Bull. Environm. Contam. Toxicol. 26, 401-406 (1981)

Brain Cholinesterase Response in Songbirds Exposed to Experimental Fenitrothion Spraying in New Brunswick, Canada

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The province of New Brunswick is currently searching for acceptable methods of protecting forests against spruce budworm (Choristoneura fumiferana) in areas of human habitation and ecological sensitivity. To that end, it conducted spray trials in the spring of 1980 to determine the efficacy of a formulation of fenitrothion (0,0-dimethyl 0-(3-methyl-4-nitrophenyl) phosphorothioate) containing a low-drift additive, applied by two types of spray hardware (boom and nozzle (B&N), and rotary atomizer (RA)). The conventional fenitrothion dosage was increased from 210 to 280 g/ha and spray applied by Cessna rather than the usual Grumman Avenger (TBM) aircraft.

Those modifications represent a major departure from the usual spraying methodology employed in that province. A considerable part of New Brunswick's spruce-fir forest lies adjacent to ecologically sensitive areas or human habitation and has in recent years been excluded from spray zones. Safe low-drift spraying thus has potential application for wide areas. As a part of long-term research on the effects of insecticide spraying on forest songbirds, we undertook to determine bird responses to the spray trials. Determination of avian brain cholinesterase (ChE) activities was used to measure exposure. It is a convenient, accurate and sensitive method which, when coupled with detailed studies of sub-lethal effects of ChE inhibition, shows much promise for spray impact assessment.

MATERIALS AND METHODS

Twelve experimental plots, each about 40 ha and located in spruce-fir forest in southwest New Brunswick, were sprayed by a Cessna 188 Ag-Truck. Fenitrothion was sprayed at a dosage of 280 g/ha. By volume, the formulation consisted of 4.73% fenitrothion (active ingredient), 0.125% Nalco-Trol^{R} (drift control agent), 0.64% Dowanol^{R} (solvent), 0.64% Atlox^{R} (emulsifier) and the remainder water (carrier), emitted at a rate of 4.67 L/ha. The aircraft was fitted with B&N equipment for treatment of six plots and with RA equipment for the remaining six plots. Within each six-plot spray system,

0007-4861/81/0026-0401 $01.20

TABLE 1

Mean brain ChE activity (mU/mg brain) in songbirds from sprayed and unsprayed plots.

	A (unsprayed)	B (RA)[1]	C (B&N)[2]	% Inhibition	
				A - B	A - C
Tennessee Warbler	33.83(10)[3]	33.15(22)	31.25(23)	-2.0(N.S.)[4]	-7.6(N.S.)
Magnolia Warbler	35.54(10)	28.17(9)	31.04(16)	-20.7(P<0.05)	-12.7(N.S.)
Blackburnian Warbler	34.04(5)	30.11(8)	-	-11.6(N.S.)	-
Bay-breasted Warbler	33.92(12)	-	22.99(7)	-	-32.2(P<0.001)
White-throated Sparrow	36.36(10)	31.85(16)	31.12(13)	-12.4(P<0.05)	-14.4(N.S.)

[1]Plots sprayed with rotary atomizer equipment.
[2]Plots sprayed with boom and nozzle equipment.
[3]Sample size in parentheses.
[4]Level of significance, Mann-Whitney U-test(N.S.= not significantly different).

three plots were sprayed with Nalco-Trol[R], a low-drift additive included in the spray formulation, and three without.

Avian response to spraying was monitored on the six plots where the low-drift additive was included in the spray formulation. Sprays were applied between 28 May and 6 June 1980. Birds were collected with a .410 shotgun 6 to 48 h after spraying. The following five most abundant species were sampled: Tennessee Warbler (*Vermivora peregrina*), Magnolia Warbler (*Dendroica magnolia*), Blackburnian Warbler (*D. fusca*), Bay-breasted Warbler (*D. castanea*) and White-throated Sparrow (*Zonotrichia albicollis*). Other birds of each species were collected from unsprayed forest at least 10 km from the nearest spraying activity. Samples were placed on dry ice (-76 C) immediately after collection and analysed later, storage time varying between two and four weeks.

Brain ChE activity was determined using the methods of ELLMAN et al. (1961), as modified by HILL and FLEMING (in press).

RESULTS

All species sampled showed some degree of brain ChE inhibition when compared with control birds (TABLE 1). Bay-breasted Warblers from B&N spray plots exhibited the most pronounced depression and Magnolia Warblers and White-throated Sparrows from the RA plots were also significantly affected. B&N spraying caused the most ChE depression, inhibiting 30% of the samples by more than 20% (TABLE 2). Three birds were inhibited more than 50% by each of the spray systems. The greatest ChE reduction (59%) was in a White-throated Sparrow from a B&N spray plot.

TABLE 2. Number and percent of birds from sprayed plots with brain ChE inhibited more than 20% and 50%.

Species	RA[1] 20%	RA[1] 50%	B&N[2] 20%	B&N[2] 50%
Tennessee Warbler	2[3](9)[4]	1 (4)	5(22)	0 (0)
Magnolia Warbler	3(33)	1(11)	5(31)	1 (6)
Blackburnian Warbler	3(37)	0 (0)	-	-
Bay-breasted Warbler	-	-	4(57)	1(14)
White-throated Sparrow	1 (6)	1 (6)	4(31)	1 (8)
Total	9(16)	3 (5)	18(30)	3 (5)

[1]Plots sprayed with rotary atomizer equipment.
[2]Plots sprayed with boom and nozzle equipment.
[3]Number of birds exceeding level of inhibition.
[4]Percent of birds exceeding level of inhibition.

There was no statistical difference in ChE values of any of the control species. Samples could therefore be lumped for analysis of differences in depression between the two spray systems and between the individual plots. B&N and RA sprayed birds both showed significant ChE inhibition (P<0.001 and P<0.02, respectively; t-test). However ChE values for B&N-sprayed birds were lower than RA-sprayed birds (P<0.02, t-test).

Birds from all B&N-sprayed plots showed significant ChE reduction (TABLE 3). One RA-sprayed plot (plot 9) showed significant depression and another (plot 1) had a low ChE value although the small sample size did not warrant statistical analysis. Birds from plot 8 showed ChE activities similar to control birds. Of the 114 birds collected, 27(24%) and 6(5%) showed ChE depression of more than 20% and 50%, respectively.

TABLE 3. Mean brain ChE activity (mU/mg brain) of unsprayed birds and birds collected from each spray plot.

	RA plots[1]			B&N plots[2]		
Unsprayed	1	8	9	2	4	11
34.9	29.3[3]	34.1*	31.0**	24.2**	21.2**	32.4**
49[4]	3	28	28	8	10	45

[1] Plots sprayed with rotary atomizer equipment.
[2] Plots sprayed with boom and nozzle equipment.
[3] Not sufficient sample size for statistical analysis.
[4] Sample size.
* Not significantly different from unsprayed birds.
** Significantly less than unsprayed birds (P<0.05, t-test).

DISCUSSION

Measurement of brain ChE activities has in recent years been successfully employed to assess the impact of organophosphate and carbamate insecticide spraying on birds (ZINKL et al. 1977, DEWEESE et al. 1979, RICHMOND et al. 1979, WHITE et al. 1979, ZINKL et al. 1979, ZINKL et al. 1980). LUDKE et al. (1975) suggested that brain ChE inhibition exceeding 20% was indicitive of exposure, and inhibition greater than 50% was sufficient for attributing the death of a bird to the insecticide. Those figures are compatible with our findings elsewhere that no mortality was associated with 15 to 20% inhibition, and death of about 20% of free-living adult White-throated Sparrows was associated with a mean ChE reduction of 42% when they were exposed to aerial spraying of fenitrothion in New Brunswick (in prep.).

The present study indicates that significant exposure of birds to fenitrothion, applied as previously described, can be expected. The B&N system appeared to cause a greater impact than the RA. All species sampled, regardless of foraging height, showed some degree of ChE depression. The relatively slight effect on RA-sprayed Tennessee Warblers appears inconsistent with reports by MOULDING (1976), ZINKL et al. (1977) and PEARCE et al. (1979) that upper canopy dwellers are the most affected. However 15 of the 23 RA-sprayed Tennessee

Warblers were collected from the one plot (plot 8) where little inhibition occurred in any species (TABLE 3). We did not witness the spraying and can only speculate that the plot received less spray than the others. The White-throated Sparrow, essentially a ground dweller, showed exposure equal to that in the upper canopy warblers. In New Brunswick, the White-throated Sparrow is primarily a bird of forest clear-cuts, and thus may be as exposed to the spray as canopy birds.

Estimates of the tolerance of birds to ChE inhibition differ considerably (LUDKE et al. 1975, ZINKL et al. 1977, 1979). Although it is generally accepted that birds eventually recover from non-lethal doses of ChE-inhibiting insecticides, possible sub-lethal effects of such poisoning have received little attention. Reduced ChE levels could increase suceptibility to predation, affect ability to establish and defend a territory, and decrease food-gathering efficiency. We have found reduced singing activity, nest desertion, increased nest predation, adult and nestling mortality, and decreased nestling growth rates and fledging weights associated with 40-50% ChE inhibition in White-throated Sparrows in fenitrothion-sprayed forest (in prep.).

Other features of forest spray operations make interpretation of our results difficult. PEARCE (1968) alluded to differences between morning and evening sprays, the former apparently having greater adverse effects on forest songbirds, presumably because the birds are then more active and thus more exposed to the sprays. The present study provides supportive evidence: ChE activity in birds from two plots sprayed with B&N equipment during the early morning was more substantially reduced than in birds exposed on the third plot to evening spraying with the same equipment. The apparently greater impact of B&N application may be only a function of spray timing as all RA plots were treated during late morning. In terms of avian activity, late morning is probably more comparable to evening. ChE levels in birds from plot 11 (B&N, evening spray) closely resemble those in birds from plots 8 and 9 (RA, late morning). Further confirmation is needed of the relative impact of the B&N and RA spray systems, and the importance of the presence or absence of drift-reducing additives in the spray formulation.

We found no dead birds and observed no abnormal avian behavior after the spraying. It seems likely that our collecting selected for birds less affected by the sprays, since sick birds would be inactive and thus more easily overlooked. Further bias may result from our sampling up to only 48 hours after spraying. ZINKL et al. (1980) showed that with some insecticides maximum ChE depression can occur three days after exposure. Our sample sizes are insufficient to assess differences between the first and second days post-spray.

Storage of birds for up to four weeks should not have affected ChE activity. ZINKL et al. (1977) reported unpublished observations that dry ice preserved the enzyme, and we have found that storage of Brown-headed Cowbird (Molothrus ater) brains on dry ice for up to 16 weeks did not significantly affect ChE activity (unpublished data).

ACKNOWLEDGEMENTS

We gratefully acknowledge the assistance of D. Kimball, J. Maxwell, D. Morton and D. Noble in collecting the birds. Special thanks are expressed to B. Pendrel, of Forest Protection Ltd., the New Brunswick forest spray agency, for cooperation throughout the study, and to J. Fleming, of Patuxent Wildlife Research Center, for advice and instruction on ChE assay techniques.

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