

A PRELIMINARY REPORT ON THE IMPACT OF AERIALLY
APPLIED ZECTRAN® (MEXACARBATE) INSECTICIDE ON
BRAIN CHOLINESTERASE (CHE) ACTIVITY OF FOREST
SONGBIRDS IN NEW BRUNSWICK, CANADA

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S.B. HOLMES

Government of Canada
Forestry Service
Forest Pest Management Institute
P.O. Box 490
Sault Ste. Marie, Ontario
P6A 5M7

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*Director
Forest Pest Management Institute
Canadian Forestry Service
P.O. Box 490
Sault Ste. Marie, Ontario
P6A 5M7*

A Preliminary Report on the Impact of Aerially Applied Zectran®
(Mexacarbate) Insecticide on Brain Cholinesterase (ChE) Activity of
Forest Songbirds in New Brunswick, Canada

A joint Canadian Forestry Service (CFS)/Canadian Wildlife Service (CWS) study to investigate the impact on forest songbirds of spraying with Zectran® (mexacarbate) insecticide was conducted near Bathurst, New Brunswick in June, 1984. This preliminary report deals with the results of the CFS (Forest Pest Management Institute) portion of the study only.

Materials and Methods

The study area was located about 35 km southwest of Bathurst, New Brunswick (Fig. 1), within the New Brunswick Uplands section of the Acadian forest region (Rowe 1972). This forest is composed mainly of balsam fir (*Abies balsamea*), black spruce (*Picea mariana*), white spruce (*P. glauca*), and white birch (*Betula papyrifera*) with eastern white pine (*Pinus strobus*) occurring commonly in mixed stands of these species. Yellow birch (*B. alleghaniensis*) is frequently present as a minor component of the forest. Near the borders of the section in the northeast, where our spray blocks were located, there are occasional ridges of sugar maple (*Acer saccharum*) and beech (*Fagus grandifolia*).

Two 300 ha spray blocks were treated by aerial application with Zectran® (mexacarbate) insecticide. One block (Z1) received a

single dose of 70 g AI (active ingredient)/ha and the other block (Z2) received two consecutive doses totalling 140 g AI/ha to simulate over swathing. The spray mixture was the same for both applications and consisted of 22% Zectran® UCZF19, 3% Triton® X-114, 1% Rhodamine red dye and 74% water by volume. Subsequent analyses of the two tank mixes revealed that the spray mixtures for Z1 and Z2 contained approximately 75% and 93% of the expected concentration of active ingredient (4.86% by weight), respectively. Technical details of the spray applications are given in Table 1.

Deposit sampling units, each comprised of a 10 x 10 cm Kromekote® card and two 7.5 x 5.0 cm glass slides, were placed at 100 m intervals along the road which bisected the spray blocks (Fig. 1). Spray droplets were counted and measured on the Kromekote® cards using an NCR Microcard reader to provide information on droplet density and size distribution of the deposited spray. The glass slides were washed with 95% ethanol and the eluate was analysed colorimetrically for dye content with a Bausch and Lomb Spectronic® 100 spectrophotometer to provide an estimate of the deposited volume of spray liquid across each block.

Birds were collected in the two spray blocks with a .410 shotgun from 1-96 hr after spraying. Control birds were collected on 19, 21 and 22 June from an area of unsprayed forest approximately 3 km east of Z2. Birds in which the brain received heavy shot damage were not included in the analyses. The species were chosen to represent all foraging levels in the forest and included Tennes-

see warblers (*Vermivora peregrina*), Cape May warblers (*Dendroica tigrina*), magnolia warblers (*D. magnolia*), yellow-rumped warblers (*D. coronata*), bay-breasted warblers (*D. castanea*), American redstarts (*Setophaga ruticilla*) and white-throated sparrows (*Zonotrichia albicollis*). Brains were immediately removed from the dead birds, split medially into right and left halves, placed in separate polyethylene serum vials and stored in liquid nitrogen (-196°C) until assayed. Cholinesterase (ChE) activity was determined colorimetrically for one half of each brain at the Forest Pest Management Institute laboratory in Sault Ste. Marie, Ontario and for the other half at the Canadian Wildlife Service laboratory in Fredericton, New Brunswick using the technique of Ellman et al. (1961) as modified by Hill and Fleming (1982). Only the results of the analyses conducted at FPMI will be discussed here.

In addition to collecting free-ranging birds, wild white-throated sparrows were caged individually in modified dog kennels in the double application block (Z2). On spray day birds were exposed to the spray at five locations across the block. Nine, 53 and 76 hours post-spray one bird from each location was scarified and its whole brain removed and stored in liquid nitrogen. These were later analysed at FPMI for ChE activity as above. Birds caged in an unsprayed area were used as a control.

Results and Discussion

The volume of spray mixture deposited on Z2 was only about

23% more than on Z1 even though the nominal application rate was twice as high on Z2 (Table 2). The droplet density on Z2 was more than five times heavier than on Z1 however. Further deposit analysis, including calculation of the NMD and VMD for each spray, should help to clarify this.

Two songbird species had brain ChE activities significantly depressed ($P < 0.05$) following the single application of 70 g AI/ha to spray block Z1 (Table 3). Brain ChE activity was depressed by 10.5% in white-throated sparrows and by 13.4% in yellow-rumped warblers, both on spray day. The greatest depression seen was in magnolia warblers from Z1 which had ChE levels 15.5% below normal on spray day and which remained depressed up to 3 days post-spray. Tennessee warblers, although they showed no depression on spray day, had lower than normal ChE activities 2 and 3 days post-spray. When change in brain ChE activity is averaged over the 4 day post-spray sampling period, it appears that magnolia warblers, Tennessee warblers and white-throated sparrows were the species most affected by the spray, with depressions of 10.7%, 3.7% and 2.8%, respectively.

Only one species showed a significant ChE depression ($P < 0.05$) following the double application of 2 x 70 g AI/ha to spray block Z2 (Table 3). The average ChE activity of Tennessee warblers was 15.2% below normal on spray day and remained depressed up to 3 days post-spray. Three other species had lower than normal ChE activities when averaged over the 4 day post-spray period. Once

again magnolia warblers appear to have been most affected (12.2% depression), followed by Tennessee warblers (10.6%), yellow-rumped warblers (8.1%) and Cape May warblers (4.7%).

There was no statistically significant difference in the ChE values of any of the species collected from the untreated control area (ANOVA, $P < 0.05$). Samples could therefore be lumped for analysis of differences in depression between the two dosage rates. Only birds collected 2 days post-spray from Z2 showed significant ChE depression ($P < 0.05$) (Table 3). When ChE activity was averaged over the 4 day post-spray period, birds from Z1 and Z2 were not significantly different from control birds. Birds from Z2 did have significantly lower ChE activity than birds from Z1 however ($P < 0.05$).

The percent change in ChE activity for all birds is presented in Table 4. Overall ChE activity was more depressed on Z2 than on Z1. In addition depressed ChE levels persisted over at least 4 days on Z2 but had recovered to normal by 2 days post-spray on Z1. When sparrows and warblers are lumped together it appears that the ChE inhibition on spray day was greater on Z1 than on Z2. There is some rationale for considering warblers (Parulidae) separately however, since not only are sparrows (Fringillidae) from a different family, but they also have a different foraging strategy and were collected from a different habitat (clearcut for sparrows and forest for warblers). For warblers alone the pattern of inhibition remains basically the same except that the depression on spray day was greater on Z2 than on Z1.

The suggestion by Ludke et al. (1975) that 20% brain ChE inhibition is indicative of exposure and that 50% inhibition is sufficient for diagnosing cause of death has gained general acceptance (DeWeese et al. 1979; Busby et al. 1981, 1982, 1983; Hamilton et al. 1981). ChE inhibition can exceed 50% under certain circumstances without mortality occurring however (Ludke et al. 1975; Zinkl et al. 1979, 1980; Fleming 1981). A modification of this concept by Zinkl et al. (1980) considered a ChE activity less than the lower of two values, the mean activity of the control birds minus 20% of the mean or the mean activity of the control birds minus 2 SD (standard deviations), to be depressed. Fifteen of the 134 birds collected from Z1 (11.2%) had brain ChE inhibition greater than 20% relative to the controls (Table 5). This is only slightly more than the proportion of control birds with ChE depression more than 20% below the average (9.3%). Magnolia warblers, yellow-rumped warblers, Tennessee warblers and white-throated sparrows accounted for 13 of the 15 birds. A magnolia warbler from Z1 3 days post-spray with brain ChE activity 51.6% below normal was the only bird inhibited by more than 50%. Twenty-eight of the 147 birds from Z2 (19%) had greater than 20% ChE inhibition. The same four species as in Z1 accounted for 24 of the 28 birds. In addition 10 birds from Z2 (6.8%) had ChE levels more than 2 SD below the mean value for controls. None of the controls had ChE levels this low.

Some birds from each area, including the control area, had higher than normal ChE activities (Table 5). In general the pro-

portion of birds with higher than normal levels of activity was greater than the proportion with lower than normal levels of activity in the control area and spray block Z1, whereas the opposite was true in spray block Z2.

There were no significant differences (Two sample t-test, $P < 0.05$) between white-throated sparrows caged in Z2 and those in the control area. None of the caged birds exposed to the spray had ChE activities depressed more than 20% or 2 SD below the mean value for controls.

Conclusions

Our preliminary results indicate that aerial spraying of Zectran® had only a slight inhibitory effect on brain ChE activity in forest songbirds, even when it was applied at twice the recommended dosage rate. We observed no birds exhibiting symptoms of anti-cholinesterase poisoning even though more than 300 man-hours of time were spent in the field in the two spray blocks. Applying the criteria that 20% brain ChE inhibition (or 2 SD, whichever is greater) indicates exposure and 50% inhibition suggests some mortality, it is likely that few, if any, birds were killed as a result of either spray and that sublethal effects were minimal. Our preliminary analysis of the data does not suggest that a dosage rate of 70 g AI/ha of Zectran® sprayed as a water emulsion for spruce budworm control exceeds an adequate margin of safety for forest songbirds.

Literature Cited

- Busby, D.G., Pearce, P.A. and Garrity, N.R. 1981. Brain cholinesterase response in songbirds exposed to experimental fenitrothion spraying in New Brunswick, Canada. Bull. Environ. Contam. Toxicol. 26:401-406.
- Busby, D.G., Pearce, P.A. and Garrity, N.R. 1982. Brain cholinesterase inhibition in forest passerines exposed to experimental aminocarb spraying. Bull. Environ. Contam. Toxicol. 28:225-229.
- Busby, D.G., Pearce, P.A., Garrity, N.R. and Reynolds, L.M. 1983. Effect of an organophosphorus insecticide on brain cholinesterase activity in white-throated sparrows exposed to aerial forest spraying. J. Appl. Ecol. 20:255-263.
- DeWeese, L.R., Henny, C.J., Floyd, R.L., Bobal, K.A. and Schultz, A.W. 1979. Response of breeding birds to aerial sprays of trichlorfon (Dylox) and carbaryl (Sevin-4-Oil) in Montana forests. U.S. Fish and Wildlife Service, Special Scientific Report-Wildlife No. 224.
- Ellman, G.L., Courtney, K.D., Andres, Jr. V., and Featherstone, R.M. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7:88-95.
- Fleming, W.J. 1981. Recovery of brain and plasma cholinesterase activities in ducklings exposed to organophosphorus pesticides. Arch. Environ. Contam. Toxicol. 10:215-229.

- Hamilton, G.A., Hunter, K. and Ruthven, A.D. 1981. Inhibition of brain acetylcholinesterase activity of songbirds exposed to fenitrothion during aerial spraying of forests. Bull. Environ. Contam. Toxicol. 27:856-863.
- Hill, E.F. and W.J. Fleming. 1982. Anticholinesterase poisoning in birds: Field monitoring and diagnosis of acute poisoning. Environ. Toxicol. Chem. 1:27-38.
- Ludke, J.L., Hill, E.F. and Dieter, M.P. 1975. Cholinesterase (ChE) response and related mortality among birds fed ChE inhibitors. Arch. Environ. Contam. Toxicol. 3:1-21.
- Rowe, J.S. 1972. The forest regions of Canada. Canadian Forestry Service Publication No. 1300.
- Zinkl, J.G., Henny, C.J. and Shea, P.J. 1979. Brain cholinesterase activities of passerine birds in forests sprayed with cholinesterase inhibiting insecticides. In Animals as Monitors of Environmental Pollutants, National Academy of Sciences, Washington. pp. 356-365.
- Zinkl, J.G., Roberts, R.B., Henny, C.J. and Lenhart, O.J. 1980. Inhibition of brain cholinesterase activity in forest birds and squirrels exposed to aerially applied acephate. Bull. Environ. Contam. Toxicol. 24:676-683.

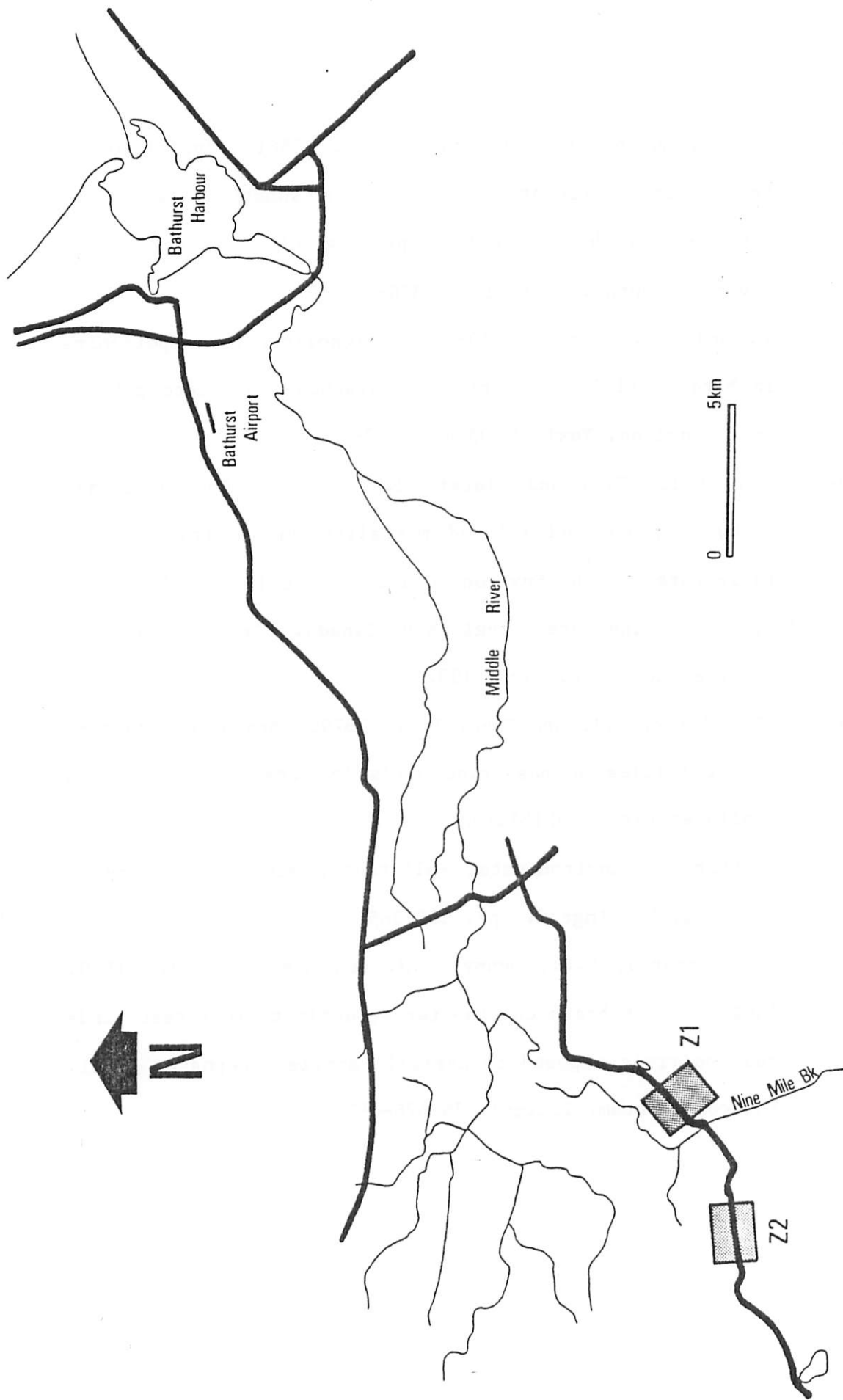


Figure 1. Map showing location of spray blocks and air strip near Bathurst, New Brunswick.

Table 1. Technical details of the spray applications to spray blocks Z1 and Z2.

	Block Z1	Block Z2
Dosage rate (active ingredient)	70 g/ha	2 x 70 g/ha
Application rate (total formulation)	1.46 L/ha	2 x 1.46 L/ha
Date and time of application	June 18, 05:54 - 06:11	June 14, 07:08 - 07:42
Aircraft	2 Cessna 188 Ag trucks	
Emission system	4 Micronair AU3000 atomizers	
	Blade angle: 30°	
	Blade CPB 252: 34 cm	
	Cage size: 13 cm	
	VRU setting: 9	
	Flow rate: 28.8 L/min	
	Swath width: 67 m	

Table 2. Deposit assessment for single application (70 g AI/ha) to spray block Z1 and double application (2 x 70 g AI/ha) to spray block Z2.

	Z1	Z2
No. of deposit samplers	16	24
Volume emitted	1.46 L/ha	2.92 L/ha
deposited	0.035 L/ha	0.043 L/ha
% deposited	2.4%	1.5%
Droplet density mean \pm SD	2.6 \pm 3.3 dr/cm ²	14.7 \pm 7.9 dr/cm ²
range	0 - 10.5 dr/cm ²	0 - 34.6 dr/cm ²

Table 3. Mean brain cholinesterase activity (mU/mg brain tissue) in forest songbirds from blocks sprayed with mexacarbate at rate of 70 g AI/ha (Z1) and 140 g AI/ha (Z2) and from an untreated control area.

Species	Block	Spray Day	Number of days post-spray			Average
			+1	+2	+3	
White-throated Sparrow	Control	--	--	--	--	27.88 ± 3.67 (23) ⁺
	Z1	24.94 ± 3.43 (9)*	27.47 ± 4.40 (12)	27.34 ± 4.90 (8)	28.37 ± 2.73 (10)	27.09 ± 3.98 (39)
	Z2	29.46 ± 5.96 (13)	28.88 ± 3.64 (10)	25.66 ± 4.38 (11)	26.96 ± 4.89 (11)	27.79 ± 4.96 (45)
Cape May Warbler	Control	--	--	--	--	28.57 ± 5.20 (10)
	Z1	29.02 ± 4.66 (5)	--	32.71 ± 7.10 (4)	28.86 ± 4.90 (4)	30.11 ± 5.39 (13)
	Z2	27.07 ± 3.01 (4)	--	27.10 ± 4.81 (10)	27.49 ± 6.33 (7)	27.22 ± 4.88 (21)
Bay-breasted Warbler	Control	--	--	--	--	24.99 ± 5.23 (10)
	Z1	25.11 ± 4.82 (8)	--	28.99 ± 6.10 (11)	29.77 ± 3.10 (11)	28.24 ± 5.05 (30)
	Z2	26.73 ± 4.73 (6)	--	26.48 ± 5.96 (11)	23.92 ± 3.13 (10)	25.59 ± 4.80 (27)
Tennessee Warbler	Control	--	--	--	--	29.88 ± 4.32 (10)
	Z1	30.24 ± 6.01 (7)	--	27.48 ± 7.09 (3)	28.19 ± 2.82 (11)	28.77 ± 4.58 (21)
	Z2	25.33 ± 3.38 (10)*	--	27.81 ± 6.37 (10)	27.03 ± 4.22 (10)	26.72 ± 4.77 (30)*
Magnolia Warbler	Control	--	--	--	--	29.79 ± 8.08 (11)
	Z1	25.18 ± 3.94 (5)	--	27.53 ± 6.48 (8)	26.53 ± 2.98 (8)	26.59 ± 4.66 (21)
	Z2	27.00 ± 5.50 (4)	--	25.42 ± 3.24 (6)	26.74 ± 16.10 (2)	26.17 ± 6.10 (12)
American Redstart	Control	--	--	--	--	27.24 ± 6.37 (9)
	Z1	27.26 ± 5.70 (5)	--	27.63 ± 0.71 (2)	--	27.36 ± 4.67 (7)
	Z2	--	--	--	--	--
Yellow-rumped Warbler	Control	--	--	--	--	29.56 ± 5.99 (11)
	Z1	25.59 ± 1.64 (2)*	--	30.47 ± 7.73 (3)	34.49 ± 6.50 (5)	30.90 ± 7.41 (10)
	Z2	28.16 ± 6.25 (4)	--	22.84 ± 7.71 (3)	28.95 ± 2.22 (5)	27.16 ± 5.49 (12)
Total	Control	--	--	--	--	28.26 ± 5.47 (84)
	Z1	26.57 ± 4.91 (41)	27.47 ± 4.40 (12)	28.66 ± 5.88 (39)	29.01 ± 3.99 (49)	28.07 ± 4.93 (141)**
	Z2	27.45 ± 4.94 (41)	28.88 ± 3.64 (10)	26.35 ± 5.23 (51)*	26.59 ± 5.03 (45)	26.90 ± 5.00 (147)**

⁺ Mean brain cholinesterase activity ± standard deviation (n).

* Mean cholinesterase activity is significantly lower than the mean for controls of that species (Two-sample t-test, $p < 0.05$).

** Values are significantly different from each other (Two-sample t-test, $p < 0.05$).

Table 4. Percent change in brain cholinesterase (ChE) activity of songbirds collected from spray blocks Z1 and Z2 relative to untreated controls.

	Block	Change in brain ChE activity Number of days post-spray				
		Spray day	+1	+2	+3	Average
Sparrows (Fringillidae)	Z1	-10.5	-1.5	-1.9	1.8	-2.8
	Z2	5.7	3.6	-8.0	-3.3	-0.3
Warblers (Parulidae)	Z1	-4.8	-	2.1	2.7	0.2
	Z2	-6.6	-	-2.6	-6.8	-5.1
Total (Fringillidae and Parulidae)	Z1	-6.0	-*	1.4	2.7	-0.7
	Z2	-2.9	-*	-6.8	-5.9	-4.8

* No value because warblers were not collected on this day.

Table 5. Number of songbirds with lower and higher than average brain cholinesterase (ChE) activity collected from spray blocks Z1 and Z2 and from an untreated control area.

Species	Block	Number of birds collected	Number with lower than average ChE activity ($\bar{x} - 0.20 \bar{x}$) ⁺ ($\bar{x} - 2 \text{ S.D.}$) ⁺⁺		Number with higher than average ChE activity ($\bar{x} + 0.20 \bar{x}$) ($\bar{x} + 2 \text{ S.D.}$)	
White-throated Sparrow	Control	23	1 (4.3) ^x	0	1 (4.3)	1 (4.3)
	Z1	39	3 (7.7)	0	2 (5.1)	2 (5.1)
	Z2	45	6 (13.3)	2 (4.4)	4 (8.9)	1 (2.2)
Cape May Warbler	Control	10	1 (10.0)	0	1 (10.0)	0
	Z1	13	1 (7.7)	0	5 (38.5)	0
	Z2	21	2 (9.5)	2 (9.5)	2 (9.5)	0
Bay-breasted Warbler	Control	10	2 (20.0)	0	3 (30.0)	0
	Z1	30	1 (3.3)	0	8 (26.7)	3 (10.0)
	Z2	27	2 (7.4)	0	3 (11.1)	1 (3.7)
Tennessee Warbler	Control	10	0	0	1 (10.0)	0
	Z1	21	3 (14.3)	0	2 (9.5)	0
	Z2	30	9 (30.0)	5 (16.7)	1 (3.3)	0
Magnolia Warbler	Control	11	2 (18.2)	0	1 (9.1)	1 (9.1)
	Z1	21	5 (23.8)	0	0	0
	Z2	12	6 (50.0)	0	1 (8.3)	0
American Redstart	Control	9	2 (22.2)	0	1 (11.1)	1 (11.1)
	Z1	7	1 (14.3)	0	1 (14.3)	0
	Z2	--	--	--	--	--
Yellow-rumped Warbler	Control	11	1 (9.1)	0	1 (9.1)	1 (9.1)
	Z1	10	2 (20.0)	0	3 (30.0)	1 (10.0)
	Z2	12	3 (25.0)	1 (8.3)	1 (8.3)	0
Total*	Control	75	7 (9.3)	0	8 (10.7)	3 (4.0)
	Z1	134	15 (11.2)	0	20 (14.9)	6 (4.5)
	Z2	147	28 (19.0)	10 (6.8)	12 (8.2)	2 (1.4)

⁺ ($\bar{x} \pm 0.20 \bar{x}$) mean ChE activity for control birds \pm 20 percent of the mean.

⁺⁺ ($\bar{x} \pm 2 \text{ S.D.}$) mean ChE activity for control birds \pm 2 standard deviations about the mean.

^x Number in parentheses is percent of sample.

* American redstarts are not included in the totals because none were collected from Z2.