Organophosphate Poisoning. Effects of Selected Organophosphate Pesticides on Plasma Enzymes and Brain Esterases of Japanese Quail (*Coturnix coturnix japonica*)

Graham E. Westlake,* Peter J. Bunyan, A. David Martin, Peter I. Stanley, and Linda C. Steed

Lethal and sublethal doses (0.33 or 0.5 × LD_{50}, LD_{50}, and 2 or 3 × LD_{50}) of five widely used organophosphate pesticides (carbophenothion, chlorfenvinphos, dimethoate, pirimiphos ethyl, and pirimiphos methyl) were administered to Japanese quail. Acetylcholinesterase (AChE), cholinesterase (ChE), and α-naphthyl acetate esterase activities were measured in plasma after 2 and 24 h or at death. AChE activities were measured in fresh brain extracts and after 7 days post-mortem. The tissue-specific enzymes glutamate dehydrogenase (GDH), glutamate oxaloacetate transaminase (GOT), and sorbitol dehydrogenase were also measured in the plasma after 2 and 24 h or at death. Both plasma AChE and ChE gave a dose–response relationship at 2 h, surviving quail at 24 h generally exhibiting less inhibition. Lethally dosed quail showed 80–85% inhibition of plasma AChE and >95% inhibition of plasma ChE. Brain AChE gave a dose–response relationship in quail that died within 2 h with the exception of the chlorfenvinphos-treated groups which all exhibited maximum inhibition. This relationship remained in survivors from the carbophenothion, dimethoate, and pirimiphos methyl treatments at 24 h. The administration of lethal doses to quail produced >60% inhibition of brain AChE activity. Plasma GOT was elevated in all quail surviving carbophenothion and pirimiphos methyl treatments at 24 h. Only carbophenothion administration gave a progressive elevation of GDH at both 2 and 24 h. Carbophenothion and pirimiphos methyl administration resulted in longer periods of plasma esterase inhibition than the other organophosphates and also produced the largest increases in plasma GOT at 24 h.

Previous studies (Bunyan and Taylor, 1966; Bunyan et al., 1968a,b, 1969, 1971; Jennings et al., 1975) have demonstrated that measurement of brain esterase levels are of value in the diagnosis of organophosphate poisoning in dead animals, while the reactivation, after electrophoresis, of brain esterases inhibited by carbamate pesticides (Bunyan and Jennings, 1976) enabled organophosphate and carbamate poisoning to be distinguished in wildlife found dead in the field. The measurement of plasma esterase inhibition has also been used (Dieter, 1974, 1975; Dieter and Ludke, 1975; Ignatov, 1976; Ludke et al., 1975; Schlinke and Palmer, 1971; Westlake et al., 1981) to demonstrate sublethal effects of organophosphate and carbamate pesticides in avian species. In addition, changes in the activity of a number of tissue-specific enzymes circulating in avian plasma have provided an indirect measure of pesticide exposure and residue accumulation (Dieter, 1974, 1975; Ignatov, 1976; Westlake et al., 1978, 1979, 1981).

This study was undertaken to investigate the degree and persistence of plasma and brain inhibition following administration of a range of doses of various organophosphate pesticides and to establish whether a correlation exists between plasma and brain esterase inhibition and changes in plasma levels of tissue specific enzymes. Such a relationship would allow further interpretation of the lethal and sublethal effects of these compounds.

The pesticides used are representatives of those of current importance. They are of widely differing chemical structures and have been shown to vary greatly in their acute toxicity in the experimental species used.

EXPERIMENTAL SECTION

Animals. Batches of six 21-day-old female Japanese quail were fed Spillers turkey starter crumbs and water ad libitum for a 21-day period prior to the start of each experiment. The birds were kept in constant environment conditions of 19 °C and 60% relative humidity with a 16-h daylight period. Weights were determined 3 days prior to dosing.

Pesticides. Five pesticides were chosen for this study, namely, carbophenothion [*S*-[[(4-chlorophenyl)thio]-methyl] *O,O*-diethyl phosphorodithionate], chlorfenvinphos [2-chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate], dimethoate [*O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate], pirimiphos ethyl [*O*-[2-(diethylamino)-6-methylpyrimidin-4-yl] *O,O*-diethyl phosphorothioate], and pirimiphos methyl [*O*-[2-(diethylamino)-6-methylpirimidin-4-yl] *O,O*-dimethyl phosphorothioate].

The pesticides were gifts from the manufacturers. Carbophenothion (analytical grade) was obtained from Stauffer Chemical Co., Ltd., chlorfenvinphos (technical, 93% pure) was from Shell Research, Ltd., dimethoate (technical) was from Murphy Chemical Co., Ltd., and pirimiphos ethyl (96.2% pure) and pirimiphos methyl (>95% pure) were from Plant Protection, Ltd.

Treatment. The experimental treatments are summarized in Table I. In the first series of treatments a single dose of the pesticides designed to approximate to one-third or half the LD_{50} dose, the LD_{50} dose, and 2 or 3 times the LD_{50} dose was administered (six birds per dose level) in corn oil solution by gelatin capsule except for dimethoate which was dosed in aqueous solution by intubation. The control groups were dosed with the same vehicle as used for each pesticide. The birds were bled by cardiac puncture 2 h after dosing unless overt symptoms of poisoning were shown earlier in which case blood samples were taken immediately before or at death. Surviving birds were bled again at 24 h and then sacrificed by cervical dislocation. At death or sacrifice each group was subdivided randomly into two, one of which was left at room temperature for 7 days before removing the brain in order to assess effects of aging. Brains were removed from the other group and processed as soon as possible after death

Tolworth Laboratory, Agricultural Science Service, Ministry of Agriculture, Fisheries and Food, Tolworth, Surbiton, Surrey, KT6 7NF, England.

0021-8561/81/1429-0772$01.25/0

Table I. A Summary of the Experimental Treatment of Japanese Quail with Organophosphate Pesticides

organophosphate	dosage level	dose, mg/kg	approx. LD_{50}	fate[a] survived to 24 h	fate[a] dead at 2 h	fate[a] died between 2 and 24 h
carbophenothion	1	28.4	0.5	4 (2)	2 (1)	0
	2	56.8	1	3 (1)	3 (1)	0
	3	113.6	2	1	5 (2)	0
chlorfenvinphos	1	74.0	0.5	4 (2)	2	0
	2	148.0	1	1	5 (2)	0
	3	296.0	2	0	6[c] (3)	0
dimethoate	1	8.3	0.33	6 (3)	0	0
	2	25.0	1	4 (2)	2	0
	3	75.0	3	0	5 (1)	1 (1)
pirimiphos ethyl	1	2.0	0.33	5 (3)	1	0
	2	6.0	1	1	5 (1)	0
	3	18.0	3	0	5[c] (2)	0
	1	2.0	0.33	6[b]	0	0
pirimiphos methyl	1	70.0	0.5	4 (2)	1	0
	2	140.0	1	2 (1)	3	0
	3	280.0	2	1	4	0 (1)
	1	70.0	0.5	6[b]	0	0

[a] Figures in parentheses refer to birds in which organ removal and esterase analysis were not carried out until 7 days post-mortem. [b] Sacrificed at 2 h in the second dosing experiment. [c] Died within 70 min of dosing.

or sacrifice. A second series of experiments were undertaken to obtain further 2-h brain esterase values. Pirimiphos ethyl and pirimiphos methyl were dosed at one-third and half the LD_{50} value, respectively. The birds were sacrificed 2 h after dosing and the brains removed immediately.

Preparation of Tissue Extracts and Enzyme Estimations. Heparinized blood was centrifuged at 2000*g* for 10 min at 4 °C. The plasma was removed and frozen at −20 °C until assayed. Brain homogenates (25%) were prepared in 1% Triton X-100 to ensure extraction of bound esterase (Bernsohn et al., 1964). The homogenates were centrifuged at 4500*g* for 15 min at 4 °C and then at 17000*g* for 40 min at 4 °C.

Acetylcholinesterase (AChE) activity was assayed by using a titrimetric method based on that described by Pickering and Pickering (1971). The reaction was carried out at 37 °C by using a Radiometer automatic titration apparatus. Enzyme (0.1 mL) was added to 1.4 mL of 0.9% saline in the reaction vessel. The pH of the sample was adjusted to approximately pH 7.7 for brain or pH 8.0 for plasma by the addition of 10 mM sodium hydroxide. The volume of 5 mM sodium hydroxide added via a syringe buret to maintain the end point pH was recorded over the next 2 min to measure the spontaneous rate of acid liberation before the reaction was started by the addition of 0.1 mL of acetylcholine chloride substrate (2.5 mM for brain or 10 mM for plasma in 0.9% saline, pH 8.0). The rate of alkali addition to maintain the end point pH was measured for a further 2 min. The nonenzymatic hydrolysis of the substrate was measured for each set of estimations by running the assay in the absence of the enzyme. α-Naphthyl acetate esterase was measured by the method of Gomori (1953) as adapted by Bunyan et al. (1968a) but incubating the enzyme for 1 h at 30 °C. Plasma cholinesterase (ChE), glutamate dehydrogenase (GDH), glutamate oxaloacetate transaminase (GOT), and sorbitol dehydrogenase (SDH) were determined by the methods of Ellman et al. (1961), Schmidt (1963), Bergmeyer and Bernt (1963), and Gerlach (1963), respectively, with modifications using the optimum conditions as described by Westlake et al. (1978).

RESULTS AND DISCUSSION

Due to the variety of the data collected, it has been presented in three different forms. Plasma and brain esterase activities are respectively expressed in the form of histograms and modified scattergrams of the percentage of the mean control values. Absolute mean control values are shown in Table II. Plasma AChE and ChE levels at 2 and 24 h after dosing or at death when within 2 h of dosing are shown in Figure 1. The direct comparison of plasma enzyme data from birds dying prior to 2 h with the plasma enzyme data from the survivors at 2 h is necessary due to the limited number of birds on test. However, the comparison must be interpreted cautiously as it is expected that enzyme activities may vary with the time of sampling. Thus, plasma esterase activities in the lower dosage groups which survived to 2 h would be expected to be slightly lower if the quail had been bled within 70 min of dosing along with the quail which died (Table I). Brain AChE levels at death, at sacrifice 24 h post-dosing, or after storage for 7 days post-mortem are shown in Figure 2. Absolute levels of tissue-specific enzymes are presented in tabular form. Plasma GDH, GOT, and SDH levels at 2 and 24 h after dosing or at death when within 2 h of dosing are shown in Table III. GOT was measured in plasma at 2 and 24 h in all treatments, but GDH and SDH were generally only measured at 24 h since effects on these enzymes are normally slow to appear (Westlake et al., 1979).

Table II. Control Esterase Levels in Japanese Quail[a]

	acetylcholinesterase[b]	cholinesterase[c]
plasma	132.9 (79)	825.0 (77)
brain (fresh)	1145.9 (22)	
brain (after 7 days post-mortem)	899.5 (18)	

[a] Mean esterase levels from the quail sample size in parentheses were taken as the respective controls (i.e., 100%). [b] Esterase activities are expressed as micromoles of NaOH used to maintain a constant pH per hour per gram of brain or per milliliter of plasma. [c] Esterase activity is expressed as International milliunits per milliliter of plasma.

Bunyan et al. (1968a) postulated that when comparing individual results with mean control values, a measured esterase value of more than two standard deviations (2 × SD) from the control mean value may be considered statistically significant. This criterion has been applied to the plasma and brain esterase results obtained for each pesticide and these are considered individually.

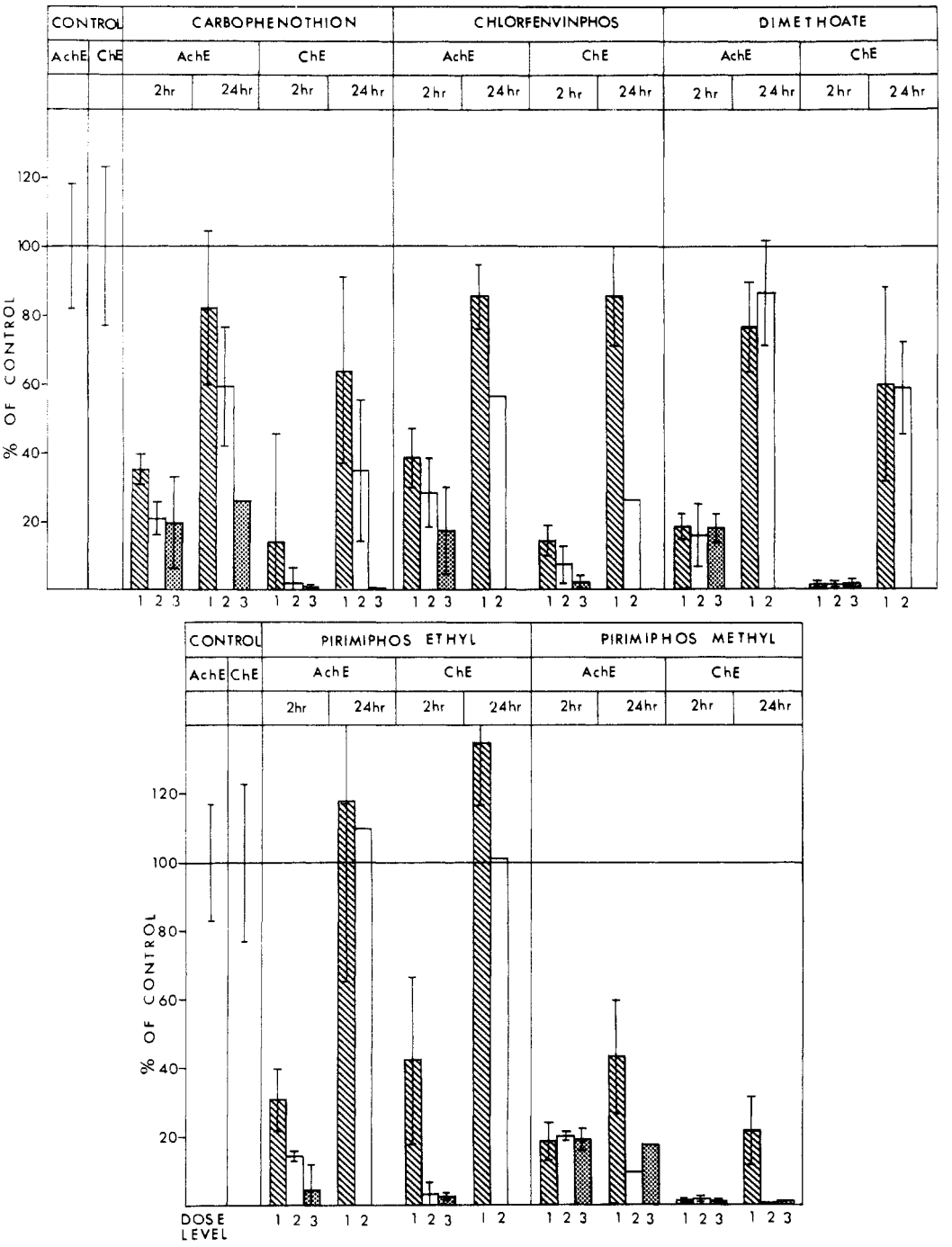

Figure 1. Plasma AChE and ChE activities in Japanese quail expressed as percentage of control mean after dosing with carbophenothion, chlorfenvinphos, dimethoate, pirimiphos ethyl, and pirimiphos methyl at various levels after 2 or 24 h or at death when within 2 h of dosing. Mean esterase activities from 79 control quail were taken as 100% (±SD). Dose levels (1, 2, or 3) are shown in Table I as milligrams of organophosphate per kilogram of body weight.

Carbophenothion. The single dose oral LD_{50} of carbophenothion to Japanese quail is 56.8 mg/kg (Jennings et al., 1975), and the doses given to the three groups were based on this figure (Table I). One bird survived the highest dose. All doses caused a significant inhibition of plasma AChE and ChE within 2 h (Figure 1). By 24 h the bird surviving the highest dose still exhibited plasma AChE and ChE inhibition in excess of 65%. AChE and ChE showed considerable recovery by 24 h in those birds given the lowest dose, but levels had not returned to normal. The group given the median dose showed significant ChE inhibition but that of AChE was less marked. Plasma GDH levels increased in all three groups after 2 h, and the increases were proportional to the dose (Table III). After 24 h the birds given the median dose showed a 2-fold increase in GOT and a 23-fold increase in GDH. The bird which survived twice this dose for 24 h had GOT and GDH levels raised approximately 20-fold and 220-fold, respectively. Plasma SDH (Table III) only showed slight increases in the LD_{50} group at 2 and 24 h.

Brain AChE was significantly inhibited in all groups after 2 h (Figure 2). In contrast, much less inhibition was found in the only fatality in the low-dose group. In this latter case, death may have been due at least in part to the trauma suffered as a result of the blood sampling. This is supported by lower AChE activities being found in two of the four birds which survived (at 24 h). The pattern of reactivation after hanging the bodies for 7 days was dose related. In those birds which died at 2 h, some reactivation of AChE occurred at the two higher dose levels although

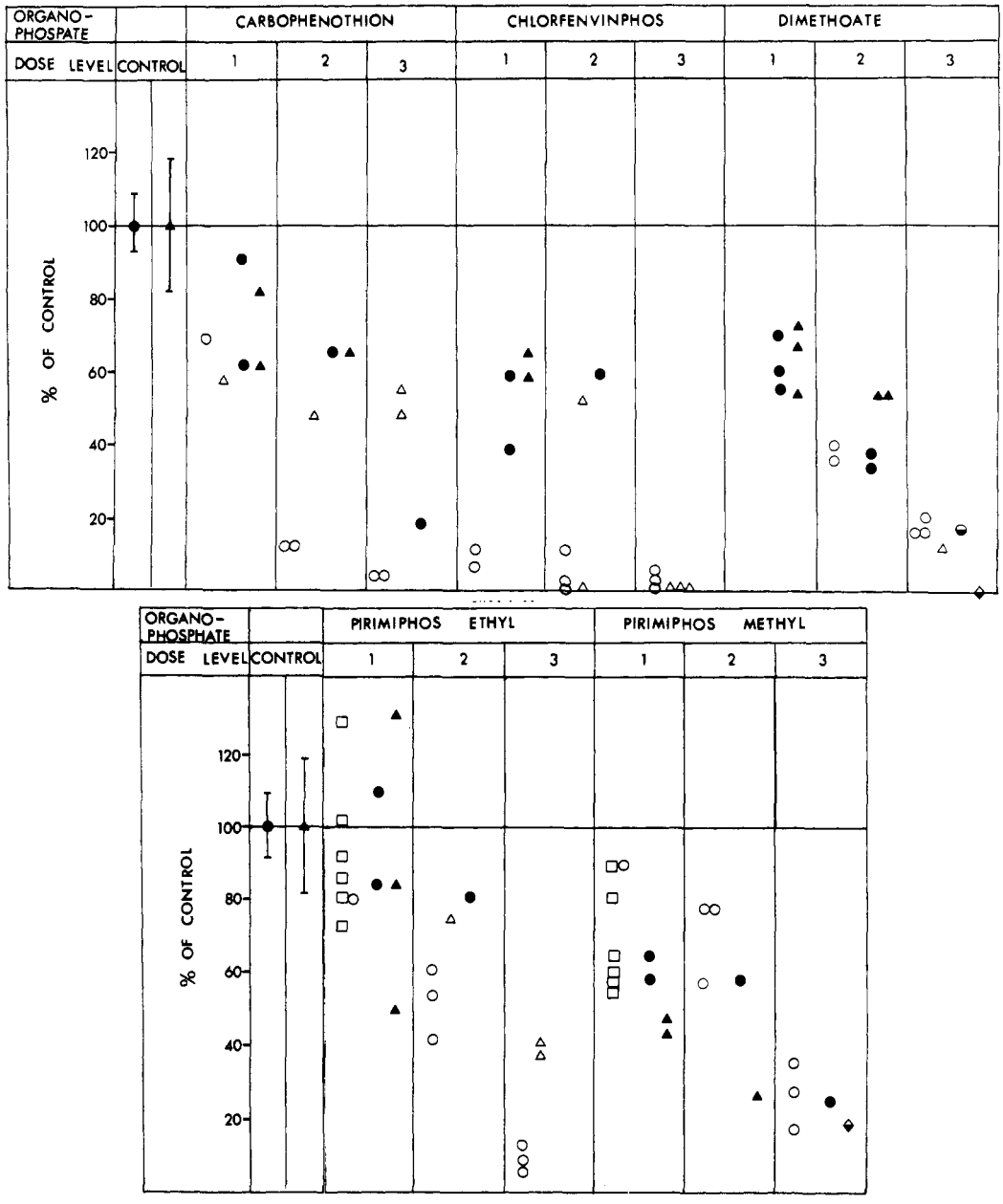

Figure 2. Brain AChE activities in Japanese quail expressed as percentage of control mean dosing with carbophenothion, chlorfenvinphos, dimethoate, pirimiphos ethyl, and pirimiphos methyl at various levels. Mean esterase activities from 22 control quail (fresh) and 18 control quail (after 7 days post-mortem) were taken as 100% (±SD). Individual results are shown as follows: (○) level measured in birds dead at 2 h or at death when within 2 h of dosing; (□) level measured in birds sacrificed at 2 h; (△) level measured 7 days post-mortem in birds dead at 2 h or at death when within 2 h of dosing; (●) level measured in birds surviving to 24 h; (▲) level measured 7 days post-mortem in birds surviving to 24 h; (⊖) level measured in birds dead during a 2–24-h period; (⊕) level measured 7 days post-mortem in birds dead during a 2–24-h period. Dose levels (1, 2, or 3) are shown in Table I as milligrams of organophosphate per kilogram body weight.

values were still outside the control range. A similar level was also found in the birds which died after receiving the low dose and was then hung for 7 days. No spontaneous reactivation was seen in those birds which were sacrificed at 24 h.

Chlorfenvinphos. The single oral LD_{50} dose of chlorfenvinphos to female Japanese quail was shown by Bunyan et al. (1971) to be 148 mg/kg. The doses given were based on this observation (Table I), and all the birds given the highest dose died within 70 min of dosing. All three dosed groups exhibited significant inhibition of both plasma AChE and ChE by 2 h (Figure 1). After 24 h, surviving quail in the low-dosed group showed a considerable recovery of AChE and ChE to within the control range of values. The surviving bird from the medium-dosed group showed some recovery of AChE and ChE overnight but then levels remained significantly inhibited. Plasma GOT levels significantly increased in the two higher dose groups (Table III) after 2 h and increased further at the lowest treatment level after 24 h. No apparent increase in plasma GDH or SDH was seen at 2 h, but plasma SDH increased slightly in survivors from the low-dose group at 24 h.

Brain AChE (Figure 2) showed a significant inhibition, in excess of 88%, in those birds which died within 2 h at all three dose levels. At 24 h significant reactivation had occurred in the surviving birds from the two lower dose groups. Only one of the two birds that died in the medium-dosed group showed reactivation 7 days post-mortem, the other bird from this group and those from the highest dose group still showed inhibition in excess of 99% of control values. One of the two surviving birds from the

Table III. Plasma GOT, GDH, and SDH Activities in Japanese Quail after Dosing with Carbophenothion, Chlorfenvinphos, Dimethoate, Pirimiphos Ethyl, and Pirimiphos Methyl at Various Levels[a]

plasma enzyme	dosage level			
	control	1	2	3
carbophenothion				
GOT: 2 h	82.68 ± 15.72 (6)	76.81 ± 25.67 (6)	74.74 ± 19.07 (6)	97.81 ± 23.91 (6)
24 h	91.03 ± 13.75 (6)	120.25 ± 99.10 (4)	156.06 ± 104.21[b] (3)	1882[d] (1)
GDH: 2 h	5.56 ± 4.69 (5)	8.06 ± 6.76 (5)	15.21 ± 17.80 (5)	24.29 ± 22.74[b] (5)
24 h	2.22 ± 2.89 (6)	5.99 ± 1.79[c] (4)	50.72 ± 13.27[d] (3)	488[d] (1)
SDH: 2 h	1.97 ± 0.60 (5)	NM	31.66 ± 61.57 (5)	1.77 ± 1.83 (5)
24 h	0.62 ± 0.89 (5)	0.53 ± 0.55 (4)	3.29 ± 1.79[c] (3)	0.87 (1)
chlorfenvinphos				
GOT: 2 h	59.28 ± 24.11 (6)	80.10 ± 94.73 (6)	101.64 ± 23.02[c] (6)	80.10 ± 7.85[b] (6)
24 h	39.21 ± 22.98 (5)	106.21 ± 76.63[b] (4)	71.13 (1)	
GDH: 2 h	7.81 ± 4.67 (6)	11.29 ± 15.87 (6)	5.99 ± 4.25 (6)	3.53 ± 3.69[b] (6)
24 h	5.56 ± 3.96 (6)	2.73 ± 1.54 (4)	4.82 (1)	
SDH: 2 h	6.17 ± 4.22 (6)	10.17 ± 15.95 (6)	5.21 ± 2.16 (6)	2.88 ± 1.80[b] (6)
24 h	0.72 ± 0.66 (6)	1.68 ± 1.44[b] (4)	0.96 (1)	
dimethoate				
GOT: 2 h	62.61 ± 3.84 (6)	69.00 ± 6.78[b] (5)	57.41 ± 7.45 (6)	77.65 ± 10.05[c] (6)
24 h	70.53 ± 12.65 (4)	58.03 ± 11.84[b] (6)	85.05 ± 21.57 (4)	
GDH: 24 h	16.16 ± 8.79 (4)	8.44 ± 8.72[b] (5)	0.36 ± 0.42[d] (4)	
SDH: 24 h	1.39 ± 0.68 (4)	1.01 ± 1.51 (5)	0.72 ± 0.71 (4)	
pirimiphos ethyl				
GOT: 2 h	86.00 ± 25.98 (6)	79.22 ± 14.78 (6)	137.17 ± 91.30 (6)	104.89 ± 18.45 (5)
24 h	91.08 ± 24.11 (6)	95.52 ± 16.06 (4)	32.06 (1)	
GDH: 24 h	2.96 ± 5.40 (6)	8.99 ± 6.53[b] (4)	NM	
SDH: 24 h	2.56 ± 3.78 (6)	0.38 ± 0.76 (4)	NM	
pirimiphos methyl				
GOT: 2 h	95.93 ± 23.22 (5)	83.00 ± 23.74 (5)	98.05 ± 15.50 (5)	66.20 ± 19.77[b] (5)
24 h	117.6 ± 24.02 (5)	110.54 ± 65.04 (3)	2270 ± 2971[b] (2)	2810[d] (1)
GDH: 24 h	2.63 ± 1.82 (5)	6.42 ± 10.81 (4)	1.93 ± 0 (2)	0.61 (1)
SDH: 24 h	0.89 ± 0.94 (5)	ND (4)	ND (2)	ND (1)

[a] Enzyme activity is in International milliunits per milliliter of plasma; mean ± SD; NM, not measured; ND, no activity detected (<0.1 mU/mL), group size in parentheses. See Table I for dosage levels in milligrams of organophosphate per kilogram of body weight. [b-d] Significant differences from the individual control values: [b] $p < 0.2$; [c] $p < 0.05$; [d] $p < 0.005$ (Student's t test).

low-dosed group which were examined 7 days post-mortem exhibited some reactivation but remained significantly inhibited.

Dimethoate. The single oral LD_{50} dose of dimethoate to sparrows has been reported as 22 mg/kg, to blackbirds as 26 mg/kg, to pheasants as 15–25 mg/kg, to chickens as 25–50 mg/kg (Sanderson and Edson, 1964), to starlings as 32 mg/kg, and to red-winged blackbirds as 6.6 mg/kg (Schafer, 1972). The LD_{50} for Japanese quail was estimated to be 25 mg/kg from these figures and dosages were based on this (Table I). Four of the birds given the highest dose of 75 mg/kg died within 2 h of dosing; the remaining birds died overnight within 24 h of dosing. Plasma AChE and ChE levels (Figure 1) both showed very significant inhibition for all three dosage groups by 2 h. However, by 24 h both enzyme activities had recovered to within the normal range for the two lower dosage groups, although ChE showed more variation in the lowest dose group. Plasma GOT levels apparently increased with the low- and high-dosage groups by 2 h, and the median-dosage group showed a slightly elevated plasma GOT level at 24 h. The apparently marked decrease in plasma GDH activity in the latter group at 24 h would appear to be due to the abnormally high control plasma GDH values at this time. Brain AChE activity (Figure 2) was reduced by 60% within 3 h of dosing in the median group and by 85% in the highest dose group. By 24 h the inhibition was related to the dose, but inhibition in the two higher dosed groups remained similar to that found at 2 h. The lowest dosed group showed ~40% inhibition at 24 h. Some reactivation of AChE was observed after the 7-day hanging period in the median group where the birds were sacrificed at 24 h. No reactivation was observable in any bird from the highest dose group.

Pirimiphos Ethyl. The single oral LD_{50} dose of pirimiphos ethyl to pheasants is 3–6 mg/kg and to pigeons is 6–12 mg/kg (Plant Protection, Ltd., 1979). The LD_{50} for Japanese quail was estimated to be 6.0 mg/kg from these figures (Table I). Three out of six quail given this dose died following symptoms characteristic of organophosphate poisoning. Birds given the highest dose died within 1 h, and in four of these six birds, the plasma AChE was below a measurable level (Figure 1). All groups showed significant depression of mean plasma AChE and ChE activities within 2 h. However, significant recovery with possibly some elevation above the control level occurred with both enzymes after 24 h in the survivors of the lowest dose group. In the one bird which survived the median dose to 24 h, both AChE and ChE activities were normal. Plasma GOT levels (Table III) were unchanged at the lowest dose level but slightly increased in the two higher dosage groups at 2 h. By 24 h plasma GDH showed a small rise in the survivors of the lowest dosed group but plasma SDH did not show a significant variation (Table III).

Brain esterases (Figure 2) showed a marked dose–response relationship at 2 h. There was significant inhibition of AChE activity at 2 h in birds given the two highest doses. The difference in activity between the three groups was also significant. At 24 h significant reactivation had occurred in the bird which survived the LD_{50} dose. Hanging for 7 days at ambient temperature resulted in a wider variation of the individual results with only the two highest dosed groups showing significant reactivation.

Pirimiphos Methyl. The acute oral LD_{50} value for quail was quoted as 140 mg/kg (Plant Protection, Ltd., 1979). Birds were dosed with 0.5 × LD_{50}, LD_{50}, and 2 × LD_{50} because of the relatively low toxicity of this pesticide. Four of the five birds which received 280 mg/kg died

within 6 h after displaying characteristic signs of organophosphorus poisoning. Those birds given 140 mg/kg displayed signs of poisoning within 2 h and three birds died shortly after bleeding. No outward signs of poisoning were exhibited by the sublethally dosed group. All doses had a marked effect on plasma AChE and ChE levels (Figure 1) after 2 h. At 24 h plasma AChE and ChE showed a significant recovery in activity in those birds given 70 mg/kg but inhibition still remained greater than 2 × SD. Plasma AChE and ChE continued to be grossly inhibited at 24 h in those birds on the two highest doses. Plasma GOT exhibited no elevation in level at 2 h, but by 24 h, the surviving quail from the LD_{50} and 2 × LD_{50} groups showed significant increases. By 24 h, no plasma SDH activity was measurable at any dosage level (Table III).

Within 2–6 h brain AChE (Figure 2) was significantly inhibited at all three dose levels and remained inhibited at 24 h. There was no indication of any tendency for AChE to reactivate after 7 days of hanging.

GENERAL DISCUSSION

The primary objective of feeding sublethal and lethal doses of a representative group of organophosphate pesticides to Japanese quail was generally achieved. Some marked differences in effect were shown between the individual pesticides examined.

The levels of plasma AChE and ChE were inhibited after 2 h with all the dosage treatments of the five organophosphate pesticides. While the depressions of ChE were generally more marked, those recorded for AChE generally followed the same dose–response relationship. The birds which died within 2 h of dosing with organophosphates generally showed >95% inhibition of plasma ChE and ~80–85% inhibition of plasma AChE. For dimethoate and pirimiphos methyl, the inhibitory response was not dose related. In those birds which survived the two lower dosage levels to 24 h, the inhibition of plasma AChE and ChE was generally less than that which had been observed at 2 h. Only the two surviving quail from the carbophenothion and pirimiphos methyl high-dose groups gave similar levels of inhibition at both 2 and 24 h. Pirimiphos ethyl produced some elevation of AChE and ChE above control levels at 24 h. The results indicate that plasma ChE measured by the method of Ellman et al. (1961) is a more sensitive indicator of the changes produced by the organophosphate pesticides than is the titrimetric estimation of AChE, although a similar pattern of effect is shown by both assays. α-Naphthyl acetate esterase was also measured in the plasma of the pesticide-treated quail. However, these results have not been presented since less inhibition (60–90%) was obtained than with plasma AChE, the standard deviations were wider, and the dose–response relationship was not consistent.

The release of abnormally high levels of specific tissue enzymes into the blood system is dependent on both the degree and type of damage exerted by the pesticide administered. The enzymes GOT, GDH, and SDH have low background levels in the plasma of normal animals which is due to aging tissue and cell replacement. Any increases outside the control range may be regarded as indicating tissue damage. Within 2 h of dosing some small increases in plasma GOT level were recorded in the groups given high doses of all the pesticides with the exception of pirimiphos methyl. By 24 h, marked elevations in plasma GOT were found in survivors from dosage with both carbophenothion and pirimiphos methyl in all groups. The response appeared to be dose related. Plasma GDH showed a progressive elevation with dose of carbophenothion at both 2 and 24 h, the enzyme response being much larger at the latter time. The other pesticide treatments did not give any significant change in GDH. Only the median-dosed carbophenothion group of quail showed a large response at 2 h in plasma SDH, and none of the pesticides tested showed any effect at 24 h. While a general correlation between plasma esterase inhibition and the level of specific tissue enzyme in the plasma is difficult to establish, it was found that both carbophenothion and pirimiphos methyl, both of which showed longer periods of plasma esterase inhibition than the other organophosphates studied, also showed the largest increases in plasma GOT at 24 h.

Brain AChE levels showed more variation with respect to the pesticides studied than plasma AChE. The birds given lethal doses of organophosphates exhibited ~60–99% inhibition of the normal mean brain AChE value. This range is lower than that reported by Bunyan et al. (1968b), who observed brain AChE inhibition in excess of 90% for ring-necked pheasants (*Phasianus colchicus*) exposed to lethal concentrations of chlorfenvinphos, demeton methyl, dimethoate, ethion, and guthion but not in pheasants dosed with diazinon. Ludke et al. (1975) considered inhibition of brain ChE in excess of 50% to be diagnostic of mortality after chronic exposure of Japanese quail to parathion while exposure to the organophosphate was indicated by 20% inhibition of brain ChE. These results suggest that the selection of a percentage inhibition of the interpretation of wildlife deaths from pesticide poisoning must be exercised with caution. Further data from avian species that have been lethally dosed with a selection of organophosphate pesticides may be useful. A dose–response relationship for brain AChE inhibition by the organophosphates was seen in the birds that died before 2 h or were killed at 2 h (pirimiphos ethyl and pirimiphos methyl) with the exception of the chlorfenvinphos groups which were all near maximum inhibition. At 24 h this dose–response relationship was still found in the surviving birds from the carbophenothion, dimethoate, and pirimiphos methyl groups.

The extent of reactivation of brain esterases in intact birds examined 7 days post-mortem was variable. Birds that died at 2 h showed reactivation in the carbophenothion and pirimiphos ethyl groups to less than 65% inhibition, but only one bird from the median-dosed chlorfenvinphos group showed reactivation. The other quail that died at 2 h in the chlorfenvinphos and dimethoate groups remained significantly inhibited without reactivation. However, surviving birds sacrificed at 24 h after dosing with carbophenothion and which were then left 7 days post-mortem did not show AChE reactivation. Dimethoate showed a varied response in the hung birds, esterase activities in the lowest dose group were unchanged and in the bird that died overnight was further inhibited but in the median-dosed group reactivation to less than 50% inhibition took place. The degree of AChE reactivation occurring after a post-mortem period may be related to the amount of protein-bound pesticide that is released (Jennings et al., 1975). The post-mortem variation found between pesticides in this work may be due to the varying amount of free pesticide remaining in brain tissue following either the release from the protein-bound state or further metabolism. The birds which were hung 7 days post-mortem before the brains were processed showed greater variation in esterase levels, and overall the esterases were lower in both the control (Table II) and test brains after this 7-day period. Brain α-naphthyl acetate esterase was also measured in addition to AChE, and in most cases parallel inhibition was found for both enzymes.

The measurement of plasma esterase inhibition has been reported to be of limited diagnostic value for pesticide exposure (Bunyan et al., 1969; Ludke et al., 1975); however, with further characterization of the elevations in level following exposure, it may be possible to relate plasma with brain esterase inhibition. Plasma cholinesterase inhibition has been recommended by the World Health Organization (1967) as an index of organophosphate exposure in humans. Bradway et al. (1977) have related blood cholinesterase activity to a range of organophosphate residues in tissues and to their metabolites in the urine of rats. The measurement of different esterases and tissue-specific enzymes in the plasma of avian species may be useful in monitoring the sublethal effects on wildlife resulting from the agricultural application of pesticides. Westlake et al. (1980) have demonstrated the value of this approach with wood mice (*Apodemus sylvaticus*) trapped on farmland following the drilling of chlorfenvinphos-treated winter wheat by relating plasma and liver esterase activities with the residue of chlorfenvinphos present in the gut tissue. For this type of trial, it is necessary to acquire the specific data on changes in the levels of relevant enzymes produced by the pesticide under test in the species to be examined or a closely associated one before moving into the field.

ACKNOWLEDGMENT

We thank C. A. Blunden and W. J. Fletcher for technical assistance and W. Mitchell for assistance in feeding and housing the birds. Gifts of pesticides from Murphy Chemical Co., Ltd., Plant Protection, Ltd., Shell Research, Ltd., and Stauffer Chemical Co., Ltd., are gratefully acknowledged.

LITERATURE CITED

Bergmeyer, H. U.; Bernt, E. In "Methods of Enzymatic Analysis", 1st ed.; Bergmeyer, H. U., Ed.; Verlag Chemie: Weinheim, 1963; p 837.

Bernsohn, J.; Barron, K. D.; Norgello, H. *Biochem. J.* **1964**, *91*, 21C.

Bradway, D. E.; Shafik, T. M.; Lores, E. M. *J. Agric. Food Chem.* **1977**, *25*, 1353.

Bunyan, P. J.; Jennings, D. M. *J. Agric. Food Chem.* **1976**, *24*, 136.

Bunyan, P. J.; Jennings, D. M.; Jones, F. J. S. *Pestic. Sci.* **1971**, *2*, 148.

Bunyan, P. J.; Jennings, D. M.; Taylor, A. *J. Agric. Food Chem.* **1968a**, *16*, 326.

Bunyan, P. J.; Jennings, D. M.; Taylor, A. *J. Agric. Food Chem.* **1968b**, *16*, 332.

Bunyan, P. J.; Jennings, D. M.; Taylor, A. *J. Agric. Food Chem.* **1969**, *17*, 1027.

Bunyan, P. J.; Taylor, A. *J. Agric. Food Chem.* **1966**, *14*, 132.

Dieter, M. P. *Toxicol. Appl. Pharmacol.* **1974**, *27*, 86.

Dieter, M. P. *Arch. Environ. Contam. Toxicol.* **1975**, *3*, 142.

Dieter, M. P.; Ludke, J. L. *Bull. Environ. Contam. Toxicol.* **1975**, *13*, 257.

Ellman, G. L.; Courtney, K. D.; Andres, V.; Featherstone, R. M. *Biochem. Pharmacol.* **1961**, *7*, 88.

Gerlach, U. In "Methods of Enzymatic Analysis", 1st ed.; Bergmeyer, H. U., Ed.; Verlag Chemie: Weinheim, 1963; p 761.

Gomori, G. *J. Lab. Clin. Med.* **1953**, *42*, 445.

Ignatov, M. *Vet.-Med. Nauki* **1976**, *13*, 14.

Jennings, D. M.; Bunyan, P. J.; Brown, P. M.; Stanley, P. I.; Jones, F. J. S. *Pestic. Sci.* **1975**, *6*, 245.

Ludke, J. L.; Hill, E. F.; Dieter, M. P. *Arch. Environ. Contam. Toxicol.* **1975**, *3*, 1.

Pickering, C. E.; Pickering, R. G. *Arch. Toxikol.* **1971**, *27*, 292.

Plant Protection, Ltd., personal communication, 1979.

Sanderson, D. M.; Edson, E. F. *Br. J. Ind. Med.* **1964**, *21*, 52.

Schafer, E. W. *Toxicol. Appl. Pharmacol.* **1972**, *21*, 315.

Schlinke, J. C.; Palmer, J. S. *Am. J. Vet. Res.* **1971**, *32*, 495.

Schmidt, E. In "Methods of Enzymatic Analysis", 1st ed.; Bergmeyer, H. U., Ed.; Verlag Chemie: Weinheim, 1963; p 752.

Westlake, G. E.; Blunden, C. A.; Brown, P. M.; Bunyan, P. J.; Martin, A. D.; Sayers, P. E.; Stanley, P. I.; Tarrant, K. A. *Ecotoxicol. Environ. Saf.* **1980**, *4*, 1.

Westlake, G. E.; Bunyan, P. J.; Martin, A. D.; Stanley, P. I.; Steed, L. C. *J. Agric. Food Chem.* **1981**, following paper in this issue.

Westlake, G. E.; Bunyan, P. J.; Stanley, P. I. *Ecotoxicol. Environ. Saf.* **1978**, *2*, 151.

Westlake, G. E.; Bunyan, P. J.; Stanley, P. I.; Walker, C. H. *Chem.-Biol. Interact.* **1979**, *25*, 197.

World Health Organization (W.H.O.) *W.H.O. Tech. Rep. Ser.* **1967**, *No. 356*, 14.

Received for review July 2, 1980. Revised March 23, 1981. Accepted March 23, 1981.