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CHOLINESTERASE ACTIVITY IN WHITE-WINGED DOVES EXPOSED TO METHYL PARATHION

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ABSTRACT: Captive white-winged doves (*Zenaida asiatica*) were exposed to various levels of methyl parathion (MP) in drinking water to determine effects on brain and blood cholinesterase (ChE) activity. We conducted two experiments to test the influence of MP dose (the amount of MP actually ingested), MP concentration (the amount of MP per unit water), and exposure duration (number of days exposed to a constant MP concentration) on ChE activity. Plasma ChE activity was not useful in predicting brain ChE activity. Methyl parathion concentration had a greater influence on plasma and brain ChE activity levels than dose or time of exposure. These results contribute to the evaluation of irrigation water as a route of exposure of wildlife to pesticides.

Key words: Cholinesterase, chronic exposure, methyl parathion, white-winged dove, *Zenaida asiatica*.

INTRODUCTION

Organophosphorus (OP) pesticides are used extensively for insect control in agriculture (Hill, 1995), in part because of their rapid rate of decomposition in the environment and their inability to bioaccumulate in animal tissues. However, wildlife species may be negatively affected by OP pesticides, even at sublethal levels (Hart, 1993). The primary toxicological effect of these pesticides is the inhibition of cholinesterase (ChE) in nervous tissue (Hill, 1995). This ChE depression causes lethargy (Grue and Shipley, 1981), anorexia (Grue, 1982), and food aversion (Evans, 1985; Bennett, 1989; Linder and Richmond, 1990).

Large-scale mortality of nesting and migrating birds has been attributed to OP pesticide toxicity (Seabloom et al., 1973; Zinkl et al., 1977; White et al., 1979, 1982; Flickinger et al., 1980) mainly because OP application to crops is often coincidental with seasonal peaks in avian numbers (King et al., 1984). At sublethal doses, lowered ChE activity in brain tissue may alter behaviors essential for survival of both the individual and dependent offspring (Hart, 1993). Brewer et al. (1988) studied the effects of OP pesticides on radio-tagged ducks in a treated and an untreated agricultural field. Nest abandonment occurred only in the OP-treated field and daily duckling loss increased.

Prior to conducting long-term studies involving chronic exposure to toxic substances, precise protocols must be established through preliminary controlled experimentation (Kendall, 1982). The experiments should emphasize understanding the effect of the specific toxin on the species involved, especially when previously unstudied routes of exposure are being investigated.

We chose to study white-winged doves (*Zenaida asiatica*) because of their economic importance in southern Texas, particularly the Lower Rio Grande Valley (LRGV). White-winged dove populations have been declining in the LRGV since the 1920's with occasional large drops in breeding pair estimates during the 1970's (George et al., 1994; Hayslette et al., 1996). We chose methyl parathion (MP) as the experimental OP pesticide because it was commonly used in the LRGV during

the white-winged dove declines of the 1970's and is still used commonly in the area.

Custer and Mitchell (1987) tested several species of birds in the LRGV for exposure to OP pesticides. Cholinesterase levels in mourning doves (*Zenaida macroura*) were significantly lower compared to controls. White-winged doves in the LRGV also have been documented with depressed ChE levels, leading Tacha et al. (1994) to suggest that significant exposure to OP pesticides had occurred. They hypothesized that exposure resulted from ingestion of contaminated irrigation water in cotton fields. Cotton field irrigation and white-winged dove reproduction occur simultaneously in the LRGV. Further, Hayslette et al. (1996) suggested that, in addition to habitat loss, pesticide exposure likely contributed to white-winged dove population declines in recent years.

We designed two dose-rate experiments to establish parameters for studies of the effects of sublethal exposure to MP via water ingestion on white-winged dove productivity. In our study, dose refers to the amount of MP actually ingested and concentration refers to the amount of MP per unit water. The objective of the first experiment was to determine the relationship between MP concentration and ChE inhibition. The objective of the second experiment was to determine the effect of duration of MP ingestion (number of days consuming a constant MP concentration) on brain and plasma ChE activity at a constant MP concentration. This experiment was conducted to allow us to more effectively design studies evaluating impacts of chronic OP pesticide exposure on white-winged dove productivity. Data from both studies were used to determine whether plasma ChE was a reliable predictor of brain ChE in this species.

MATERIALS AND METHODS

General

White-winged doves used in this study were post-hatching-year and wild-caught in Kingsville, Texas, USA (27°32′N, 97°51′W). Birds were captured between 13 June 1995 and 10 January 1996 and housed in facilities at Texas A&M University-Kingsville (Kingsville, Texas, USA). Doves were provided a nutritionally complete pelleted feed (Purina Mills Corporation, St. Louis, Missouri, USA) and water *ad libitum* and were allowed ≥8 wk to adapt to captivity before beginning each experiment. The doves were handled in accordance with Texas Parks and Wildlife Department permit number SPR-0496-773 (Austin, Texas, USA) and U.S. Fish and Wildlife Service permit number PRT-800477 (Albuquerque, New Mexico, USA). All activities were approved by the Texas A&M University-Kingsville Animal Welfare and Care Committee.

Experimental methods

Birds in both experiments were placed in individual cages (25 × 25 × 50 cm) and randomly assigned to a treatment group. Treatment water was prepared from commercially available MP (Setre Chemical Company, Memphis, Tennessee, USA) in liquid form at a concentration of 0.54 kg MP/L xylene. The pesticide was diluted in distilled water, then poured through number 1 Whatman filter paper to remove insoluble particles of xylene (with adhering MP particles) from the solution, yielding a 12.98 ppm MP solution. Other treatment solutions were obtained by dilution of this stock solution in the appropriate amount of distilled water. The control group in both experiments was provided *ad lib* distilled water. Water intake (and subsequent MP ingestion) was calculated daily by weight (±0.05 g.). Four control water bottles accounted for evaporation. Treatment water was administered in amber glass and changed daily to minimize decomposition of the MP.

Experiment 1

On 12 March 1996, 40 birds were assigned to one of five treatment groups categorized as 0 (control), 2.6, 5.2, 7.8, and 10.4 ppm MP in drinking water. These MP concentrations were chosen to provide a range of sublethal ChE inhibition. A subsample of stock solution was chemically verified ($n = 5$, $\bar{x} \pm SE = 12.98 \pm 1.04$). An additional subsample was chemically verified following 24 hr of exposure to experimental conditions and did not differ significantly from the stock solution ($t = -0.58$, $P = 0.58$). The treatment lasted 5 days before birds were euthanized, blood samples were taken, and carcasses frozen.

TABLE 1. Brain and plasma ChE activity, water intake, and methyl parathion (MP) intake of white-winged doves in a 5 day trial in Kingsville, Texas.

MP concentration (ppm)	n	ChE activity				Water intake (mL/day)		MP dose (μg/day)	
		Brain (μmol/min/g)		Plasma (mU/mL)					
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
0.0	8	21.0A[a]	1.8	1,223.6A	189.0	29.6A	7.3	0.0[b]	0.0
2.6	8	14.3B	4.5	821.1B	288.5	20.3B	2.3	52.9A	5.9
5.2	8	14.2B	7.1	921.5AB	470.0	18.0BC	5.4	88.6B	28.2
7.8	8	7.5C	2.5	815.0B	356.7	14.7CD	3.9	114.4B	30.3
10.4	7	4.6C	1.7	95.4C	151.8	10.5D	3.9	109.3B	40.7

[a] Means within a column sharing the same letter do not differ significantly ($P > 0.05$).
[b] The control (0.0 ppm MP) was not included in analysis of MP dose.

Experiment 2

On 3 May 1996, 60 white-winged doves were assigned to three groups of 20 birds. One group was used as a control and the other two groups received water containing 0.87 and 3.9 ppm of MP, respectively. At 3-day intervals over 15 days, four birds from each group were randomly selected, euthanized, and assayed for plasma and brain ChE activity. Percent enzyme inhibition was calculated based on control ChE activity for the corresponding period.

Analysis

Brain and plasma ChE activities were determined following the techniques described by Ellman et al. (1961), Pilz (1974), Ludke et al. (1975), Hill and Fleming (1982), and Hill (1988). Bird carcasses were frozen for 3 days, thawed, and the brains were excised and added to Tris buffer (pH 8) at a ratio of 0.5 g brain/5 ml buffer, and homogenized for 30 sec (Tissue Tearor, model 985-370, Biospec Products, Inc., Bartlesville, Oklahoma, USA). Three ml of dithiobisnitrobenzoic acid (DTNB) and 100 μl of acetylthiocholine iodide (ACTH) were combined with 20 μl of brain homogenate in a spectrophotometer cuvette. The cuvette was covered with parafilm, gently inverted to mix the reagents, and placed in the spectrophotometer (Milton Roy Spectronic 401, Milton Roy Company, Rochester, New York, USA) programed to a wavelength of 405 nm. The spectrophotometer was allowed 30 sec to stabilize, then change in absorbance was recorded for 2 min.

For plasma ChE, we collected blood in EDTA-treated collection vials that were centrifuged to separate plasma from red blood cells. Cholinesterase activity was determined in the same manner as the brain samples except that 20 μl of plasma were used in place of brain homogenate.

Cholinesterase activities were measured as μmoles acetylthiocholine hydrolyzed/min/g brain or ml plasma. Plasma ChE activity was described as mU/ml and brain activity as μmol/min/g for ease of interpretation. All assays were conducted at a room temperature of 25 C. Data were analyzed with least-squared regression and analysis of variance (ANOVA) techniques. Polynomial and transformed data regressions were tested with only a minimal increase in regression fit. Fisher's Protected Least Significant Difference test was used for multiple comparisons when the overall ANOVA was significant (Zar, 1984). We analyzed actual ChE activity and % inhibition compared with the control mean. Water consumption was compared across MP concentration to evaluate taste aversion.

RESULTS

Experiment 1

One bird in the 10.4 ppm group died on day 3 of the experiment with brain ChE activity inhibited 88% from the control mean. This individual was excluded from statistical analyses. Body mass was not analyzed by treatment because of high variability resulting primarily from differences in crop contents.

Brain and plasma ChE activity (Table 1) were more closely related to MP concentration ($r^2 = 0.64$, $P < 0.01$ and $r^2 = 0.33$, $P < 0.01$, respectively) than to mean MP dose/day ($r^2 = 0.38$, $P < 0.01$ and $r^2 = 0.29$, $P < 0.01$, respectively). Daily water intake (averaged over the trial for each individual) varied among treatments ($P < 0.01$) and declined with increasing MP dose (Table 1). The temporal pattern of

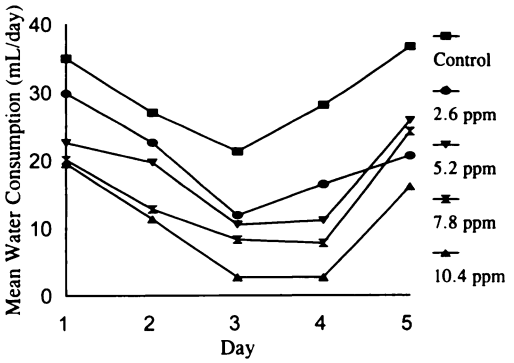

FIGURE 1. Average water consumption of white-winged doves in Kingsville, Texas expressed by methyl parathion (MP) treatment group and day (n = 8/point; except n = 7 in the 10.4 ppm group).

water consumption did not vary by day across groups (P = 0.85) (Fig. 1). Overall, the mean daily dose of MP increased with increasing MP concentration in the water ($P < 0.01$), but was not different between the three highest concentrations because of the decreased water intake as MP concentration increased (Table 1). Plasma and brain ChE activities were related, although the relationship was not strong enough for reliable prediction of brain ChE from plasma ChE (r^2 = 0.44, $P < 0.01$).

Experiment 2

Brain and plasma ChE activity did not change significantly over the 15 day trial for either the 0.87 ppm treatment (P = 0.18 and P = 0.19, respectively) or the 3.9 ppm treatment (P = 0.22 and P = 0.29, respectively) (Fig. 2). Multiple regression indicated that brain ChE activity was more closely related to the concentration of MP in the water ($P < 0.01$) than to daily dose (averaged over the 3 days before the bird was euthanized; P = 0.92) or period of exposure (P = 0.61). Plasma ChE activity was also influenced more by the concentration of MP in the water ($P < 0.01$) than by dose (P = 0.66) or period of exposure (P = 0.14). As in the first experiment, plasma and brain ChE activity were related although the relationship was even weaker (r^2 = 0.18, $P < 0.01$).

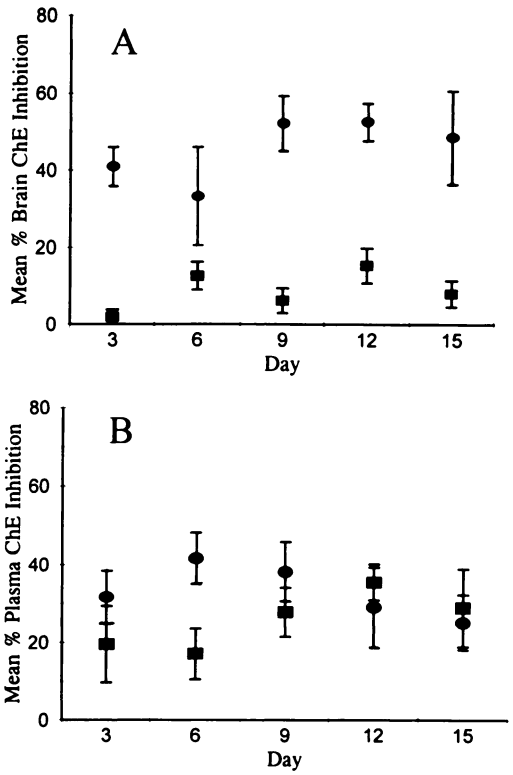

FIGURE 2. Percent brain (A) and plasma (B) cholinesterase inhibition ($\bar{x} \pm 1$ SE) in white-winged doves over time at 2 levels (squares: 0.87 ppm; circles: 3.9 ppm) of methyl parathion in drinking water (n = 4 doves/day) in Kingsville, Texas.

DISCUSSION

Numerous studies exist on the effects of OP pesticides on avian ChE activity levels and the subsequent effects of the enzyme inhibition. These studies (e.g., Rattner et al., 1982a, 1982b; Hunt et al., 1995; George et al., 1995), conducted in the field or the lab, focused on food (e.g., contaminated grain, insects) as the route of exposure. Several studies have been conducted using intubation (Keplinger and Diechman, 1967; Johnston et al., 1994) or topical contamination (Hunt et al., 1995).

Few studies of OP effects on ChE activity have used drinking water as a carrier (Brust et al., 1971), despite the potential for exposure of wildlife to pesticides through consumption of irrigation water in treated fields. The negative impact of toxins on water consumption makes deter-

mination of a causal relationship between dose and ChE activity difficult if birds are allowed *ad libitum* access to treated water. However, such relationships may not be as important when drinking contaminated water exclusively because the concentration of the pesticide appears to have a greater influence on ChE activity than dose (Koshakji et al., 1973). Such a relationship suggests that factors which decrease the concentration of pesticide in irrigation water would reduce impacts on wildlife, somewhat independent of the amount consumed.

Our results suggest that white-winged doves limit consumption of MP. In the first experiment, MP ingestion peaked at approximately 110 μg MP/day as a result of doves limiting water consumption as MP concentration increased. Whether this limit on MP consumption is a result of simple taste aversion or a more complicated postingestive feedback mechanism (Provenza, 1995) cannot be determined by our study; however, if birds can detect pesticides and limit their consumption of contaminated water, the likelihood of debilitating exposure to pesticides from water ingestion in the field is reduced.

Studies in which a given level of ChE activity must be maintained (i.e., during chronic exposure to OP pesticides) require knowledge of ChE activity response to OP exposure over time. The results of our second experiment suggested that ChE activity stabilized within 3 days and remained consistent for 15 days. Similar results were reported by Brust et al. (1971) and Fleming (1981). Such a relationship simplifies studies of the effects of chronic exposure to OP pesticides.

Plasma and brain ChE activity were related, but the relationship was weak and only useful for detecting large changes in brain ChE activity. Others have noted this poor relationship (e.g., Fleming, 1981) and discussed the physiology that contributes to the poor relationship (Lotti, 1995). Future research may benefit by focusing on acetylcholinesterase activity in brain and plasma, thus removing variation resulting from butrylcholinesterase and subsequent masking of a statistically significant relationship.

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