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Oral and Dermal Toxicity of Organophosphate Pesticides in the Domestic Pigeon (*Columba livia*)

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Low levels of blood and brain cholinesterases (ChE) are widely accepted biomarkers of exposure to anticholinergic pesticides, especially organophosphate (OP) esters (Ludke et al., 1975; Fleming, 1981; Wilson et al., 1992). The relatively rapid recovery of ChE levels due to hydrolysis of OP pesticide-ChE complexes and synthesis of plasma ChEs often lead investigators to infer that the exposures were both acute and recent. During the course of studying exposures of raptors to dormant sprays in orchards (Hooper et al., 1989; Wilson et al., 1991; Fry et al., 1993; Seiber et al., 1993), we undertook research on pigeons (*Columba livia*) as surrogates for wild birds such as Red-tailed Hawks (*Buteo jamaicensis*). This report presents oral and dermal toxicities of commonly used orchard sprays, ethyl parathion, diazinon and methidathion, to this readily available experimental animal.

MATERIALS AND METHODS

Eight to 16 wk old domestic pigeons were purchased from squab breeders (either Stuart's Farm Fresh, Sacramento, CA or D & G Squab Farm, Hughson, CA), and fitted with leg bands for identification. The birds were housed individually in stainless steel cages under constant environmental conditions (70°F, 14 hr light:10 hr dark cycle). The birds were given Purina® Pigeon Chow® Checkers and water *ad libitum*. Pigeons were maintained and studied under Animal Care Protocol #4131 approved by the Office of the Campus Veterinarian.

Diazinon technical [O,O-diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate], purity 85%, and methidathion [O,O-dimethyl phosphorodithioate S-ester with 4-(mercaptomethyl)-2-methoxy-Δ^2-1,3,4-thiadiazolin-5-one], purity 99%, was obtained from Ciba-Geigy Corporation, Greensboro, North Carolina. Ethyl parathion [O,O-diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate], purity 93%, was obtained from Chem Services, West Chester, Pennsylvania. Purities were determined by gas chromatographic analysis.

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Feed was withdrawn from the birds the night before oral dosing to be certain that their crops were empty. Dosing solutions were made with a polyethylene glycol (PEG) vehicle, and were delivered into the esophagus with a gavage cannula. Controls were given PEG. Antidotes, such as atropine and oximes, were not used in this study. In the oral dose plasma ChE time course experiment shown in Figure 1, there were two groups of three birds dosed with each OP. This was done so that blood sampling could be alternated between groups and reduce stress to the pigeons during the frequent early sampling times. The n values (3 or 6) at each sampling time are indicated in the figure caption.

Dermal dosing was carried out by delivering a set volume of test chemical to both feet. The top of the foot was used to reduce loss of chemical by abrasion which might occur on the foot bottom. A 30 µl volume was the maximum that could be delivered at one time without the chemical dripping off of the foot. Test chemicals were applied undiluted (neat), except methidathion (a solid at room temperature) which was dissolved in acetone. Control birds used in the methidathion experiment had acetone applied to their feet. The other control birds were untreated.

Approximately 0.5 to 1.0 ml aliquots of blood were drawn from wing or leg veins into heparinized syringes. Blood was centrifuged for 10 minutes at 1000xg, 4°C, and the supernatant plasma was stored at -70°C. Pigeons were sacrificed at the end of the experiments by decapitation. The brains were removed by dissection and stored at -70°C. Brains were homogenized in 4 volumes of 0.5% Triton X-100, 0.1 M sodium phosphate, pH 8 buffer with a Polytron tissue homogenizer, and then diluted 100-fold in 0.1 M sodium phosphate, pH 8 buffer for ChE assays. ChE activity in plasma and brain was determined by the colorimetric method of Ellman, et al. (1961), modified for use with an automated microplate reader. Acetylthiocholine was used as the substrate for all samples because it is as good a substrate as butyrylthiocholine for non-specific ChE (BChE) of birds (Hill and Fleming, 1982). The protein concentration of brain samples was determined by the method of Lowry, et al. (1951), using bovine serum albumin as a standard protein.

Linear regression analysis was used to estimate dose levels that resulted in 50% inhibition (ID_{50}) of brain acetylcholinesterase (AChE). Paired t-tests were used to test for statistically significant ($P<0.05$) differences from the initial plasma ChE activity in the time course experiments shown in the figures (Sokal and Rohlf, 1981). The error bars of the standard deviations are displayed in only one direction for the sake of clarity.

RESULTS AND DISCUSSION

Dose-dependent gross symptoms of OP poisoning usually appeared within a half-hr in pigeons dosed orally. These ranged from none to mild (nodding, drowsy appearance, head resting on breast, leg weakness, labored breathing) to severe (convulsions, salivation). With few exceptions, birds that showed the severe symptoms died shortly thereafter (Table 1). The lethal dose ranges and brain

Table 1. Mortality in orally dosed pigeons.

Compound	Dose (mg/kg)	Mortality
Parathion	0.5	0/6
	0.6	1/8
	0.7	1/4
	0.75	3/4
	0.8	2/4
	1	3/3
Diazinon	1	0/6
	2	0/4
	3	1/4
	5	6/6
Methidathion	10	0/6
	15	0/2
	30	1/2
	40	2/2
	50	2/2

Table 2. Estimated oral toxicity of pesticides.

Compound	Lethal Dose Range (mg/kg)	Estimated ID_{50}	r Value
Parathion	0.6 - 1	0.6	0.91
Diazinon	3 - 5	2	0.79
Methidathion	30 - 40	20	0.84

ID_{50} is the dose that inhibits 50% of brain AChE.
r is the linear correlation coefficient for the ID_{50} calculation.

AChE ID_{50} values indicate the order of oral toxicity was parathion > diazinon > methidathion (Table 2). There was a rapid depression in plasma ChE levels within two hr of dosing, a rapid recovery from two to twelve hr and complete recovery by three to five days (Figure 1).

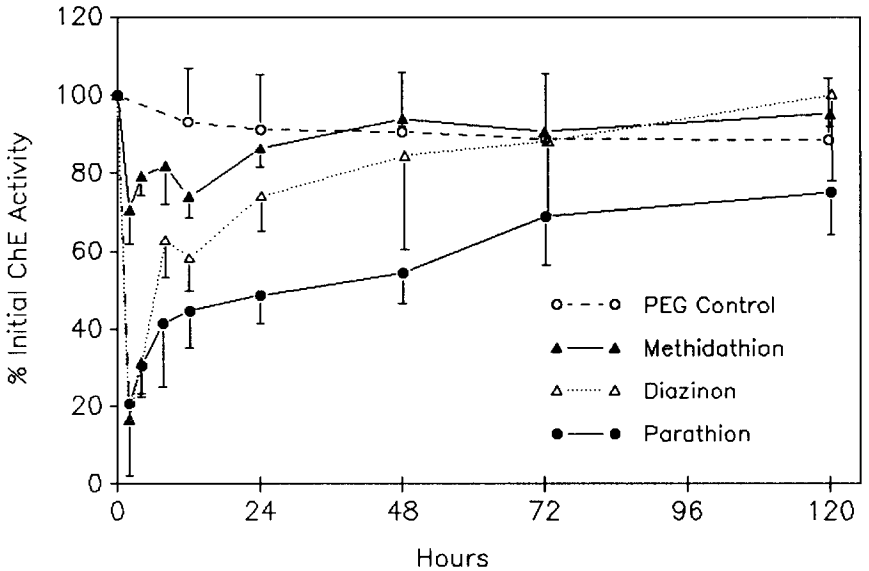

Figure 1. Plasma ChE activity in pigeons after an oral dose of organophosphate pesticide. Polyethylene glycol (PEG) was the vehicle (n = 9). Doses were 10 mg/kg methidathion, 1 mg/kg diazinon, and 0.5 mg/kg parathion (n = 6 at 0, 96, 120 hr; n = 3 at 2, 4, 8, 12, 24, 48, 72 hr for the three OP groups). Activity is average $\pm$ sd. Significant difference ($P<0.05$) from initial values: control had none; methidathion at all points except 8 hr; diazinon from 2 to 12 hr; parathion at all points.

Dermal treatments were carried out by applying the pesticides to the feet of the birds because absorption through the feet of hawks perched in orchards was suspected to be a route of exposure during winter dormant spraying (Fry et al., 1993). The onset of toxic effects of the pesticides applied dermally was less rapid than after oral treatments. Clinical symptoms did not appear until one to two days, and birds did not die until three to four days after dosing. Only birds treated with parathion died: 1 of 3 birds in the high dose group (113 mg/kg) died after three days, and 2 of 3 birds died from the mid-dose group (59 mg/kg) after four days.

Plasma ChE activity in dermally exposed birds was almost completely inhibited by 24 hr after parathion and diazinon treatment (Figure 2 and 3). There was no detectable inhibition of plasma ChE with methidathion (Figure 4), although two of three birds in the higher dose group (37 mg/kg) displayed mild symptoms of OP poisoning. These data and the brain AChE data in Table 2 suggest methidathion is a better AChE inhibitor than a BChE inhibitor in the pigeon, as has been reported in mammals (Chang et al., 1992). Pigeon plasma ChE is composed almost entirely of BChE, while many avian species have a mixture of AChE and BChE (unpublished observation). Whether the significant increases in

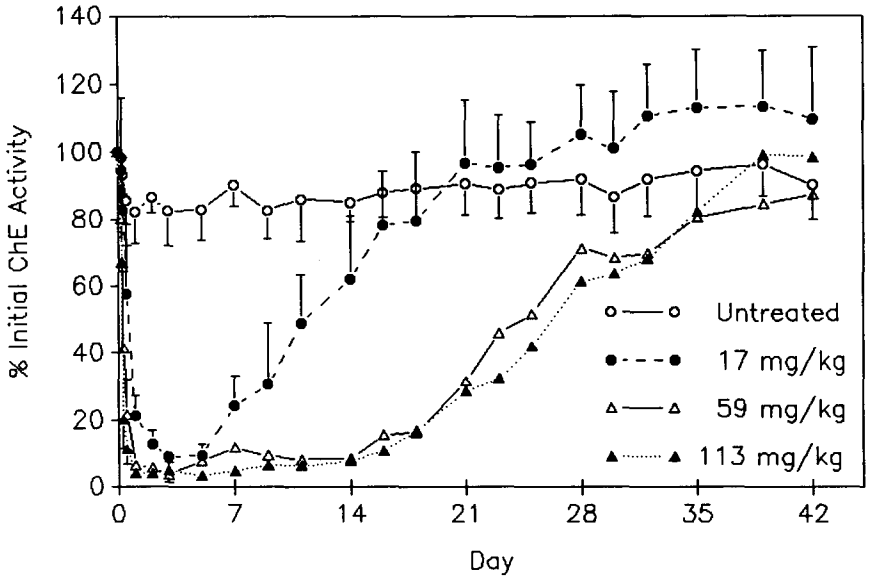

Figure 2. Plasma ChE activity in pigeons dosed dermally with parathion. Doses shown are the average dose in each group (n = 3). Activity is average ± sd. Significant difference (P<0.05) from initial values: control at day 2; 17 mg/kg from 1 to 11 d; 59 mg/kg from 5 hr to 3 d (2 birds died on day 4); 113 mg/kg from 8 hr to 2 d (1 bird died day 3).

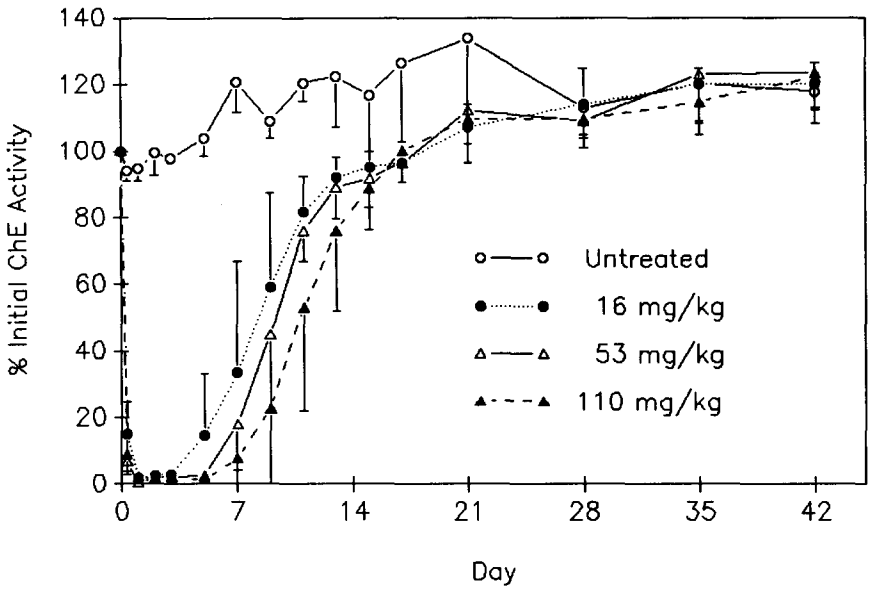

Figure 3. Plasma ChE activity in pigeons dosed dermally with diazinon. Doses shown are the average dose in each group (n = 3). Activity is average ± sd. Significant difference (P<0.05) from initial values: control at day 11; 16 mg/kg from 8 hr to 5 d, 35 to 42 d; 53 mg/kg from 8 hr to 7 d, 11 and 21 d; 110 mg/kg from 8 hr to 9 d, 35 d.

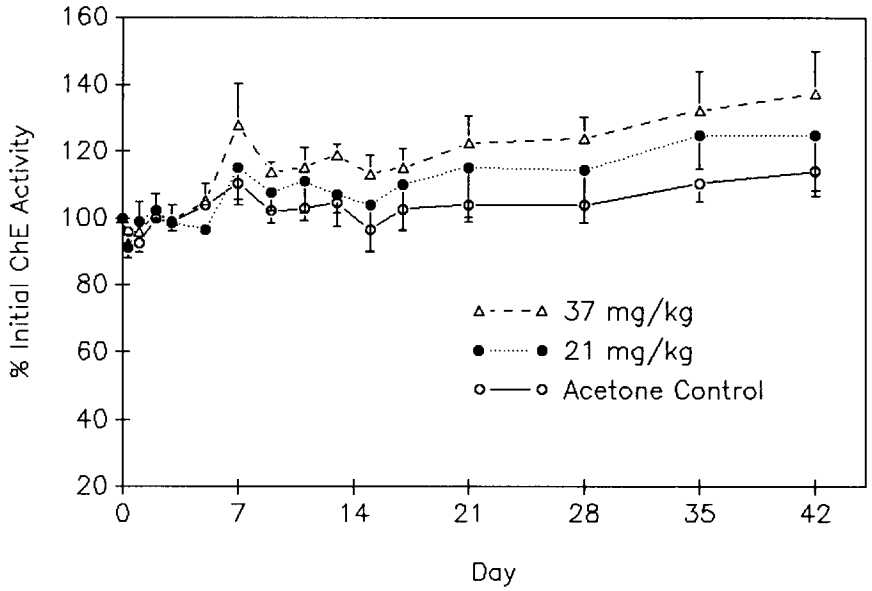

Figure 4. Plasma ChE activity in pigeons dosed dermally with methidathion. Doses shown are the average dose in each group (n = 3). Activity is average ± sd. Significant difference ($P<0.05$) from initial values: control at day 1 and 28; 21 mg/kg at 8 hr, from 3 to 5 d; 37 mg/kg at 8 hr, 9 to 13 d, 17 to 21 d, 35 to 42 d.

ChE activity following dermal methidathion exposure are due to a stimulation of ChE synthesis, and whether these increases have any physiological significance, deserve further study.

The order of dermal toxicity of the pesticides is the same as the previously stated order of oral toxicity: parathion > diazinon > methidathion.

Recovery of plasma ChE after dermal exposure in parathion and diazinon dosed pigeons was very slow. Plasma ChE activity in birds that survived the high and mid doses of parathion did not begin to recover until two wk after application and ChE activities had not returned to normal until 6 wk had passed (Figure 2). The two survivors of the high dose parathion group displayed mild clinical symptoms five to seven days after dosing. Plasma ChE levels in birds given the low dose of parathion (17 mg/kg) and all three diazinon doses (16, 53 and 110 mg/kg) began to recover five to seven days after dosing, and recovery was complete by three wk (Figures 2 and 3). Of these, only one of the three birds in each of the mid and high dose groups displayed mild clinical symptoms.

OP chemicals are expected to fall to low levels in the blood of birds in a matter of hours or a few days due to excretion, metabolism, esterase hydrolyses and binding to target sites, as they do in mammals (Qureshi et al., 1992). Levels of ChE activity usually return to normal in one to two days after oral treatment in

chickens, depending on the agent and the dose applied (Henderson et al., 1992). Oral application of the pesticides followed this pattern, but dermal foot applications did not. The data suggest the pesticides were stored in some depot, gradually appearing in the blood stream at concentrations sufficient to inhibit plasma ChE. Whether the pesticides persist on the skin (e.g. under the scales) or in some depot within the body such as extracellular lipids (Stromberg et al., 1990) is under study. Little is known concerning the dermal absorption of pesticides in birds (Driver et al., 1991; Cisson and Wilson, 1983).

Although the levels of pesticides tested in this study may be somewhat higher than would be expected to occur in the field, the persistence of low cholinesterase activities after foot exposures raises the possibility that birds can accumulate OPs by repeatedly roosting at exposed sites such as branches in pesticide sprayed orchards or perches treated to discourage roosting (Hunt et al., 1991). Moreover, the findings caution against concluding that low levels of blood and brain cholinesterase activity in wild birds necessarily indicate recent exposure to esterase-inhibiting agents.

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