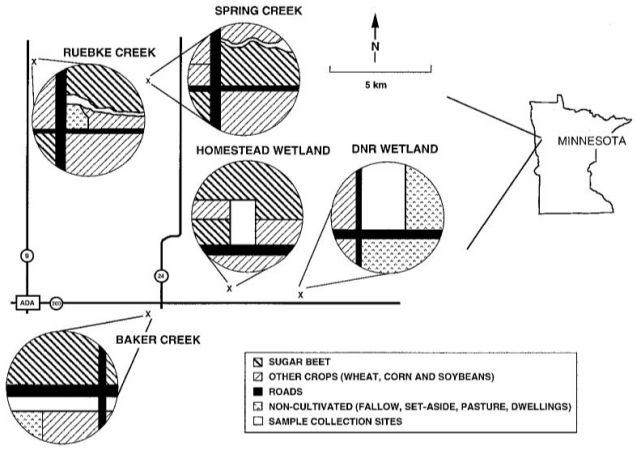

Fig. 1. Location of the study site and land-use patterns at sample collection sites in Norman County, Minnesota, June 1992.

sugar beet root maggot (*Tetanops myopaeformis*) (Rder.) outbreak (D. Berglund, personal communication). The OP insecticide Lorsban® (Dow Elanco, Indianapolis, IN, USA) is commonly applied to control this pest. All field sites were located in Norman County, within 18 km of the town of Ada (Fig. 1). We identified possible study sites through evaluation of maps of the area and by driving roads searching for sites close to sugar beet fields where red-winged blackbird were present and likely to nest.

Reference sites

DNR Wetland. This wetland was located on a 64-ha wildlife area managed by the Minnesota Department of Natural Resources (DNR) in Section 12 of Lake Ida Township. The wetland comprised the majority of the site, but was bordered by shrubby and grassy vegetation. Agricultural land near this site included grass and hay fields, fallow land with minimal vegetation (set aside), and wheat fields. There were no sugar beet fields or other crops commonly treated with OP insecticides or carbamates adjacent to or within 300 m of this wetland. We visited 25 red-winged blackbird nests at the southern end of the wetland.

Ruebke Creek. Ruebke Creek was located in the southwest corner of Section 10 in Pleasant View Township and comprised approximately 1 ha of land dominated by sedges and grasses, with a shallow creek winding through it. A sugar beet field (within 100 m of the monitored nests) was north and adjacent to the creek and to the southwest across a paved road was another large beet field (~80 ha); the other adjacent fields were planted in wheat. One house sparrow and five red-winged blackbird nests were monitored at this site: red-winged blackbird nests were constructed in cattails (*Typha* spp.) or small shrubs (<3 m tall). The sugar beet field north of Ruebke Creek was not treated with chlorpyrifos during this study.

Treatment sites

Baker Creek. This site was located in Section 13 of McDonaldsville Township, including part of Judicial Ditch Number 51. The ditch was a shallow, channelized waterway flowing westward, parallel to State Highway 200. A cornfield and cow pasture bordered the creek to the south and Highway 200 bordered it to the north. A large sugar beet field (~112 ha) was located directly north of the highway. Red-winged blackbird nests at this site were constructed in cattail in the creek bed, or in willow (*Salix* spp.) or other woody plants on the banks of the waterway. Samples were also collected from house sparrows in a nest under a road bridge at this site. Along this creek, we monitored 17 nests, located approximately 15 to 30 m from the edge of the beet field, which was sprayed from a plane with chlorpyrifos on June 9, 1992.

Homestead Wetland. The Homestead Wetland was on an abandoned home site in the southwest quarter of Section 9 in Lake Ida Township. The wetland was small (diameter approximately 15 m) and cattail and stinging nettle (*Urtica dioica*) were the dominant plant species. The wetland was at the center of a 16-ha plot of nonagricultural land, and was primarily dominated by grasses and low shrubs, although several hectares were dominated by deciduous trees. Three sides of the site were bordered by sugar beet fields and nests were approximately 150 to 250 m from the borders of these fields. Most (7 of 11) red-winged blackbird nests at the site were constructed in cattail, although we found four nests in small shrubs (about 1 m high) in the upland surrounding the wetland. The sugar beet field to the north of this site was band sprayed with chlorpyrifos on June 6, 1992 and fields to the west were broadcast sprayed with chlorpyrifos on June 8, 1992.

Spring Creek. The Spring Creek site was in the southwest quarter of Section 7 in Green Meadow Township. Nests at this site were found along a creek (approximately 2 m wide) with containment dikes built on either side of the waterway. At this site willow and other shrubs covered approximately 50% of the banks; the other 50% was dominated by sedges and grasses. In addition, there were two small cattail wetlands (6–10 m diameter) within 10 m of the creek. A wheat field was located

Table 1. Summary of nestling plasma samples and nestling carcasses collected from passerine birds in Norman County, Minnesota, in June 1992. All nestling plasma and brain tissue samples from carcasses were analyzed for 2-PAM ChE reactivation and all carcasses were tested for chloropyrifos residue. Samples are from red-winged blackbirds unless otherwise noted.

Site	No. of nests sampled	No. of plasma samples (no. individuals)	No. of carcasses	Ages (range in days)	Dates of sampling	Dates of chlorpyrifos application	No. of plasma reactivations	No. of carcasses with chlorpyrifos residue	No. of samples with brain reactivation
Reference									
DNR Wetland	6	10 (10)	1	5–10	9 June 11 June	—	2	0	0
Ruebke Creek[a]	1	2 (2)	0	5	20 June	—	0	0	0
Treatment									
Homestead Wetland	4	9 (9)	2	4–10	19 June 20 June	6 June 8 June	4	0	0
Spring Creek	4	12 (9)	2	5–10	13 June 18 June 20 June	3 June 10 June 12 June	2	2	0
Baker Creek[b]	5	20 (20)	6	5–12	10 June 12 June 18 June 20 June	9 June	8	0	1

[a]These values are for brown-headed cowbird nestlings.
[b]Four plasma samples and one carcass are from house sparrow nestlings.

across the road to the east and north and two more beet fields were south and west of the monitored nests, within 1 km of the site. Eleven red-winged blackbird nests monitored at this site were approximately 50 to 100 m from the nearest beet field. The field to the south of Spring Creek was band sprayed with chlorpyrifos on June 3 and 12, 1992. Large fields to the southwest and south of the site were treated aerially with chlorpyrifos on June 10, 1992.

Sample collection

We located nests by searching wetland vegetation and observing adult red-winged blackbird behavior. Once located, nests were visited at 2-to 5-d intervals and the numbers of eggs and chicks were recorded at each visit. Individual nestlings were marked for identification with black permanent marker (Sanford's Marker De Luxe, Sanford's, Bellwood, IL, USA) on feathers on the back or wings.

Nestlings were weighed with a spring scale and the length of the tarsus, culmen, and wing chord were measured with a calipers (0.1 mm resolution). Blood was drawn from the jugular vein of nestlings with heparinized 1-ml disposable syringes with 27-gauge needles. The skin and feathers on the neck near the jugular vein were swabbed with alcohol prior to drawing blood. Collected blood was stored on wet ice until centrifuged, within 1 h of collection, for 10 min at 3,200 rpm in a portable centrifuge (Vulcon Technologies, Grandview, MO, USA) powered by a car battery. We sampled three birds from Spring Creek a second time 5 d after the first samples were drawn; in all other cases birds fledged or disappeared before a second blood sample was obtained. Eleven birds were euthanatized via cervical dislocation (Table 1) after blood was drawn, for ChE analysis of brain tissue and residue analysis of the gastrointestinal tract and carcass washes. Carcasses and plasma were stored in a freezer (at approximately −10°C for up to 5 d) until shipped on dry ice for analysis.

Age estimates

Hatching date was determined by visiting nests at 2-to 5-d intervals and by observing indications of recent hatching (damp down on nestlings, only one of several eggs hatched when those eggs later hatched, presence of eggshell in the nest, etc.). Young observed with signs of recent hatching were considered to have hatched on the day they were first observed. Hatching dates of siblings could subsequently be determined because red-winged blackbird eggs within a clutch hatch within 24 h of one another [23]. Mass and culmen, wing chord, and tarsus lengths of nestlings were used to develop regression equations to estimate age of nestlings when hatching date was not apparent. Blood samples were collected from red-winged blackbirds that were from 5 to 11 d old because nestlings <5 d old were too small for drawing blood, and most had fledged by d 11 or 12. Red-winged blackbirds generally fledge between 10 and 14 d after hatching [23,24].

Wing chord length and the length of remiges have been shown to increase linearly with age during most of the nestling period for several bird species (e.g., house martins [*Delichon urbica*] [25], northern harriers [*Circus cyaneus*] [26], and red-tailed hawks [*Buteo jamaicensis*] [27,28]) and we developed a similar model for nestling red-winged blackbirds. The number of days since hatch in nestling red-winged blackbirds of unknown age was determined based on wing measurements from known-age birds. Nestlings estimated to be from 4 to 6 d old were treated as 5-d-old nestlings in analyses. Similarly, nestlings estimated at 9 to 11 d old were included in analyses as 10 d old.

Insecticide application

Landowners and persons farming the property adjacent to study sites were contacted for information regarding insecticide application. During the study, insecticide was applied as a spray to sugar beets either aerially or from the ground. Foliar insecticide application at all sites where nestling blood was collected is summarized in Table 2. No additional independent monitoring of insecticide application was attempted, and we did not attempt to estimate rate of application of chlorpyrifos.

In addition to foliar application of insecticides, sugar beets in northwestern Minnesota receive application of ChE-inhibiting

Table 2. Application of chlorpyrifos insecticide on study sites in Norman County, Minnesota, in June 1992. Application was determined by direct observation and through landowner and farmer contact.

Site	Number of applications	Application method	Distance of nests from chlorpyrifos application (m)
Reference			
DNR Wetland	0	—	>300
Ruebke Creek	0	—	>300
Treatment			
Homestead Wetland	2	Band and broadcast	150–250
Spring Creek	3	Band and aerial	50–100
Baker Creek	1	Aerial	15–30

insecticides at planting. Virtually all sugar beet fields in the study area were treated with granular application of terbufos (as Counter®, American Cyanamid, Wayne, NJ, USA [S[[(1.1-dimethylethyl) thio] methyl] *O,O*-di-ethylphosphorodithioate]) or chlorpyrifos at planting. Other crops in the area generally did not receive insecticide application at planting (D. Berglund, personal communication). In 1992 most sugar beet fields near Ada were planted during or near the first week in May. During late May and June 1992 (our sampling period) foliar application of ChE-inhibiting insecticides on crops other than sugar beets was unlikely; however, up to 5% of wheat fields may have received application of OP or carbamate insecticides during our sampling period (D. Berglund, personal communication).

Chemical analyses of samples

Chemical and biochemical analyses were performed at The Institute of Wildlife and Environmental Toxicology, Clemson University, Pendleton, South Carolina, USA. For a more detailed description of chemical and biochemical analyses, see Hoff [29].

Tissue preparation. Samples were stored for approximately 3 months at −80°C. Plasma samples were thawed and diluted 40-fold with 0.05 M tris buffer (pH 7.4) prior to analysis. Brain tissue was removed from carcasses, mixed with 0.05 M tris buffer and homogenized for 30 s in a homogenizer (VirTis Co., Gardiner, NY, USA). The homogenate was diluted again to 300-fold in the same buffer. Brain and plasma preparations were separated into three 500-μl aliquots. One aliquot was placed on wet ice, for approximately 35 min, until it was assayed for ChE activity levels. The other two aliquots were used to test for ChE reactivation in the presence of pyridine-2-aldoxime methochloride (2-PAM; Sigma Chemical Company, St. Louis, MO, USA). One of the aliquots was spiked with 2-PAM (final concentration, 10^{-4} M) and the other with distilled water, and these samples were assayed after a 30-min incubation period at 25°C. Mean activities from triplicate measures of each aliquot were compared using an upper-tailed *t*-test to determine if there was a significant increase in ChE activity in the sample incubated with 2-PAM compared to the sample incubated without 2-PAM. Those samples found to have a significant increase of ChE activity after 2-PAM incubation were considered to contain OP-inhibited ChE and, therefore, birds from which the sample had been collected had been exposed to an OP insectide (see description of statistical analyses, below).

Cholinesterase activity assay. Diluted brain and plasma samples were spectrophotometrically assayed on a Vmax 96-well Kinetic microplate reader (Molecular Devices Corporation, Palo Alto, CA, USA) using a modification [30] of the method of Ellman et al. [21]. All samples were run in triplicate at 25°C. The substrate, acetylthiocholine iodide (ASCh, 10^{-4} M, Sigma), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB, Sigma), tris 8.0 buffer, and enzyme dilution were added to microplate wells. Cholinesterase activity was expressed as μmoles ASCh hydrolyzed/min (or ''units'')/ml plasma, or per gram of brain tissue [21]. Total plasma ChE was separated into acetycholinesterase (AChE) (and other nonbutyrylcholinesterase [non-BChE], nonspecific esterases) and butyrylcholinesterase (BChE) activities using the selective BChE inhibitor, tetraisopropylpyrophosphoramide (iso-OMPA, Sigma) [31,32]. Activity remaining after a 5-min incubation with 10^{-4} iso-OMPA was considered to be from AChE, and BChE activity was calculated as the difference between measured total ChE and AChE activity. Brain ChE is almost entirely AChE [33,34], thus BChE activity is not reported for brain tissue.

Residue extraction. Residue extractions were performed on gastrointestinal-tract (GI-tract) tissues and contents and on solvent used to wash carcasses. The entire GI-tract, rather than the gut contents or the crop, was analyzed for chlorpyrifos residue to maximize the opportunity for identifying residue, if present. Entire GI-tracts were dispersed using a VirTis homogenizer and then samples were extracted with a 3:1 hexane : acetone solvent mixture on an orbital shaker. The volume of solvent was reduced by nitrogen evaporation. Samples greater than approximately 5 g were divided into samples of ≤5 g each and homogenized, extracted, and analyzed separately. A 3:1 solution of hexane : acetone was also used to wash carcasses, and the resulting wash was subsequently analyzed through gas chromatography [29]. Carcasses were washed by placing them in a sealed glass container with 50 ml of hexane : acetone (3:1) and shaking the container for 2 min to ensure the carcass surface was completely saturated. Resulting solvents were passed through dried sodium sulfate in a glass funnel lined with 41 Whatman filter paper (Whatman International, Maidstone, UK) and nitrogen evaporation was used to reduce volume prior to gas chromatographic analysis.

Statistical analysis

Mean ChE, AChE, and BChE activity among sample sites for each age group (5-and 10-d-old nestlings) were compared using one-way analysis of variance (ANOVA) and significantly different mean activity among sites was distinguished using a Tukey test. We used Kruskal–Wallis tests to compare the magnitude of reactivation among collection sites and nonparametric association analysis to detect trends in enzyme activity in relation to nestling age. Enzyme activity levels from 5-and 10-d-old nestlings were evaluated using diagnostic thresholds (DTs) as described by Hill [35]. Samples that exhibited activities two standard deviations below the mean of birds collected at reference sites were considered to have been exposed to a ChE-inhibiting insecticide. Enzyme reactivation was considered to be significant when a sample incubated with 2-PAM had an increase in activity of >5% compared to the paired sample without 2-PAM and when the means of the three replicates of each sample were significantly different (upper-tailed *t*-test, $p < 0.05$). Error in assaying two replicates of enzyme, each in triplicate on the plate reader, can lead to significant differences up to approximately 4%. Therefore, we set 5% as a lower limit

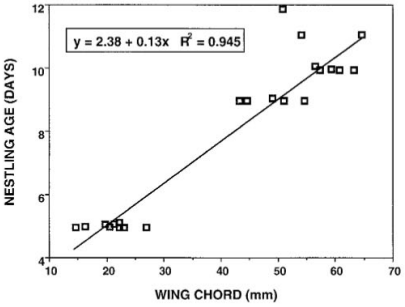

Fig. 2. Simple linear regression of age versus wing chord length for red-winged blackbird nestlings (n = 23) of known age, sampled in Norman County, Minnesota, June 1992.

to avoid false positives. Parametric statistical procedures follow those outlined in Snedecor and Cochran [36] and nonparametric procedures are after Gibbons [37].

RESULTS

Sample collection

Fifty-three blood samples were collected from 50 nestlings (five house sparrows, four brown-headed cowbirds, and 41 red-winged blackbirds). In addition, 10 red-winged blackbirds and one house sparrow from which blood had been collected were euthanized for measurement of brain ChE activity and pesticide residue analyses of GI-tracts and carcass surfaces. (The plasma sample from one euthanized red-winged blackbird was too small for analysis.) Sample collection is summarized in Table 1.

Age estimates

Wing chord length was the best predictor of age (among morphometric variables measured) of nestling red-winged blackbirds of known hatching date (n = 23), based on the proportion of variance explained by the simple linear model, and by the pattern of regression residuals (Fig. 2). Inclusion of mass or culmen or tarsus length did not significantly ($p > 0.05$) improve the fit of the regression model. We thus estimated age of unknown-age nestling red-winged blackbirds from wing chord measurements [AGE = 2.38 + WING · 0.13, where AGE is measured in days since hatching and WING is wing chord length in mm ($R^2 = 0.945$, $p = 0.000$)]. The regression sample of birds of known age included 5-, 9-, 10-, and 11-d-old nestlings.

Plasma analyses

Cholinesterase activity levels. Blood samples from the Spring Creek site (treatment) had significantly lower ChE ($F_{3,19} = 12.80$, $p < 0.001$) and BChE ($F_{3,19} = 10.35$, $p < 0.001$) activities than samples from the DNR Wetland (reference) and Homestead sites (treatment) for red-winged blackbird nestlings that were approximately 5 d old. Acetylcholinesterase activity levels from 5-d-old red-winged blackbird nestlings on the Spring Creek site were lower ($F_{3,19} = 3.15$, $p = 0.049$) than those from the Homestead site. Total ChE ($t = -2.51$, $d.f. = 21$, $p = 0.033$) and BChE ($t = -2.56$, $d.f. = 21$, $p = 0.043$) activity were lowest in 5-d-old nestlings at sites adjacent to treated beet fields. There were no differences among sites in ChE, AChE, or BChE activity for 10-d-old nestlings (all ps > 0.375, $n = 19$). Across sites, ChE activity levels in nestlings increased with age (Spearman's $\rho = 0.402$, $p = 0.008$), AChE activity ($\rho = -0.328$, $p = 0.032$) decreased with age, and BChE activity ($\rho = 0.477$, $p = 0.001$) increased with age.

Diagnostic thresholds. We calculated diagnostic thresholds (two standard deviations below the mean of reference samples) for ChE, AChE, and BChE activity for treatment sites for plasma samples drawn from red-winged blackbirds that were approximately 5 d old. At the reference sites (DNR Wetland and Ruebke Creek, see below), two plasma AChE samples reactivated in the presence of 2-PAM, indicating exposure to an OP insecticide. Diagnostic thresholds were calculated with and without reference samples that reactivated with the addition of 2-PAM. Mean ChE, AChE, and BChE activity for the samples ($n = 6$) collected from 5-d-old nestlings at the reference sites were 1.82 (standard deviation [SD] = 0.367), 0.30 (SD = 0.117), and 1.52 (SD = 0.470) units/min/ml plasma, respectively, and excluding samples that reactivated ($n = 2$), the means were 1.69 (SD = 0.357), 0.34 (SD = 0.088), and 1.35 (SD = 0.424) units/min/ml plasma, respectively. The threshold values for ChE were 1.086 and 0.977 units/min/ml plasma calculated for all reference samples and for only reference samples with no reactivation. The comparable values for AChE were 0.168 and 0.063 units/min/ml plasma and for BChE they were 0.583 and 0.501 units/min/ml plasma.

At the Baker Creek site (treatment) ChE activity in one sample was below both calculated diagnostic thresholds. At the Spring Creek site (treatment) three samples had ChE activity below the threshold for all reference samples and two were below the threshold calculated excluding reactivating samples. One sample from Spring Creek exhibited AChE activity below both thresholds and BChE activity from one sample was below the threshold for all samples, but not for the threshold calculated excluding reactivating samples. No samples were below the calculated diagnostic threshold at the Homestead site (treatment). In total, four samples exhibited ChE activities and one sample exhibited BChE activity below the threshold calculated from all reference samples and AChE activity in one sample was below both calculated thresholds.

2-PAM reactivations. Sixteen (31%) of 52 plasma samples exhibited a statistically significant ($p < 0.05$) increase of ≥ 5% in AChE activity after the addition of 2-PAM (Fig. 3). Seventeen percent of the samples from the DNR Wetland and Ruebke Creek sites combined (distant from chlorpyrifos application) reactivated. Seventeen percent of the samples from the Spring Creek site reactivated, 42% of the samples from Baker Creek reactivated, and 44% from the Homestead site reactivated. Nestlings from sites that were near fields where chlorpyrifos was applied were more likely to exhibit plasma AChE reactivation than nestlings from reference sites where OP or carbamate insecticide application was improbable ($\chi^2 = 3.805$, $d.f. = 1$, $p \sim 0.05$). Total ChE reactivated in 30% of samples from Baker Creek, and in all cases where ChE reactivated, AChE also reactivated ≥ 5%. The magnitude of AChE reactivation at Baker Creek also appeared higher than at other sites (Kruskal–Wallis $H = 7.65$, $d.f. = 3$, $p = 0.054$). Nest-specific reactivation occurred on both the Baker Creek and Homestead sites, with individuals from the same nest exhibiting AChE reactivation ≥ 5%. The greatest increases in AChE reactivation occurred within 1 to 3 d after insecticide application (Fig. 4), although there

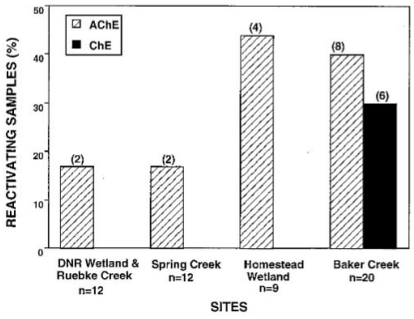

Fig. 3. Percent of nestling plasma samples in which significant reactivation of acetylcholinesterase (AChE) and total cholinesterase (ChE) was observed. The total number of birds sampled at each site (n) is noted below site names and the number of samples that reactivated is above the bars. Red-winged blackbird, house sparrow, and brown-headed cowbird nestlings were sampled in Norman County, Minnesota, in June 1992 (see Table 1).

was no significant relationship between days postapplication and magnitude of reactivation ($r = -0.298$, $p = 0.301$).

Plasma samples that exhibited significant reactivation after incubation with 2-PAM did not appear to fall outside of the range of ChE activity levels for samples from other similar-aged birds. In plasma from individuals that were sampled twice, AChE from one bird at 5 d old reactivated 14% with the addition of 2-PAM, but plasma from the same bird showed no significant reactivation 5 d later. No samples that exhibited reactivation with 2-PAM were also below calculated diagnostic thresholds.

Brain tissue analyses

Brain tissue from 10 nestling (5–11-d-old) red-winged blackbirds and one house sparrow (Table 1) was analyzed for ChE activity and reactivation with the addition of 2-PAM. Total ChE reactivated significantly in one brain from a red-winged blackbird nestling from the Baker Creek site; ChE and AChE also

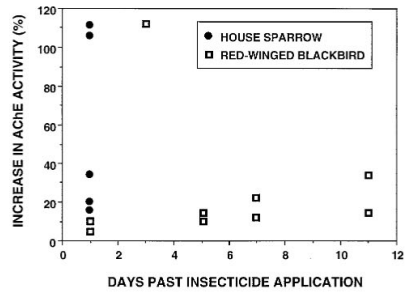

Fig. 4. Percent increase in acetylcholinesterase (AChE) activity where significant (≥5%) reactivation occurred in plasma samples of nestling red- winged blackbirds ($n = 9$) and house sparrows ($n = 5$) as a function of days past chlorpyrifos application. Nestlings were sampled in Norman County, Minnesota, in June 1992.

reactivated in plasma from this individual. In addition, one plasma sample from a house sparrow from which brain tissue had been sampled exhibited significant reactivation of both AChE and ChE, and two additional plasma samples from red-winged blackbirds exhibited significant reactivation of AChE. Cholinesterase activity (primarily AChE, $n = 10$) in brain tissue increased significantly ($r = 0.695$, $p = 0.026$) with age, although only three nestlings sampled were >6 d old.

Residue analyses

Chlorpyrifos residues were identified from one carcass wash and two GI-tracts from the six nestlings collected at Baker Creek. Chlorpyrifos was recovered from the carcass wash (0.135 μg) and GI-tract material (0.538 μg) in one house sparrow, which also exhibited reactivation of plasma ChE and AChE. Trace levels (<0.1 μg) of chlorpyrifos were identified in the GI-tract of one red-winged blackbird, which also exhibited 2-PAM reactivation of ChE in brain tissue and ChE and AChE in plasma.

DISCUSSION

Insecticides

In 1992 an outbreak of sugar beet root maggots occurred in northwestern Minnesota; approximately 80% of the sugar beet fields in the Ada area were sprayed with chlorpyrifos during the 1992 growing season, in contrast to most years when only about 3% of fields receive foliar application of an insecticide (D. Berglund, personal communication). Thus, we felt confident that nests near beet fields were near insecticide application, and we were able to document insecticide application in fields adjacent to where nests were located. In this context, we used several different laboratory and statistical procedures for evaluating exposure of nestling birds to the foliar application of chlorpyrifos in sugar beet fields.

Plasma analyses

We were able to sample nestling birds in an agricultural landscape, and evaluate the feasibility of monitoring exposure to ChE-inhibiting insecticides through measuring blood plasma and brain ChE activity. Total ChE and BChE activity levels were lowest for 5-d-old red-winged blackbirds at the Spring Creek site (treatment) compared to other sites. Combining samples from sites where insecticide application occurred, ChE and BChE activity levels were lower at treated sites compared to reference sites. Plasma AChE activity did not differ between samples collected at treated versus reference sites. These data suggest that when treated and nontreated sites are compared, plasma ChE and BChE activity levels may be more sensitive to chlorpyrifos exposure, and perhaps exposure to other ChE-inhibiting insecticides, than plasma AChE activity.

In birds, the proportion of plasma ChE that is BChE or AChE varies for different species [38] and different species have different age-dependent patterns of AChE and BChE activity. The significance of these patterns in relation to ChE inhibition is not well understood. In eastern bluebirds (*Sialia sialis*) and European starlings (*Sturnus vulgaris*) [30], plasma AChE declined slightly and BChE increased over the nestling period. In a comparable study, Grue et al. [39] observed an increase in brain ChE activity, an increase in plasma BChE activity, and a decrease in plasma AChE activity in nestling bluebirds and starlings over the nestling period. We observed a similar pattern of plasma AChE and BChE activity in red-winged blackbirds. Although sample sizes were small, our results are consistent

with the results of other studies of altricial birds, suggesting that our sampling and laboratory procedures were comparable to those of previous studies. Unlike controlled laboratory studies, however, our analyses of ChE inhibition in nestlings were complicated by the fact that many of the sampled birds were exposed to OP insecticides at different times and at different ages. Gard and Hooper [30] suggested that the increase in plasma BChE activity during the nestling period may be responsible for decreased sensitivity to ChE inhibitors that occurs in some birds during the same period [40]. In other studies, nestling starlings have been shown to be more sensitive to exposure to OP insecticides than adults [40,41].

In our study, the diagnostic threshold method of evaluating exposure to ChE-inhibiting insecticides did not appear to be a sensitive indicator of exposure to chlorpyrifos. The activity of six ChE samples fell greater than two standard deviations below the mean of reference samples, and none of these exhibited significant reactivation in the presence of 2-PAM, although we did not know whether other inhibitors less responsive to 2-PAM reactivation may have been present in these samples. Five samples were within two standard deviations of the mean ChE activity in reference samples, yet exhibited significant reactivation in the presence of 2-PAM. However, this analysis was hindered by the relatively small number of similar-aged birds used for calculation of diagnostic thresholds, and the uncertain relationship between nestling age and cholinesterase activity levels.

Reactivation of ChE in plasma samples appeared to be a more sensitive indicator of insecticide exposure in this study than diagnostic thresholds; nestlings from sites that were near fields where chlorpyrifos was applied were more likely to exhibit ChE reactivation than nestlings from reference sites where OP or carbamate insecticide application was improbable (Fig. 3). The magnitude of AChE reactivation was highest within 1 to 3 d of insecticide application, although reactivation was measured up to 11 d after application of chlorpyrifos (Fig. 4). Acetylcholinesterase activity levels seemingly decreased with nestling age, suggesting that inhibition of plasma cholinesterase activity was short-lived. In general, AChE reactivation appeared to be a more sensitive indicator of exposure to ChE-inhibiting insecticides than ChE reactivation. In all cases where ChE reactivated, AChE also reactivated, although the reverse was not true. One particularly important characteristic of the 2-PAM reactivation technique is the comparison of a single sample's ChE activity with and without the addition of 2-PAM. Thus, results are independent of factors such as age and species differences that influence the initial level of enzyme activity [42], and this method also removes the requirement of a nonexposed reference sample.

It is unclear why two samples from the reference sites distant from ChE-inhibiting insecticide application showed plasma reactivation. Perhaps these birds were exposed to an insecticide application of which we were unaware. In another study of 2-PAM reactivation of plasma from birds [42] unexposed birds showed low rates and levels of reactivation. However, it is impossible to rule out the possibility that nestlings at all sites, including the reference site, were exposed to ChE-inhibiting insecticides other than foliarly applied chlorpyrifos. Drift from aerial pesticide application has been shown to occur over distances of at least 1 to 2.5 km [13,43], and aerially applied herbicide has reportedly drifted up to 24 km [43]. Studies of chlorpyrifos have estimated the half-life in soils to be from 8 to 279 d, averaging 69 d [44].

Brain tissue analyses

Only 1 of 11 (9%) brain samples collected exhibited significant ChE reactivation. Three (27%) plasma samples collected from euthanatized birds exhibited significant ChE reactivation, and five (46%) plasma samples exhibited significant AChE reactivation. Both ChE and AChE reactivated from plasma collected from the nestling where brain ChE reactivated, and AChE reactivated in all three cases where ChE reactivated. These results suggest that plasma AChE reactivation may be a more sensitive indicator of ChE-inhibiting insecticide exposure than either plasma ChE reactivation or brain ChE reactivation. Previous studies indicate that plasma ChE inhibition generally occurs at lower levels of OP insecticide exposure than does brain ChE inhibition [1,20,45,46]; this may explain why a higher proportion of plasma than brain samples reactivated.

Residue analyses

Residue analyses of GI-tracts and wash samples indicated exposure to chlorpyrifos in 2 of 11 (18%) carcasses, one red-winged blackbird and one house sparrow. In the red-winged blackbird, plasma ChE and AChE and brain ChE exhibited significant reactivation. Total ChE and AChE in plasma from the house sparrow exhibited significant reactivation; however, brain ChE in this sample did not reactivate. These results suggest that nestlings were exposed to insecticides by adults that foraged where prey had been exposed, or that adults themselves were exposed directly and transferred the insecticide to nestlings. The single nestling that had detectable levels of chlorpyrifos residues in both its GI-tract and in a wash sample was a house sparrow from a nest located beneath a bridge at the Baker Creek site, where direct overspray or drift were highly unlikely. Additionally, we observed the female red-winged blackbird from the nest where brain ChE reactivated in a nestling, foraging in a beet field that had been treated with chlorpyrifos. Both of these observations suggest that adults may forage in areas where exposure to insecticides is likely, and that in turn, nestlings can be exposed to insecticides through ingestion of prey delivered by adults and through direct contact with parents. Several studies [40,41] have shown that in some altricial species nestlings are significantly more sensitive to OP insecticides than adults. Thus, it is possible that adults could continue to forage in fields with contaminated insects and remain behaviorally unaffected, while delivering doses of insecticides to their nestlings that cause ChE inhibition.

Our results suggest that red-winged blackbird and house sparrow nestlings near fields where insecticide application occurred were exposed to a ChE-inhibiting compound, probably chlorpyrifos applied as Lorsban® to sugar beet foliage. The route of exposure may have been via the adult, either through direct contact or from food items delivered to the young. Of the methods we evaluated, the 2-PAM reactivation technique in plasma seemed to most frequently indicate that nestlings were exposed to chlorpyrifos. The benefits of comparing a single sample's ChE activity before and after the addition of 2-PAM rather than collecting reference samples is important because it is often difficult to identify with certainty birds that have not been exposed to ChE-inhibiting chemicals. It also reduces the number of birds that may need to be sampled. The most significant drawback is that this technique has not commonly been applied to this type of problem and the frequency of false positives has not been established. For example, in this study two of the reference plasma samples reactivated ($\bar{x}$ = 15.0%) and it is not

obvious how those birds may have been exposed to ChE inhibitors.

This study was not designed to address the consequences of exposure, although some studies suggest that behavioral and physiological effects of sublethal exposure may impact bird survival [6,47–49]. For the purpose of monitoring, it is important to continue to develop the technique of 2-PAM reactivation (or similar methodologies) and to differentiate between carbamate and OP insecticide exposure [50,51]. Finally, it is necessary to understand the occurrence of reactivation in reference samples and to standardize the methods and interpretations of these results.

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REFERENCES

1. **Ludke, J.L., E.F. Hill** and **M.P. Dieter.** 1975. Cholinesterase (ChE) response and related mortality among birds fed ChE inhibitors. *Arch. Environ. Contam. Toxicol.* **3**:1–21.
2. **Grue, E.C., G.V.N. Powell** and **C.H. Gorsuch.** 1982. Assessing effects of organophosphates on songbirds: Comparison of a captive and a free-living population. *J. Wildl. Manage.* **46**:766–768.
3. **Powell, G.V.N.** 1984. Reproduction by an altricial songbird, the red-winged blackbird, in fields treated with the organophosphate insecticide fenthion. *J. Appl. Ecol.* **21**:83–95.
4. **Meyers, S.M., J.L. Cummings** and **R.S. Bennett.** 1990. Effects of methyl parathion on red-winged blackbird (*Agelaius phoeniceus*) incubation behavior and nesting success. *Environ. Toxicol. Chem.* **9**:807–813.
5. **Martin, P.A., K.R. Solomon** and **H.J. Boermans.** 1991. Effects of carbofuran ingestion on mallard ducklings. *J. Wildl. Manage.* **55**:103–111.
6. **Martin, P.A., K.R. Solomon, D.J. Forsyth, H.J. Boerman** and **N. Westcott.** 1991. Effects of exposure to carbofuran-sprayed vegetation on the behavior, cholinesterase activity and growth of mallard ducklings (*Anas platyrhynchos*). *Environ. Toxicol. Chem.* **10**: 901–909.
7. **Seabloom, R.W., G.L. Pearson, L. Woring** and **J.R. Reilly.** 1973. An incident of fenthion mosquito control and subsequent avian mortality. *J. Wildl. Dis.* **9**:18–20.
8. **Mendelssohn, H.** and **U. Paz.** 1977. Mass mortality of birds of prey caused by azodrin, an organophosphorus insecticide. *Biol. Conserv.* **11**:163–170.
9. **Frank, R., P. Mineau, H.E. Braun, I.K. Barker, S.W. Kennedy** and **S. Truedeau.** 1991. Deaths of Canada geese following spraying of turf with diazinon. *Bull. Environ. Contam. Toxicol.* **46**:856–858.
10. **Smith, G.J.** 1987. Pesticide use and toxicology in relation to wildlife: Organophosphorus and carbamate compounds. *U.S. Fish Wildl. Serv. Res. Publ.* **170**:1–171.
11. **White, D.H., J.T. Seginak** and **R.C. Simpson.** 1990. Survival of northern bobwhites in Georgia: Cropland use and pesticides. *Bull. Environ. Contam. Toxicol.* **44**:73–80.
12. **U.S. Department of Agriculture.** 1991. Agricultural chemical usage, 1990 field crops summary. National Agricultural Statistics Service, Washington, DC.
13. **Deeley, G.M.** 1980. Pesticide drift and its effect on cholinesterase levels of birds in non-target areas of the Buena Vista Marsh. M.S. thesis. University of Wisconsin, Stevens Point, WI, USA.
14. **DeWeese, L.R., L.C. McEwen, L.A. Settimi** and **R.D. Deblinger.** 1983. Effects on birds of fenthion aerial application for mosquito control. *J. Econ. Entomol.* **76**:906–911.
15. **Grue, C.E., W.J. Fleming, D.G. Busby** and **E.F. Hill.** 1983. Assessing hazards of organophosphate pesticides to wildlife. *Trans. N. Am. Wildl. Nat. Resour. Conf.* **48**:200–220.
16. **McEwen, L.C., L.R. DeWeese** and **P. Schladweiler.** 1986. Bird predation on cutworms (Lepidoptera: Noctuidae) in wheat fields and chlorpyrifos effects on brain cholinesterase activity. *Environ. Entomol.* **15**:147–151.
17. **Hooper, M.J., P.J. Detrich, C.P. Weisskopf** and **B.W. Wilson.** 1989. Organophosphorus insecticide exposure in hawks inhabiting orchards during winter dormant spraying. *Bull. Environ. Contam. Toxicol.* **42**:651–659.
18. **Wilson, B.W., M.J. Hooper, E.E. Littrell, P.J. Detrich, M.E. Hansen, C.P. Weisskopf** and **J.N. Seiber.** 1991. Orchard dormant sprays and exposure of red-tailed hawks to organophosphates. *Bull. Environ. Contam. Toxicol.* **47**:717–724.
19. **O'Brien, R.D.** 1976. Acetylcholinesterase and its inhibition. In C. F. Wilkinson, ed., *Insecticide Biochemistry and Physiology*. Plenum, New York, NY, USA, pp. 271–291.
20. **Hill, E.F.** and **W.J. Fleming.** 1982. Anticholinesterase poisoning of birds: Field monitoring and diagnosis of acute poisoning. *Environ. Toxicol. Chem.* **1**:27–38.
21. **Ellman, G.L., K.D. Courtney, V. Andres, Jr.** and **R.M. Featherstone.** 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* 7:88–95.
22. **Martin, A.D., G. Norman, P.I. Stanley** and **G.E. Westlake.** 1981. Use of reactivation techniques for the differential diagnosis of organophosphorus and carbamate pesticide poisoning in birds. *Bull. Environ. Contam. Toxicol.* **26**:775–780.
23. **Payne, R.B.** 1969. Breeding seasons and reproductive physiology of tricolored blackbirds and redwinged blackbirds. *Univ. Calif. Publ. Zool.* **90**:1–115.
24. **Harrison, C.** 1984. *A Field Guide to the Nests, Eggs, and Nestlings of North American Birds.* Collins Publishers, Toronto, Ontario, Canada.
25. **Bryant, D.M.** 1975. Breeding biology of house martins (*Delichon urbica*) in relation to aerial insect abundance. *Ibis* **117**:180–216.
26. **Saunders, M.B.** and **G.L. Hansen.** 1989. A method for estimating the ages of nestling northern harriers (*Circus cyaneus*). *Can. J. Zool.* **67**:1824–1827.
27. **Petersen, L.R.** and **D.R. Thompson.** 1977. Aging nestling raptors by fourth primary measurements. *J. Wildl. Manage.* **41**:587–590.
28. **Bechard, M.J., B.W. Zoelick** and **M. Nickerson.** 1985. Accuracy in determining the age of nestling red-tailed hawks. *J. Wildl. Manage.* **49**:226–228.
29. **Hoff, D.J.** 1992. Organophosphorus pesticide exposure to American kestrels (*Falco sparverius*) in a southeastern Iowa agroecosystem. M.S. thesis. Clemson University, Clemson, SC, USA.
30. **Gard, N.W.** and **M.J. Hooper.** 1993. Age-dependent changes in brain and plasma cholinesterase activities of eastern bluebirds and European starlings. *J. Wildl. Dis.* **29**:1–7.
31. **Aldridge, W.N.** 1953. The differentiation of true and pseudo cholinesterase by organo-phosphorus compounds. *Biochem. J.* **53**:62–67.
32. **Fairbrother, A., B.T. Marden, J.K. Bennett** and **M.J. Hooper.** 1991. Methods used in determination of cholinesterase activity. In P. Mineau, ed., *Cholinesterase Inhibiting Insecticides: Their Impact on Wildlife and the Environment.* Elsevier, New York, NY, USA, pp. 35–71.
33. **Hill, E.F.** 1989. Divergent effects of postmortem ambient temperature on organophosphorus- and carbamate-inhibited brain cholinesterase activity in birds. *Pestic. Biochem. Physiol.* **33**:264–275.
34. **Walker, C.H.** and **H.M. Thompson.** 1991. Phylogenetic distribution of cholinesterases and related esterases. In P. Mineau, ed., *Cholinesterase Inhibiting Insecticides: Their Impact on Wildlife and the Environment.* Elsevier, New York, NY, USA, pp. 1–17.
35. **Hill, E.F.** 1988. Brain cholinesterase activity of apparently normal wild birds. *J. Wildl. Dis.* **24**:51–60.
36. **Snedecor, G.W.** and **W.G. Cochran.** 1980. *Statistical Methods,* 7th ed. Iowa State University Press, Ames, IA, USA.
37. **Gibbons, J.D.** 1985. *Nonparametric Methods for Quantitative Analysis,* 2nd ed. American Sciences Press, Columbus, OH, USA.
38. **Hooper M.J.** and **B.W. Wilson.** 1987. Oxime reactivation of cho-

linesterase: Monitoring organophosphate exposure in endangered species. Final Project Report. Contract C-1523. State of California Department of Fish and Game, Sacramento, CA, USA.
39. **Grue, E.C., G.V.N. Powell** and **N.L. Gladson.** 1981. Brain cholinesterase (ChE) activity in nestling starlings: Implications for monitoring exposure of nestling songbirds to ChE inhibitors. *Bull. Environ. Contam. Toxicol.* **26**:544–547.
40. **Grue, C.E.** and **B.K. Shipley.** 1984. Sensitivity of nestling and adult starlings to dicrotophos, an organophosphate pesticide. *Environ. Res.* **35**:454–465.
41. **Hooper, M.J., L.W. Brewer, G.P. Cobb** and **R.J. Kendall.** 1990. An integrated laboratory and field approach for assessing hazards of pesticide exposure to wildlife. In L. Somerville and C.H. Walker, eds., *Pesticide Effects on Terrestrial Wildlife.* Taylor & Francis, London, UK, pp. 271–283.
42. **Hooper, M.J.** 1988. Avian cholinesterases: Their characterization and use in evaluating organophosphate insecticide exposure. Ph.D. thesis. University of California, Davis, CA, USA.
43. **Akesson, N.B.** and **W.E. Yeates.** 1964. Problems relating to application of agricultural chemicals and resulting drift residues. *Annu. Rev. Entomol.* **9**:285–318.
44. **Marshall, W.K.** and **J.R. Roberts.** 1978. Ecotoxicology of chlorpyrifos. Report 16079. National Research Council of Canada, Ottawa, ON.
45. **Fleming, W.J.** 1981. Recovery of brain and plasma cholinesterase activities in ducklings exposed to organophosphorus pesticides. *Arch. Environ. Contam. Toxicol.* **10**:215–229.
46. **Fleming, W.J.** and **C.E. Grue.** 1981. Recovery of cholinesterase activity in five avian species exposed to dicrotophos, an organophosphorus pesticide. *Pestic. Biochem. Physiol.* **16**:129– 135.
47. **Patnode, K.A.** and **H. White.** 1991. Effects of pesticides on songbird productivity in conjunction with pecan cultivation in southern Georgia: A multiple-exposure experimental design. *Environ. Toxicol. Chem.* **10**:1479–1486.
48. **Galindo, J.C., R.J. Kendall, C.J. Driver** and **T.E. Lacher, Jr.** 1985. The effect of methyl parathion on susceptibility of bobwhite quail (*Colinus virginianus*) to domestic cat predation. *Behav. Neural Biol.* **43**:21–36.
49. **Rattner, B.A.** and **J.C. Franson.** 1984. Methyl parathion and fenvalerate toxicity in American kestrels: Acute physiological responses and effects of cold. *Can. J. Physiol. Pharmacol.* **62**:787–792.
50. **Martin, A.D., G. Norman, P.I. Stanley** and **G.E. Westlake.** 1981. Use of reactivation techniques for the differential diagnosis of organophosphorus and carbamate pesticide poisoning in birds. *Bull. Environ. Contam. Toxicol.* **26**:775–780.
51. **Smith, M.R., N.J. Thomas** and **C. Hylse.** 1995. Application of brain cholinesterase reactivation to differentiate between organophosphorus and carbamate pesticide exposure in wild birds. *J. Wildl. Dis.* **31**:263–267.