Transactions of the Illinois State Academy of Science
(1993), Volume 86, 1 and 2, pp. 63 - 69

Screening for Anticholinesterase Pesticide Poisoning in Illinois Raptors

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ABSTRACT

To determine the utility of archived reference values of species specific normal brain cholinesterase (ChE) values and the prevalence of organophosphate and carbamate (antiChE) pesticide poisoning of raptors, 105 raptor [great horned owl (*Bubo virginians*), screech owl (*Otus asio*), barred owl (*Strix varia*), red-tailed hawk (*Buteo jamaicensis*), and kestrel (*Falco sparvenius*)] carcasses were collected from central and southern Illinois. Specimens were necropsied and brain tissue assayed for ChE activity. Cause of death determined for 83 specimens included cachexia, trauma, and disease. Mean brain ChE activity was calculated for each species from adultspecimens with a known proximal cause of death (Cooperative Wildlife Research Laboratory (CWRL) data set). Based on published mean brain ChE values 4 of the 105 specimens would be considered suspect. Based on normal ChE values generated in the present study (CWRL data set), 6 of the 105 birds would have been considered suspect. The close agreement in determination of suspect specimens based on the data from this study and archived reference data indicates the utility of published reference values in screening birds for antiChE poisoning.

INTRODUCTION

Agricultural and silviculture applications of organophosphate and carbamate pesticides with anticholinesterase (antiChE) properties have been responsible for mortality of songbirds, gamebirds, gulls, and waterfowl (Finley, 1965; Hill and Fleming, 1982; White and Mitchell, 1983; White and Kolbe, 1985). Raptors are susceptible to primary poisoning and also to secondary poisoning by feeding on incidentally or intentionally poisoned birds or mammals (Balcomb, 1983; Henny et al., 1987). Raptor mortality due to antiChE pesticide exposure may be underestimated because animals often die some distance away from the pesticide source.

Diagnosis of antiChE pesticide poisoning requires demonstration of ≥50% depression in normal brain ChE activity and detection of antiChE pesticide residue in or on the dead

animal (Hill and Fleming, 1982). Detection of pesticide residue is costly and more difficult than determination of brain ChE activity, therefore, it would be desirable to use reduction in ChE activity as a screening test for antiChE pesticide poisoning. Calculation of percent ChE inhibition requires knowledge about normal ChE activity for a species. Concurrently collected control specimens handled in the same manner as suspect specimens would provide the best comparative data for determination of normal brain ChE activity (Hill and Fleming, 1982). However, it may not always be feasible or, in the case of endangered or threatened species, desirable to obtain suitable control specimens. Therefore, it has been suggested that a reference file of normal ChE activities be developed from wildlife species collected for other projects or opportunistically salvaged specimens where cause of death was not believed to be due to antiChE pesticides (Westlake et al., 1981; Hill, 1988a).

Utilization of a reference file of normal ChE activity may be limited because of variation in specimen handling, assay equipment or other inter-laboratory differences (Hill and Fleming, 1982; Fairbrother and Bennett, 1988). The purpose of this study was to determine brain cholinesterase activity in dead raptors collected in Illinois and compare these values to published reference values of normal ChE activities for these species as a screening test for exposure to anticholinesterase compounds. If published reference values did not exist, estimates were made from the current data base.

MATERIALS AND METHODS

During 1986-87, 105 dead or moribund raptors of 5 species [great horned owl (*Bubo virginianus*), screech owl (*Otus asio*), barred owl (*Strix varia*), red-tailed hawk (*Buteo jamaicensis*), and kestrel (*Falco sparvenius*)] were collected from central and southern Illinois by personnel of Treehouse Wildlife Center, Illinois Department of Conservation, Jackson County Humane Society, and Cooperative Wildlife Research Laboratory, SIU-C. Moribund birds were euthanized. Bird carcasses were handled by a variety of methods and were received for analysis in different stages of autolysis. Once in our laboratory, all carcasses were frozen at -4°C. Heads were removed, without thawing and returned to -4°C. Thawed, decapitated specimens were necropsied. Sex, general physical condition and age were assessed. Diagnosis of cause of death (Table 1) was based on gross lesions and histopathology when necessary. Trauma diagnosed included animals gun shot, hit by car, and caught in traps. Cause of death was not determined for 22 specimens. Histories were not available on all specimens, however, 2 birds (classified unknowns) were believed to have been poisoned based on reported clinical signs. ChE values for all unknowns and birds of undetermined sex (n=2) were not included in statistical analysis.

Brain tissue was assayed for ChE activity by the modified method of Ellman (Ellman et al., 1961) as outlined by Hill and Fleming (1982). In addition to bird samples, positive and negative controls using rat brains were included. Proper assay performance was monitored by inter-assay determination of brain ChE activity of sub-lethally poisoned rats (positive controls) and normal (negative) controls. Five male Sprague-Dawley rats (150 g), topically treated on a shaved area of their backs with one third the dermal LD_{50} of fenthion (Kenaga and End, 1974 as cited in Buck et al., 1976) 24 hours prior to sacrifice, served as positive controls. Five other rats received sham treatment and were used as negative controls. Rats were killed in a CO_2 chamber, decapitated and heads frozen.

Rat and bird heads were thawed and brains removed no more than 24 hours prior to assay. Thawed brains were refrigerated until a 0.8 g sub-sample was homogenized in a hand operated Dounce tissue grinder and assayed at room temperature (22±1°C). A 20 μl aliquot of the sample, substrate and chromogen were incubated 2.5 to 3.0 min.; absorbance was recorded every 30 sec. after the first 30 sec. Absorbance was measured at a wavelength of 405 nm on a Turner model 350 spectrophotometer fitted with a 400 to 600 nm filter. Mean ChE activity and standard deviation for each species was determined from adult specimens with a known proximal cause of death (CWRL data set).

Significance of inter-assay differences between positive and negative control rats was determined by t-test for related samples. The significance of intra-specific age and sex differences was determined by t-tests (SAS Institute Inc. 1987). Affect of autolysis on ChE activity was determined by assaying a second sub-sample from 10 raptor brains held for 22 to 24 hours at room temperature. Paired comparison t-test (t statistic and probability value for null hypothesis that group mean difference is equal to zero) was used to determine affect of autolysis. Significance level for all tests was 0.05.

RESULTS

ChE values for positive control rats were significantly lower than their intra-assay negative control (5.41±SE 0.61, 9.18±SE 1.01, respectively). Mean ChE activity did not vary significantly between adult and juvenile great horned owls, screech owls and barred owls. Juvenile red-tailed hawks and kestrels differed significantly from their adult counterparts, but in both cases, the juvenile sample size was small. No significant differences were found between sex of adult birds of the same species.

Sub-samples from brains allowed to autolyze at room temperature differed significantly from the pre-autolysis sub-samples. One autolyzed sample increased in ChE by 3.4 μmol/min/g (22%), wet wt., over its initial value. All other autolyzed samples decreased ChE activity significantly (5 to 49%; -3.62±SE 1.56) over their pre-treatment values.

Mean ChE activity for adults of all bird species ranged from 18 to 29 μmol/min/g, wet wt. (Table 2). Species mean brain ChE activity as determined from the CWRL data set were similar to those determined by Hill (1988a); however, variation was higher in the Illinois birds. Four of the 105 birds examined would have been recommended for further analysis for possible pesticide residue detection based on 50% depression from the means reported by Hill (1988a). Six of the 105 birds would have triggered further analyses based on mean data from the CWRL's data set (Table 3). The great horned owl and a kestrel submitted as poisoning suspects had brain ChE levels depressed 69% and 52%, respectively from their species mean in the CWRL data set.

DISCUSSION

Six of the 105 raptors surveyed had brain ChE activity suggestive of antiChE pesticide poisoning. Although not a high percentage, it does suggest exposure of Illinois raptors to antiChE compounds. Although the proximal cause of death of all suspect specimens was attributed to causes other than antiChE poisoning, sublethal exposure could not be

discounted as a contributing factor. Experienced raptor rehabilitators had noted signs of neural poisoning in a great horned owl and kestrel that had greater than 50% inhibition of brain ChE. Altered behavior, as a result of chemical exposure, may increase the vulnerability of an animal to mortality factors, such as predation or accidents.

In general, ChE activity of raptors reported in this investigation was more variable than data presented by Hill (1988a). Fairbrother and Bennett (1988) outlined a variety of reasons for between or within laboratory variation in ChE determination including differences in homogenization technique and settling variation in brain homogenate. Our spectrophotometer, unlike Hill's (1988a), was not equipped with a circulating water bath for precise temperature control. Therefore, some intra-specific ChE variation may be due to inter-assay temperature variation. Differences in degree of autolysis can also affect ChE activity. Although none of our continued autolysis sample values fell below 50% of the species normal mean as determined by us or Hill (1988a), they did differ significantly from their pre-autolysis samples. Highly variable ChE values might logically be expected from specimens that vary greatly in degree of autolysis.

The greatest utility of published reference values for normal ChE activity is in monitoring for organophosphate/carbamate exposure or investigating localized die-offs where tentative results could determine whether more costly pesticide residue identification is warranted. Although there are many factors to consider in comparison of inter-assay and inter-laboratory results (Hill and Fleming 1982, Fairbrother and Bennett 1988, Hill 1988b), we believe the close agreement between specimens classified as suspect based on the CWRL data and those classified suspect based on published reference values (Hill 1988a) support the use of archived reference values. Assays conducted in a modestly equipped field laboratory such as ours can help resolve the incidents of wildlife mortality of unknown cause. ChE determination from opportunistically collected suitable "clean" specimens, especially of rare or endangered species, is encouraged. Future studies should include controls such as the ChE standard suggested by Fairbrother and Bennett (1988). Using a ChE standard would increase confidence in inter-laboratory and -assay comparisons.

ACKNOWLEDGMENTS

We thank the personnel of Treehouse Wildlife Center, Illinois Department of Conservation, Jackson County Humane Society and Cooperative Wildlife Research Laboratory, SIU-C for raptor carcasses and their interest. We thank M. McKee for critical review of this manuscript.

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Table 1: Cause of death determined by necropsy and histopathology for 105 raptor specimens.

Species	Cachexia*	Trauma	Diseased	Unknown
Great Horned Owl	6	27	4	4
Screech Owl	0	8	0	0
Barred Owl	2	20	1	14
Red-tailed Hawk	0	10	1	3
Kestrel	1	2	1	1

*Cause unknown

Table 2: Species, sample size [n], mean cholinesterase value and standard deviation (SD) for adult raptors as determined in this study (CWRL) and by Hill (1988a).

	Mean ChE Activity μmol/min/g, wet wt. (SD) [n]		
Species	CWRL	Hill	CWRL Range
Great Horned Owl	20 (5.5) [33]	16 (2.5) [19]	9.3-30.3
Screech Owl	20 (3.6) [6]	19 (2.5) [11]	16.6-27.0
Barred Owl	18 (6.1) [18]	–	9.2-34.4
Red-tailed Hawk	22 (7.3) [9]	19 (3.2) [15]	13.0-35.9
Kestrel	29 (1.7) [3]	27 (2.8) [11]	26.9-30.2

Table 3: Comparison of suspect specimens based on CWRL and Hill's (1988a) means.

Species	Age[1]	Presumptive Diagnosis	Brain ChE	% Depressed from mean	
				CWRL	Hill's
Great Horned Owl	A	Trauma	9.36	53	41
Great Horned Owl	A	Poisoning Suspect	6.13	69	61
Red-Tailed Hawk	SA	Trauma	10.07	54	47
Screech Owl	SA	Trauma	7.93	60	58
Kestrel	SA	Cachexia	13.52	53	50
Kestrel	A	Poisoning Suspect	13.00	52	50

[1] A = adult, SA = sub-adult