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Inhibition of Brain Cholinesterase Activity in Forest Birds and Squirrels Exposed to Aerially Applied Acephate

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During June 1977, the U.S. Forest Service in cooperation with the Idaho Department of Public Lands sprayed 4 experimental plots of about 500 hectares each with the organophosphorus insecticide acephate (Orthene 75 S^R) for control of Western spruce budworm (Choristoneura occidintalis). Acephate was applied at a rate of 0.57 kg/ha. In previous studies, acephate had inhibited brain cholinesterase (ChE) activity of passerine birds exposed during spraying of forests, (ZINKL et al. 1979.). Since brain residue had not been determined in those studies, brain ChE activity and residue concentrations were determined in birds and squirrels collected from the sprayed areas in this study.

MATERIALS AND METHODS

On four 500 ha plots of the Payette National Forest located near McCall, Idaho, 0.57 kg AI of acephate/ha was applied by helicopter early in the morning. Three plots sprayed on June 23, 25 and 27, 1977 were used for bird and mammal collections.

Birds and mammals used for determination of brain ChE activity were collected between 3 and 6 h after the spray (day 0) and on post-spray days 1, 3, 6, 25, and 26. Birds were collected by mist netting or by shooting with shotguns. Birds collected in the mist nets were killed by asphyxiation with carbon dioxide sublimed from dry ice. Mammals were collected by shooting with shotguns. Animals used as controls were collected prior to and after the first spray date from habitats close to and similar to those of the sprayed plots.

Immediately after collection, the animals were frozen on dry ice, transported to the laboratory at McCall, Idaho where they were stored until processed for analysis. All ChE analyses were performed within 3 days of collection by previously published methods (ELLMAN et al. 1961, DIETER & LUDKE 1975, ZINKL et al. 1979).

ChE activities were calculated in milliunits/mg (mU/mg) of brain. A unit is defined as the conversion of one mole of substrate to product(s) in one min. The mean control brain ChE activity and standard deviation of the mean was calculated for each species.

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Since activities of animals from treatment areas were evaluated for depression individually, it was necessary to establish criteria by which activities could be judged normal or depressed. To do this, 2 values were calculated, the mean less 2 standard deviations ($\bar{X}$-2 S.D.) and the mean less 20% of the mean ($\bar{X}$-0.2$\bar{X}$) (LUDKE et al. 1975, ZINKL et al. 1979). When a ChE activity was less than the lower of these calculated values, it was judged as depressed.

Residues of acephate and methamidophos were determined in the brains of the western tanagers, dark-eyed juncos, Swainson's thrushes and Columbian ground squirrels (Scientific names are given in Table 1.). After ChE analysis the remaining 1:5 dilution of the brain homogenates was stored on dry ice or at -70°C in a deep freezer until just prior to analysis. After thawing, the brain homogenate weight was determined. The homogenate was then transferred to a 250-mL beaker and blended by sonication with 20 mL of distilled water. While continuously blending, 100 mL of redistilled ethyl acetate was added followed by 50 g of sodium sulfate. This was followed by two 50 mL additions and one 25 mL addition of ethyl acetate. Blending was continued for about 1 min after each ethyl acetate addition. The mixture was then poured over 100 g of sodium sulfate in a funnel and allowed to drain into a 1000-mL rb flask. When the mixture had stopped draining an additional 15 mL of ethyl acetate was poured over the sodium sulfate. After the ethyl acetate had stopped dripping from the sodium sulfate, it was reduced to dryness by rotary evaporation under reduced temperature and pressure. The sample was transferred from the rb flask with three 5 mL rinses of ethyl acetate to a scintillation vial containing 1 g of activated charcoal. The charcoal was thoroughly mixed with the ethyl acetate with a vortex mixer. The mixture was centrifuged for 10 min at 10,000 rpm at -10°C. A 10 mL aliquot was transferred to a 5-dram vial and evaporated to dryness with a stream of nitrogen at 40°C. The sample was reconstituted with 3 mL of ethyl acetate for gas chromatographic (GC) analysis.

Analysis for acephate and methamidophos was performed on a gas chromatograph equipped with an alkali flame ionization detector and a 1.8 m x 2 mm (id) glass column packed with 1% Reoplex 400 coated on 100/120 mesh Gas Chrom Q. Operating temperatures in C° were: column 175, injector 210 and detector 210. Gas flows in mL/min were: helium 50, hydrogen 36 and air 235. Limits of detection were about 0.2 ng for acephate and 0.01 ng for methamidophos. Recovery for acephate was 83% when total amount was below 0.1 µg and 53% when above. Recovery for methamidophos was 67%.

RESULTS

Sufficient data useful for evaluation of the effects of acephate on brain ChE inhibition activities were obtained from 11 avian and 2 squirrel species (Table 1). On the day of spray 11 of 22 birds (50%) had depressed brain ChE activities. One day later, 14 of 26 (54%) were depressed, at 3 days, 13 of 14

TABLE 1. Brain ChE activities of Birds[a] and Squirrels[a] Collected from Unsprayed Forests

Species	Scientific Name	N	$\bar{X}$	S.D.	$\bar{X}$-2 S.D.	$\bar{X}$-0.2 $\bar{X}$
American robin	*Turdus migratorius*	6	26.06	1.12	23.82	20.85
Western tanager	*Piranga ludoviciana*	6	29.74	2.04	25.66	23.79
Evening grosbeak	*Hesperiphona vespertina*	9	29.16	1.33	26.50	23.33
Swainson's thrush	*Catharus ustulata*	3	17.81	1.58	14.65	13.54
Chipping sparrow	*Spizella passerina*	6	20.30	1.97	16.36	16.24
Dark-eyed junco	*Junco hyemalis*	4	31.41	2.32	26.77	25.13
Pine siskin	*Carduelis pinus*	5	21.45	2.15	17.15	17.16
Empidonax spp.[b]		2	22.46	3.06	16.34	19.97
Yellow-rumped warbler	*Dendroica coronata*	2	33.12	1.81	29.50	26.50
Cassin's finch	*Carpodacus cassinii*	2	22.97	1.12	20.73	18.38
Mountain chickadee	*Parus gambeli*	1	32.56	-	-	26.05
Columbian ground squirrel	*Spermophilus columbianus*	3	16.51	1.65	13.21	13.21
Red squirrel	*Tamiasciurus hudsonicus*	5	25.20	2.66	19.88	20.16

[a]Excludes young of the year

[b]Includes both *E. oberholseri* and *E. hammondii*

(93%) were depressed, at 6 days, 18 of 27 (67%) were depressed, and at days 25-26 three of 19 (16%) were depressed. The maximum observed depression (57%) occurred in a chipping sparrow 6 days after the spray. Brain ChE activity was depressed 25-32% in western tanagers 25 and 26 days after spray (Table 2). Brain ChE activity was inhibited in Columbian ground squirrels on days 3, 6 and 25-26 and in red squirrels on days 1, 3 and 6 (Table 2).

Residue analysis for acephate and methamidophos in the brains of western tanagers, dark-eyed juncos and Swainson's thrushes indicated that only a western tanager collected on day 0 and one from day 3 contained detectable residues of acephate and methamidophos in the brain. No residues were detected in the brains of the dark-eyed juncos or Swainson's thrushes. One Columbian ground squirrel collected on day 0 contained residues of acephate and methamidophos; two collected on day 3 and one collected on day 6 contained acephate only (Table 3).

DISCUSSION

The results of this study and of our 1976 studies (ZINKL et al. 1979) and those of others in 1977 (JULIN & GRAMLICH 1978) show that depression of brain ChE activity occurs in birds exposed to acephate in amounts being considered for use in spray projects for control of forest insects. However, birds exposed to 0.57 kg/ha in this study and in that of JULIN & GRAMLICH (1978) were less affected than reported for a 1.14 kg/hectare application (ZINKL et al. 1979), because brain ChE was inhibited less and the percentage of birds affected was smaller. The time of year that exposure occurs may be of importance because the studies conducted in the fall (ZINKL et al. 1979) show less brain ChE depression than in this and other (ZINKL et al. 1979) summer studies at the same spray concentrations of acephate. However, in the fall, transients from adjacent areas may have been included with resident birds from the spray areas and food habits and weather conditions may have influenced the exposure of the birds.

The maximum brain ChE inhibition (57%) occurred in a chipping sparrow 3 days after the spray was applied. Several other birds had brain ChE inhibition of about 50%. This magnitude of depression may be detrimental since LUDKE et al. (1975) found that Japanese Quail (Coturnix coturnix) that died after being fed 1400 mg parathion/kg feed for up to 5 days had 50% brain ChE inhibition. In a 5 day feeding study with acephate on dark-eyed juncos, we have found that brain ChE was inhibited about 60% in those birds that died, but in an acute LD_{50} study with acephate, brain ChE inhibition was greater than 80% in dark-eyed juncos that died (ZINKL et al. submitted). Ninety percent inhibition occurred in ring-necked pheasants (Phasianus colchinus) that died from a single oral dose of various organophorous insecticides (BUNYAN et al. 1968). Brain ChE in ring doves (domestic Straeptopelia risoria) that died from 42 mg trichlorfon/kg was inhibited 95% and that of homing pigeons (domestic rock doves-Columba livia) that died from 195 mg trichlorfon/kg was inhibited 85% (ZINKL & HUDSON, unpublished).

TABLE 2. Inhibition of Brain ChE Activity of Birds[a] and Squirrels[a] Collected from Forests Sprayed with 1/2 lb AI Acephate per Acre.

Species	Days After Spray									
	0		1		3		6		25-26	
American robin	2[b]/5	24[c] 23	4/5	38 34 22 22	1/2	32	2/2	32 30	-	
Western tanager	0/2		5/6	39 37 23 22 21	2/2	53 32	4/4	50 39 34	3/7	32 30 25
Evening grosbeak	2/2	28	1/4	31	1/1	29	1/3	37	0/4	
Swainson's thrush	0/1		0/1		3/3	42 31 30	1/5	32	0/2	
Chipping sparrow	2/4	35 33	0/5		2/2	57 45	2/4	27 30	0/5	
Dark-eyed junco	0/3		-		1/1	40	3/3	37 28 23	-	

TABLE 2. (continued) Inhibition of Brain ChE Activity of Birds[a] and Squirrels[a] Collected from Forests Sprayed with 1/2 lb AI Acephate per Acre.

Species	Days After Spray									
	0		1		3		6		25-26	
Pine siskin	1/1	36	0/1		-		-		0/1	
Empidonax spp.	2/2	51	1/1	44	-		2/3	41		
		34						39	-	
Yellow rumped warbler	-		1/1	21	1/1	43	2/2	32	-	
Cassin's finch	1/1	33	-		-		-		-	
Mountain chickadee	1/1	28	2/2	39	2/2	45	1/1	32	-	
				29		31				
Columbian ground squirrel	0[b]/1		0/1		2/2	31	2/2	34	1/5	27
						29		31		
Red squirrel	0/1		1/2	27	1/3	27	1/3	33	0/3	

[a]Excludes young of the year

[b]Numeratory = number of birds and squirrels with depressed ChE activity

[c]Percent depression

TABLE 3. Concentrations of Acephate and Methamidophos in Animals Collected from Areas Previously Sprayed with Acephate

Species	N	Day	ChE Depression (%)	Residue Concentration (µg/g) Acephate	Metamidophos
Western Tanager	2/21[a]	0	ND[b]	0.21	ND[c]
		3	32	0.32	0.055
Dark-eyed junco	0/7				
Swainson's thrush	0/12				
Columbian ground squirrel	4/10	0	ND	0.27	0.032
		3	31	0.091	NF[c]
		3	29	0.13	NF
		6	31	0.033	NF

[a]Number of animals with detectable residues/number of animals analyzed

[b]ND = not depressed

[c]ND = less than .005 µg/g

These data suggest that brain ChE inhibition of at least 80% is required before birds will be killed with a single oral dose of organophosphorus insecticide but birds may die with only 50% inhibition when continuously exposed through feed. Assuming that the birds of this study were continuously exposed through feed and perhaps through air and dermally, it is possible that they were in a life-threatening situation.

The brains of only 2 of 21 western tanagers, none of 12 Swainson's thrushes, none of 7 dark-eyed juncos, and 4 of 10 Columbian ground squirrels contained any acephate or its deacetylated metabolite methamidophos (Table 3). The lack of insecticide residues in birds having brain ChE depression is difficult to explain. The recent suggestion that methamidophos could be oxidized to the potent anti-cholinesterase agent dimethyl phophoramidothiolate S-oxide (ETO et al. 1977) suggests that this very toxic metabolite or other metabolites might be the ultimate cause of brain ChE inhibition. These products are not detected by the methods we used for analysis of acephate and methamidophos. Nevertheless, brain ChE inhibition occurred in birds and squirrels exposed to an aerial application of 0.57 kg/ha. Since we observed no bird or squirrel mortality or signs of organophosphate insecticide poisoning in the field in this study, we cannot assess the importance of the brain ChE inhibition we observed. Laboratory studies recently completed suggest that inhibition in some birds may have been sufficient to be life threatening (ZINKL et al., submitted).

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