Brain Cholinesterase Activities of Birds from Forests Sprayed with Trichlorfon (Dylox) and Carbaryl (Sevin-4-oil)

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SUMMARY

Brain cholinesterase activities were determined in birds from forests sprayed with Dylox[2] at 1.13 kg/hectare (1 lb/acre - active ingredient [a.i.]) or Sevin-4-oil[2] at 1.13 kg/hectare (1 lb/acre - a.i.) for up to 5 days postspray. Of ten bird species evaluated from the Dylox spray area, four species represented by six individuals had values which were depressed more than 2 standard deviations below the mean. Three of these activities (two species) were about 20% less than the mean. Of 12 species evaluated from the Sevin-4-oil spraying, three individuals representing three species had depressed values. One value was depressed greater than 20% below the mean. Half of the depressed activities were in canopy-dwelling birds collected on the day of spray.

INTRODUCTION

During summer 1975, in the Beaverhead National Forest of southwestern Montana, we studied the impact of dimethyl(2,2,2-trichloro-1-hydroxyethyl) phosphonate (trichlorfon, Dylox) and 1-naphthyl N-methylcarbamate (carbaryl, Sevin-4-oil) on resident breeding bird populations (DEWEESE and HENNY 1976, ZINKL et al. 1976). The study plan included: (1) an evaluation of reproductive performance (nesting success), (2) the estimation of breeding pair density before and after spray within major habitats, (3) estimation of total birds at fixed stations in each major habitat, (4) exploration into the food habits of the resident birds as they related to the spruce budworm and other important insect groups, and (5) determination of brain cholinesterase activities from abundant and diverse avian species. The latter is the topic of this report.

Details of the plot locations, dates sprayed, application rates, formulation of the insecticides and operational summaries

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[2]Reference to trade names does not imply U.S. Government endorsement of commercial products.

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are given elsewhere (DEWEESE and HENNY 1976). Briefly, among nine 400-800-ha (1,000-2,000-acre) plots, three were sprayed by helicopter with a Sevin-4-oil formulation, three with Dylox and three were untreated. Each was a single application made early in the morning at a calculated rate of 1.13 kg/hectare (1 lb/acre - a.i.).

Since Dylox and Sevin-4-oil are organophosphate and carbamate insecticides, respectively, they inhibit cholinesterase enzymes. By inhibiting acetylcholinesterase, they interfere with cholinergic nerve transmissions. Signs of cholinesterase inhibitor poisoning include myosis, salivation, lacrimation (muscurinic effects), muscle twitching, dyspnea, paralysis and clonic convulsions (nicotinic effects). Death is due to asphyxiation from paralysis of respiratory muscles and/or inhibition of the central respiratory center (O'BRIEN 1967:56).

Since cholinesterase activity is easily measured, its measurement can be used to determine if an animal has been poisoned with organophosphate or carbamate insecticides (STICKEL 1974). However, certain precautions must be taken in order to assure that the results are valid. The cholinesterase activity of birds suspected to have been poisoned with cholinesterase inhibitors must be compared with that of unpoisoned birds of the same species because of the great variation of activity between species (STICKEL 1974). Secondly, storage of the enzyme-containing tissue should be such as not to cause any deterioration of enzyme between the time of death and the time of analysis (STICKEL 1974, LUDKE et al. 1975). With these precautions in mind, brain cholinesterase activities were determined in birds collected from Montana forest areas sprayed with either Dylox or Sevin-4-oil.

MATERIALS AND METHODS

Birds were collected by mist netting or by shooting with shotguns. The birds collected with mist nets were killed by asphyxiation in CO_2. Either whole birds or heads were frozen on dry ice until the brains were dissected for analysis. Occasionally, the brain of a bird collected by gun was discarded because of excessive damage and tissue loss. This precaution was taken because different areas of bird brains have different cholinesterase activities (KNITTLE and TUCKER 1974).

Control birds and treatment birds (spray area) were collected from similar habitats. Control birds were collected before spraying and during the time of spraying in order to determine if a short-term temporal change in cholinesterase activity occurred. Since both sexes were collected, it was also possible to determine if there were any differences due to sex. All analyses were carried out within 12 hr after collection.

After removal from the calvarium, brains were homogenized in cold 0.1 M phosphate buffer (pH 7.4) at a 1:5 dilution. They were then diluted to either 1:50 or 1:100 with the phosphate buffer just

prior to analysis. The Ellman (ELLMAN et al. 1961) method was adapted to determine brain cholinesterase activity (DIETER and LUDKE 1975). The reagents for this technique were obtained in kit form from BMC Corporation, Dallas, Texas. A Spectronic 88 spectrophotometer (Bausch & Lomb) fitted with a water-jacketed micro flow-through cell was used for determining the activity. Optical density readings were taken every 30 seconds for 3 minutes in order to assure that the reaction was linear. All analyses were carried out at 25°C.

RESULTS

Cholinesterase activities of 25 species of the orders Passeriformes (22 species) and Piciformes (3 species) taken from unsprayed forests were determined. Although sufficient data were obtained for only 14 species from the spray areas to be useful in evaluating the effects of the spray, no short-term temporal effects or sex differences were found. The brain cholinesterase activities of birds from control areas are given in Table 1. The two species with the highest activities were both woodpeckers, the yellow-bellied sapsucker and the hairy woodpecker (47.2 and 42.5 mU/mg brain, respectively) (Table 1).

TABLE 1

Brain cholinesterase activities[a] of birds from unsprayed forests

Taxonomic classification	No. of birds	Mean±S.D
Order Piciformes (woodpeckers, jacomars, toucans and barbets)		
Family Picidae (woodpeckers and wrynecks)		
Dendrocopos villosus (hairy woodpecker)	2	42.5±1.2
Sphyrapicus varius (yellow-bellied sapsucker)	4	47.5±4.3
Colaptes auratus (common flicker)	3	24.8±1.2
Order Passeriformes (perching birds)		
Family Tyrannidae (tyrant flycatchers)		
Empidonax spp.[b]	8	22.2±2.6
Family Fringillidae (grosbeaks, finches, sparrows and buntings)		
Hesperiphona vespertina (evening grosbeak)	6	31.9±2.1
Carpodacus cassinii (Cassin's finch)	1	20.7
Spinus pinus (pine siskin)	10	22.2±2.4
Zonotrichia leucophrys (white-crowned sparrow)	6	31.4±2.8
Spizella passerina (chipping sparrow)	7	23.5±1.8
Junco hyemalis (dark-eyed junco)	9	33.2±1.1
Melospiza lincolnii (Lincoln's sparrow)	5	25.2±1.4
Chlorura chlorura (green-tailed towhee)	3	35.7±4.3
Passerina amoena (lazuli bunting)	5	31.6±2.7

TABLE 1 (cont'd)

Family Thraupidae (tanagers)		
Piranga ludoviciana (western tanager)	8	28.3±2.9
Family Hirundinidae (swallows)		
Iridoprocne bicolor (tree swallow)	5	21.2±3.0
Family Vireonidae (vireos)		
Vireo gilvus (warbling vireo)	10	33.8±3.9
Family Parulidae (wood warblers)		
Dendroica petechia (yellow warbler)	1	36.2
Dendroica coronata (yellow-rumped warbler)	6	33.0±2.9
Oporornis tolmiei (MacGillivray's warbler)	5	34.5±1.9
Family Troglodytidae (wrens)		
Troglodytes aedon (house wren)	2	36.0±0.8
Family Paridae (titmice, verdins and bushtits)		
Parus gambeli (mountain chickadee)	3	33.8±1.3
Family Sylviidae (old world warblers, gnatcatchers and kinglets)		
Regulus calendula (ruby-crowned kinglet)	1	31.0
Family Turdidae (thrushes, solitaires and bluebirds)		
Hylocichla ustulata (Swainson's thrush)	1	20.9
Turdus migratorius (American robin)	10	26.6±3.9
Sialia currucoides (mountain bluebird)	5	33.2±2.3

[a] Activities expressed as mU/mg brain.

[b] *Empidonax* spp. were primarily *Empidonax oberholseri* (dusky flycatcher).

Sufficient data were obtained from ten species of birds to evaluate the effect of Dylox on brain cholinesterase activity. One dark-eyed junco, one evening grosbeak, two mountain chickadees, and two western tanagers had values which were at least 2 standard deviations (S.D.) below the mean (Table 2). Both western tanagers' activities were more than 20% below the mean (26.5% and 20.5%) while the evening grosbeak's activity was depressed nearly to that level (19.8%). These western tanagers were collected on the day of spray, while the evening grosbeak was collected 3 days after spraying.

Of the 12 species of birds evaluated from the Sevin-4-oil spray areas, three individuals representing three species had values depressed greater than 2 S.D. below the mean (Table 3). They were a mountain chickadee, an evening grosbeak and a Lincoln's sparrow. Only the evening grosbeak's brain cholinesterase activity was more than 20% below the mean (21.3%). This evening grosbeak was collected on the day of spray.

TABLE 2

Brain cholinesterase activities[a] of birds taken from areas sprayed with Dylox

Species	Control[b]	Normal range ($\bar{x} \pm 2$ S.D.)	Day 0[c]	Day 1	Day 2	Day 3	Day 5	Abnormally low values	
Empidonax spp.[d]	22.2±2.6 (8)	17.0 - 27.4	24.6±0.5 (2)	-	-	-	-		
Evening grosbeak	31.9±2.1 (6)	27.7 - 36.1	33.1 (1)	30.9 (1)	30.3 (1)	28.1±3.5 (2)	30.4±2.8 (3)	25.6 (day 3)	
Pine siskin	22.2±2.4 (10)	17.4 - 27.0	25.8±1.1 (2)	-	-	22.0 (1)	25.5±1.5 (3)		
Chipping sparrow	23.5±1.8 (7)	19.9 - 27.1	24.5±1.3 (4)	24.4±3.5 (2)	23.4±1.1 (3)	24.7±1.9 (3)	24.2±1.4 (5)		
Dark-eyed junco	33.2±1.1 (9)	31.0 - 35.4	35.6±1.8 (8)	36.7±1.4 (3)	32.3±1.0 (4)	35.6±1.7 (6)	35.6±2.5 (3)	30.9 (day 2)	
Lazuli bunting	31.6±2.7 (5)	26.2 - 37.0	31.0 (1)	-	-	32.0 (1)	-		
Western tanager	28.3±2.9 (8)	22.5 - 34.1	21.7±1.2 (2)	30.0±2.3 (3)	30.1±1.6 (4)	30.2±1.3 (3)	29.7 (1)	22.5 (day 0)	20.8 (day 0)
Warbling vireo	33.8±3.9 (10)	26.0 - 41.6	35.0±1.5 (3)	-	29.3 (1)	31.6 (1)	-		
Mountain chickadee	33.8±1.3 (3)	31.2 - 36.4	30.9±3.2 (2)	34.6±2.9 (4)	32.3±2.0 (2)	31.4 (1)	33.2±0.6 (3)	28.6 (day 0)	30.9 (day 2)
American robin	26.6±3.9 (10)	18.8 - 34.4	29.6±3.4 (3)	28.1±2.2 (3)	28.0 (1)	29.7±0.3 (3)	31.5±2.6 (4)		

[a] Activities expressed as mU/mg brain.

[b] Mean and standard deviation (numbers of birds).

[c] Days after spraying.

[d] Empidonax spp. were primarily dusky flycatchers.

TABLE 3

Brain cholinesterase activities[a] of birds taken from areas sprayed with Sevin-4-oil

Species	Control[b]	Normal range ($\bar{x} \pm 2$ S.D.)	Day 0[c]	Day 1	Day 2	Day 5	Abnormally low values
Yellow-bellied sapsucker	47.5±4.3 (4)	38.6 - 55.8	50.7±2.6 (2)	-	-	-	
Common flicker	24.8±1.2 (3)	22.4 - 27.2	25.7±0.5 (2)	-	-	-	
Empidonax spp.[d]	22.2±2.6 (8)	17.0 - 27.4	24.2±1.0 (4)	-	-	28.4 (1)	
Evening grosbeak	31.9±2.1 (6)	27.7 - 36.1	27.7±2.3 (3)	31.2±2.3 (2)	-	32.4±2.4 (4)	25.1 (day 0)
Pine siskin	22.2±2.4 (10)	17.4 - 27.0	22.6±1.3 (7)	23.0 (1)	-	22.1±0.9 (3)	
Chipping sparrow	23.5±1.8 (7)	19.9 - 27.1	24.2±1.6 (5)	-	-	26.4±0.5 (3)	
Dark-eyed junco	33.2±1.1 (9)	31.0 - 35.4	33.5±1.1 (6)	-	31.9 (1)	36.2±0.8 (4)	
Lincoln's sparrow	25.2±1.4 (5)	22.4 - 28.0	24.1±2.5 (4)	24.9 (1)	-	24.8±0.7 (2)	21.6 (day 0)
Western tanager	28.3±2.9 (8)	22.5 - 34.1	29.8±2.5 (6)	-	-	-	
Warbling vireo	33.8±3.9 (10)	26.0 - 41.6	32.7±2.6 (4)	-	-	33.0 (1)	
Mountain chickadee	33.8±1.3 (3)	31.2 - 36.4	32.6±0.8 (3)	-	30.9±1.2 (3)	-	29.5 (day 2)
American robin	26.6±3.9 (10)	18.8 - 34.4	28.9±2.9 (6)	29.0±0.4 (4)	28.8±1.5 (3)	30.5±1.9 (5)	

[a] Activities expressed as mU/mg brain.

[b] Mean and standard deviation (numbers of birds).

[c] Days after spraying.

[d] *Empidonax* spp. were primarily dusky flycatchers.

DISCUSSION

Previous work in our laboratory with starling (Sturnus vulgaris) brains and sera showed that storage in dry ice (~ -76°C) preserves cholinesterase enzyme activity for up to 5 weeks (ZINKL and HUDSON 1975). KNITTLE and TUCKER (1974) have shown that storage at -40°C and -68°C preserves the enzyme. However, deterioration does occur at -18°C (KNITTLE and TUCKER 1974) or -22°C (LUDKE et al. 1975). In this study it is unlikely that there was any loss of activity from the time of collection until analysis because the brains were stored in dry ice and the analyses were carried out soon after collection (within 12 hr).

A considerable difference of opinion exists among investigators regarding what degree of brain cholinesterase depression is diagnostic as the cause of death. LUDKE et al. (1975) showed that 50% inhibition occurred in Japanese quail (Coturnix c. japonica) that died after being fed up to 1,400 ppm parathion for up to 5 days. BUNYAN et al. (1968) found that pheasants (Phasianus colchicus) dying from a single dose of a variety of organophosphates had at least 90% brain cholinesterase depression. In our laboratory, ring doves (domestic [Streptopelia risoria]) given a single dose of 21.2 mg Dylox/kg B.W. had 83% brain cholinesterase depression when sacrificed 2 hr after dosing. Others given this amount survived. Ring doves that died after being given 42.4 mg Dylox/kg B.W. had 95% depression. Homing pigeons (domestic rock doves [Columba livia]) given 195 mg Dylox/kg B.W. died within 45 minutes after dosing. Their brain cholinesterase activities were 83% inhibited. Others given 78.1 mg Dylox/kg B.W. survived for 18 hr before being sacrificed. Their activities were depressed 68% at that time even though they were showing few signs of organophosphate toxicity. Ring doves given 1,000 mg Sevin-4-oil/kg B.W. had brain cholinesterase activities that were decreased 56% when sacrificed 2 hr after dosing. Other birds given the same dose survived (ZINKL and HUDSON 1975).

Therefore, even using the most stringent criterion (50% depression), no birds in the present study were in danger of dying from direct poisoning by either Dylox or Sevin-4-oil. However, at least 4 of the birds had activities depressed about 20% below the mean of the species. This indicates exposure had occurred (LUDKE et al. 1975). Five more birds had activities depressed greater than 2 S.D. below the mean. Of the 5 species having depressed activities ($\bar{x}$ - 2 S.D.), 3 are canopy dwellers (mountain chickadee, evening grosbeak, and western tanager). The latter birds represented 7 of the 9 depressed values, and they were the most depressed values, probably reflecting greater exposure of these species rather than greater susceptibility to the chemicals.

Although we are not aware of any experimental work concerning the effects of sublethal cholinesterase inhibition on birds, it is possible that the levels found in our study might have increased susceptibility to predation or decreased ability to obtain food

(e.g., fly-catching). Nevertheless, birds with decreased cholinesterase activity represent a small part of the total specimens we evaluated.

In conclusion, spraying coniferous forest habitat with Dylox or Sevin-4-oil at 1.13 kg/hectare (1 lb/acre - a.i.) had little effect on brain cholinesterase activities in birds. Thus, only minimal exposure occurred, a finding similar to that of KURTZ and STUDHOLME (1974) who determined residues in birds from eastern forests sprayed with Dylox and Sevin.

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