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Effects of Field Application of the Anti-Cholinesterase Insecticide Methomyl on Brain Acetylcholinesterase Activities in Wild *Mus musculus*

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Carbamate insecticides are well-known inhibitors of the enzyme acetylcholinesterase (AChE) responsible for degrading the neurotransmitter acetylcholine (O'BRIEN 1967). Field studies of the effects of anti-cholinesterase insecticides on wild species have been inconclusive, however. Some studies of avian species have shown little or no acute mortality with slight impacts on the population (CONNER 1960, MULLA et al. 1966, DOANE & SCHAFFER 1971, PILLMORE et al. 1971) while others (McLEOD 1967, FOWLE 1972, McEWEN et al. 1972, MOULDING 1976, BART 1979) have found varying degrees of mortality or AChE inhibition following anti-cholinesterase insecticide application. Differences in results were probably influenced by such factors as species, compound used and its formulation, size of the area relative to home ranges of species, and sampling frequency, intensity and methodology.

Few systematic studies have been conducted in which the sub-acute effects of anti-cholinesterase insecticides on small mammals have been measured. SMITHSON & SANDERS (1978) found that brain AChE inhibition in cottontail rabbits (*Sylvilagus floridanus*) taken from fields sprayed with methyl parathion, *phosphorothioic acid O, O-dimethyl O-(4-nitrophenyl) ester,* ranged from 7.0 to 32.4%. ZINKL et al. (1980) found that red squirrels (*Tamiasciurus hudsonicus*) collected in forests sprayed with acephate, *acetylphosphoramidothioic acid O, S-dimethyl ester,* had mean depressions of AChE of 33%.

The present study was undertaken to measure AChE inhibition in small mammals exposed to the carbamate insecticide methomyl, *N[(methylcarbamoyl) oxy] thioacetimidic acid methyl ester,* during agricultural application. Sampling was designed to evaluate temporal effects of methomyl on AChE activity during the first 3 days after spraying because this is the period during which small mammals are most sensitive to AChE inhibitors under laboratory conditions of direct oral dosing (MONTZ & KIRKPATRICK 1982).

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METHODS AND MATERIALS

The study area was approximately 6 km west of Deltaville in Middlesex County, VA. Small mammals were live-trapped in and around soybean fields aerially treated with a mixture of *Lannate* 1.8L (505 g methomyl/ha) and toxaphene 6EC (1683 g active ingredient/ha) both applied at the rate of 2.3 l/ha. Spraying was completed before 0700 hours on 9 September 1982.

Trapping was conducted on 3 consecutive nights after spraying (Sept. 9-11, 1982, hereafter referred to as days S+1, S+2, and S+3, respectively). Sherman live-traps were baited with a mixture of peanut butter and rolled oats. Traps were placed in straight lines at 5 m intervals along the edges of two fields (27.4 and 93.9 ha) which were bordered by mowed strips composed primarily of orchard grass (*Dactylis glomerata*). Rows of 5 traps 5 m apart were also set in lines perpendicular to those bordering the field. Rows placed within the field were 25 m apart. Traps were set on the evening of 9 September and checked on mornings and evenings of the 3 following days.

No animals were trapped in evenings. Trapped animals were removed from the traps each morning (0700-0900 E.D.T.) following discovery, killed via cervical dislocation, and their brains were removed in the field. Brains and carcasses were packaged separately in Whirl-pak bags (Nasco, Fort Atkinson, WI) and immediately placed on ice. Within 1 hour, all samples were placed in a commercial freezer (approx. -5C) and frozen until trapping was concluded. The samples were stored in a cooler on ice during 6 hours of transport back to the laboratory at V.P.I. & S.U. where they were kept frozen at -10C until analyzed.

Controls were live-trapped locally on the farms of V.P.I. & S.U. from areas known not to have been subjected to any recent pesticide treatment. traps were checked each morning and captured animals moved to the laboratory where they were killed by cervical dislocation, their brains removed, and handled thereafter identically to those taken in the treatment fields.

After thawing, brains were prepared for AChE determination by removing the cerebral hemispheres and wet weight measured $\pm$0.1 g. Each brain was thoroughly homogenized in 0.1 M pH 8 phosphate buffer (1 part brain: 19 parts buffer; w:v) using a motor driven glass homogenizer with teflon coated pestle. Brains were

kept on ice through the procedure. Reagents were prepared as described by ELLMAN et al. (1961) except that the concentration of acetylthiocholine iodide was increased from 0.075 M to 0.100 M. For each assay, the following reagent volumes were placed in a ground quartz spectrophotometer curvette; 2.9 ml 0.1 M pH 8 phosphate buffer, 0.1 ml DTNB reagent, 0.02 ml acetylthiocholine iodide reagent and 0.1 ml brain homogenate. The reaction mixture was vortexed and reaction rate recorded using a Hitachi Model 100-30 spectrophotometer equipped with a Kipp-Zonen strip chart recorder. Absorbances were recorded continuously for at least 3 min at 412 nm. The mean of duplicate assays was taken as the AChE activity for a particular sample.

The Statistical Analysis System (SAS, HELWIG & COUNCIL 1979) computer package was used to evaluate differences in AChE activities. The results were analyzed by analysis of variance (ANOVA) using the general linear model (GLM) procedure available in SAS. Duncan's Multiple Range Test was used to distinguish differences in cell means.

RESULTS

Mus musculus was the principal species caught. Two *M. musculus* which died in the traps were not included in the analysis because the time from death to recovery could not be determined. The AChE activities of the *Peromyscus leucopus* and *Rattus norvegicus* caught were not included in data analysis because sample sizes were too small from both treated and control areas. Overall, mean AChE activities of the *M. musculus* from the treated fields were significantly ($P < 0.10$) lower when compared with control groups and were depressed 11.2% below controls. Mean AChE activities of mice from treated fields differed significantly ($P < 0.10$) with date of capture being lowest on S+3 (Table 1). Mean AChE activities of mice caught on S+1 and S+3 were significantly ($P < 0.10$) lower than those of controls.

DISCUSSION

Mammals exposed to methomyl could be expected to show depressions in AChE activity. Because toxaphene intoxicates by a distinctly different mode of action than methomyl (O'BRIEN 1967), compounding effects on AChE activities are not expected. Data herein shows that the *M. musculus* residing in and around treated fields received sufficiently large doses of methomyl to depress AChE activities. High variability of AChE activities associated with such factors as sex, season, age, and type of compound has been noted (COPE 1971).

TABLE 1. Mean brain acetylcholinesterase activities of *Mus musculus* from control and methomyl treated areas.

Group	Dates	N	Mean (±S.E.) Activity a
Control	9/29-10/6	8	4.34±0.17 c
S+1 b	9/9	10	3.85±0.09 de
S+2 b	9/10	11	4.24±0.12 cd
S+3 b	9/11	7	3.81±0.25 e

a: AChE activities expressed as micromoles acetylthioiodide transformed per minute per mg wet weight of brain tissue.

b: S+1, S+2, S+3, are days 1, 2, and 3 after spraying, respectively.

c,d,e: Means with different superscripts were significantly ($P < 0.10$) different.

Mean AChE activities of wild-trapped mice did not differ significantly with date of capture. The mean activity of mice trapped on S+1 and S+3 was significantly ($P < 0.10$) lower than that of control mice. These data essentially agree with those of ZINKL et al. (1980) who found maximal AChE inhibition in several species 3 days after spraying of acephate. Laboratory mice orally dosed with anti-cholinesterase insecticides in the laboratory have had relatively short AChE response periods of 6 hours or less with some insecticides (MONTZ unpublished data). The longer response period seen in this field study could have been caused by a requirement that relatively large quantities of contaminated food be eaten and/or air breathed before AChE activities become depressed. Food habits studies of *M. musculus* by HOUTCOOPER (1978) have shown that soybean leaves and lepidopterous larvae contribute substantially to the diet when available. Therefore, consumption of contaminated food was probable.

These data show that the technique of live-trapping soon after spraying can be used to capture mammalian subjects of exposure to anti-cholinesterase insecticides. This method is biased insofar as it samples only those animals still healthy enough and willing to enter live-traps. Results of AChE assays of animals obtained by live-trapping must be considered conservative because of the above sampling bias. Live-trapping eliminates those human biases associated with

sampling by shooting as a shooter is likely to select highly visible or vocal animals and thus unconsciously bias the sample by taking animals exhibiting little or no effects from spraying.

The present data show widespread sub-acute AChE inhibition may occur in small mammals following the application of anti-cholinesterase insecticides in agricultural areas. The present work did not allow for recovery of victims of lethal effects or of severely intoxicated animals and accordingly probably underestimates effects on AChE. Neither did it fully establish the temporal pattern of AChE inhibition under field conditions because trapping was discontinued as planned after 3 days.

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