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EFFECT OF PESTICIDES ON MEDITERRANEAN FISH MUSCLE ENZYMES

DJELOVANJE PESTICIDA NA ENZIME MIŠIĆA MEDITERANSKIH RIBA

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The effect of the pesticides aldrin, dimethoate and permethrin on muscle pyruvate kinase, lactate dehydrogenase, malate dehydrogenase, succinate dehydrogenase and cytochrome oxidase was investigated in the Mediterranean fish, *Boops boops, Coryphaena hippurus* and *Mugil cephalus.* Aldrin and permethrin stimulated the extramitochondrial enzymes studied. Whilst permethrin inhibited the mitochondrial enzymes, aldrin inhibited only cytochrome oxidase. Dimethoate did not affect the extramitochondrial enzymes but inhibited the mitochondrial ones.

INTRODUCTION

The *in vitro* effects of organochlorine compounds on cellular respiration have been reported in both invertebrate and vertebrate tissues. Endrin has been shown to inhibit succinate dehydrogenase and cytochrome oxidase activity in liver homogenates of the catfish, *Ictalurus punctatus* (Calvin and Philips quoted by McCorkle and Yarbrough, 1974). McCorkle and Yarbrough (1974) studied the effect of mirex on succinate dehydrogenase activity in tissue preparations from insecticide-resistant *Gambusia affinis* and the green sunfish, *Lepomis cyanellus* which is susceptible to insecticides. In *G. affinis,* succinate dehydrogenase activity of brain and liver was inhibited by 10 uM mirex. This result was not consistent with the pattern of effect reported for other organochlorine compounds on enzyme activity in resistant species (Boyd and Ferguson, 1964). In *L. cyanellus* the same concentration of mirex inhibited enzyme activity. *Salmo irideus* fry were shown to be highlly sensitive to lindane treatment. The sensitivity was found to increase with development. The effect was marked by changes in the histological structure of the liver and muscle and in the levels of the glycolytic enzymes: lactate dehydrogenase, pyruvate kinase, aldolase and phosphoglucomutase (Boulekbache and Spiess, 1974). There is some evidence to indicate that the effect of pesticides is

not always inhibitory. Following exposure to sub-lethal doses of hydrocarbons glycogenic enzymes of *Mercenaria mercenaria* showed stimulation. Low concentrations of mirex were found to stimulate succinate dehydrogenase and cytochrome oxidase activity in *G. affinis* and in *L. cyanellus* (McCorkle and Yarbrough, 1974). The present paper reports the effects of three pesticides on the key enzymes of muscle metabolism from three species of Mediterranean fish. Three insecticides of different types have been chosen (1) poorly metabolised organochlorine — aldrin (2) organothiophosphate — dimethoate (3) pyrethroid a class which are rapidly metabolised — permethrin. Enzyme effects were studied *in vitro*. White and red muscle were investigated in view of metabolic differences between the two types of muscle. No pesticide investigations appear to have been carried out on Mediterranean fish.

MATERIALS AND METHODS

All substrates and chemicals were obtained from Sigma Chemical Co., U. S. A. The pesticides, aldrin and dimethoate were supplied by the Department of Agriculture, Malta. Permethrin was a generous gift from Wellcome Laboratories, Berkhamsted, England.

Fish of the species *Boops boops* (bogue), *Coryphaena hippurus* (dolphinfish) and *Mugil cephalus* (grey mullet) were obtained from the Fisheries Department, Malta. White and red muscle samples were taken from the three species. About 1 g of muscle was minced with scissors and disintegrated with a homogenizer of the Potter-Elvehjem type in 10 ml of ice-cold buffer, as used for the enzyme assays. Homogenisation was continued for 1 minute after which the homogenates were centrifuged at 1,000 g for 10 minutes in an MSE Mistral 4L Centrifuge at 2°C. The supernatants were retained in an ice-bath for immediate enzyme assays. For pyruvate kinase a partial purfication as described by Randall and Anderson (1975) and by Bannister and Anastasi (1976) was carried out.

Activity determinations were conducted spectrophotometrically at about 23°C in an Eppendorf photometer with recorder (Eppendorf Geratebau, Germany) using 3 ml of assay mixture in a 1-cm cuvette. With the exception of succinate dehydrogenase and cytochrome oxidase, all enzyme activities were measured at 366 nm. The activity of succinate dehydrogenase was determined at 405 nm and that of cytochrome oxidase at 546 nm. The assay mixture for each of the measured enzymes was as follows:

Lactate dehydrogenase (EC. 1.1.1.27) : 33 mM phosphate buffer, pH 7.4, 0.27 mM NADH and a maximum concnetration of 1.33 mM pyruvic acid.

Pyruvate kinase (EC. 2.7.1.40) : 2 mM adenosine diphosphate, 8 mM magnesium chloride, 75 mM potassium chloride, 3.6 U of lacate dehydrogenase and 0.15 mM NADH in 50 mM Tris-NC1 buffer, pH 7.5. Phosphenolpyruvate was used at a maximum concentration of 3 mM. In a separate set of assays, 0.5 mM fructose 1,6-diphosphate (FDP) was added as allosteric modifer.

Malate dehydrogenase (EC. 1.1.1.37) : 0.23 M Tris-HC1 buffer, pH 7.4, 0.05 mM NADH and a maximum concentration of 0.25 mM of freshly prepared oxaloacetate.

Succinate dehydrogenase (EC. 1.3.99.1): The enzyme was assayed by measuring the reduction of potassium ferricyanide in the presence of sufficient potassium cyanide to inhibit the cytochrome oxidase activity (Slater and Bonner, 1952). To a 1 cm cell were added 0.2 ml each of 0.1 M neutralised potassium cynaide and 0.01 M potassium ferricyanide and 0.2 M sodium succinate solution. The maximum final concentration of succinate was 20 mM. 33 mM phosphate buffer, pH 7.2 was added to give a total volume of 3.0 ml.

Cytochrome oxidase (EC. 1.9.3.1) : 33 mM-phosphate buffer, pH 7.4 and maximum concentration of 17.5 μM reduced cytochrome c.

In all assays the reaction was started by the addition of the enzyme sample. After the control assays 1 ppm and 5 ppm of aldrin, dimethoate or permethrin was included in the incubation mixture and the enzyme activity determination over a range of substrate concentrations was repeated. The activities of pyruvate kinase, lactate dehydrogenase and malate dehydrogenase were calculated using the extinction coefficient of 3.30×10^6 litre. $mol^{-1} \cdot cm^{-1}$ for NADH at 366 nm. Activities were expressed as umoles NADH oxidised/litre/minute.

For calculating succinate dehydrogenase activity the change in absorbance units/litre/minute was determined. A change in absorbance of one unit was arbitratily defused as an unit of enzyme activity. For cytochrome oxidase the oxidation of cytochrome c was followed by measuring the decrease in absorbance at 546 nm. The change in the concentration of reduced cytochrome c (per minute) was calculated as a fraction of the total measurable change for the cytochrome c concentration in the cuvettes.

The protein concentration of the homogenates was measured with the Folin reagent using crystalline bovine serum albumin as standard (Lowry *et al.*, 1951).

RESULTS

The experimental data for these enzymes indicated an overall picture of Michaelis-Menten kinetics. The Michaelis constants and maximum velocities determined for the enzymes are given in Tables 1—15 together with their standard errors. Comparison of the computed standard errors with the values of the kinetic parameters as given in Tables 1—15 shows that the Michaelis constant (Km) was fitted less precisely than the maximum velocity (V_{max}). It is therefore to be considered that the estimates of V_{max} are more reliable than those of Km and can be used for statistically significants effects of the pesticides of the enzymes. In general, however, comparable Km values were obtained for the white and red muscle preparations of the enzymes, except in the case of cytochrome oxidase. Comparable values were also obtained for each enzyme among the three fish species. The results obtained indicate that no significant effect of the pesticides on the Km values of lactate dehydrogenase, pyruvate kinase, malate dehydrogenase, succinate dehydrogenase and cytochrome oxidase. The trend of statistically

significant pesticide effect was the elevation of the maximum velocity of lactate dehydrogenase, pyruvate kinase and malate dehydrogenase and depression of the maximum velocity of succinate dehydrogenase and cytochrome oxidase. This result corresponds to an elevation of the maximum velocity of the extramitochondrial enzyme. The present work did not distinguish between mitochondrial and cytosol malate dehydrogenase. Depression of the maximum velocities of the mitochondrial enzymes, succinate dehydrogenase and cytochrome oxidase, took place. There was no special effect of any of the pesticides in any of the enzymes. Whereas aldrin and permethrin showed both elevation and depression of maximum velocities, as statistically significant effects according to enzyme, dimethoate showed only depression of maximum velocities.

DISCUSSION

The results do not exibit a constant trend in the effect of any of the pesticides. Of the three pesticides studied, aldrin showed on the average a stimulatory effect on lactate dehydrogenase, while dimethoate and permethrin showed no effect on this enzyme. This stimulatory effect of aldrin is at variance with the results of previous studies using other organochlorine pesticides. Hendrickson and Bowden (1975) have demonstrated inhibition of rabbit muscle lactate dehydrogenase activity by dieldrin, DDT, mirex and kepone.

Khaikina *et al.* (1970) have shown that DDT and lindane cause depression of activity of total rat serum lactate dehydrogenase and disruption in the normal distribution of lactate dehydrogenase isozymes. Gertig *et al.* (1970) demonstrated the inhibition of human serum lactate dehydrogenase activity by aldrin, dieldrin and lindane. Boulekbache and Spiess (1974) showed a marked decrease in liver and muscle lactate dehydrogenase in *Salmo irideus* that had been exposed to lindane.

The other glycolytic enzyme, pyruvate kinase, showed increased activity in the presence of aldrin. This effect was also observed with permethrin but there was no effect with dimethoate. The stimulatory effect of the organochlorines was also observed after elevation of V_{max} by fructose 1,6-diphosphate. Boulekbache and Spiess (1974) showed an inhibitory effect on *S. irideus* liver and muscle pyruvate kinase with lindane.

While the glycolytic enzymes were stimulated by aldrin, the tricarboxylic acid cycle enymes were on the average unaffected by this pesticide. The other organochlorine, permethrin was not constant in its effect. It stimulated malate dehydrogenase but depressed succinate dehydrogenase activity. Dimethoate also depressed succinate dehydrogenase activity. All the three pesticides, however, inhibited cytochrome oxidase activity. Previous studies on succinate dehydrogenase and cytochrome oxidase have given contradictory results. Aldrin and dieldrin at 10 mM concentration completely inhibited cytochrome oxidase activity in *in vitro* muscle studies on the *Periplaneta americana* (Morrison and Brown, 1954). Johnston (1950) found no inhibition of succinate dehydrogenase in rat heart homogenates with DDT, DDA and HCH at concentrations of 0.1 mM and 0.01 mM. Endrin has

been shown to inhibit both succinate dehydrogenase and cytochrome oxidase activity in liver homogenates of the catfish (Calvin and Phillips, quoted in McCorkle and Yarbrough, 1974). McCorkle and Yarbrough (1974) observed inhibition of succinate dehydrogenase activity in brain and liver mitochondria from resistant and susceptible mosquito fish after disruption of the mitochondrial membrane. These workers postulated a mitochondrial membrane barrier to pesticides in resistant species.

Stimulation of enzyme activity by the two organochlorine pesticides, aldrin and permethrin is difficult to explain. The apparent increase in glycolytic enzyme activity would indicate an activation of the glycolytic pathway if the response occurs *in vivo*. Possibly the stimulation represents a pre-response to inhibition at higher pesticide concentrations. The decrease in activity observed for the two mitochondrial enzymes, succinate dehydrogenase and cytochrome oxidase might be related to a decrease in accessibility of substrate. The pesticides may affect membrane permeability allowing less substrate to penetrate the outer mitochondrial membrane.

The general *in vitro* effects of pesticides on enzymes are difficult to relate to what actually happens *in vivo*. Further experiments are in progress to determine *in vivo* effects.

CONCLUSIONS

The muscle enzymes from three Mediterranean fish species showed similar responses, indicating uniform susceptibility to pesticides. No effect of pesticides on K_m was observed. Aldrin raised the V_{max} of pyruvate kinase and lactate dehydrogenase, permethrin raised the V_{max} of pyruvate kinase and malate dehydrogenase whilst the V_{max} of succinate dehydrogenase was depressed by both dimethoate and permethrin. All three pesticides lowered the V_{max} of cytochrome oxidase.

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KRATAK SADRŽAJ

Proučeno je djelovanje pesticida aldrina, dimetoata i permetrina na piruvat kinazu, laktat dehidrogenazu, malat dehidrogenazu, sukcinat dehidrogenazu i citokrom oksidazu mediteranskih riba, *Boops boops*, *Coryphaena hippurus* i *Mugil cephalus*. Aldrin i permetrin stimuliraju ekstramitohondrijalne enzime. Dok permetrin inhibira mitohondrijalne enzime, dotle aldrin inhibira samo citokrom oksidazu. Dimetoat nije djelovao na ekstramitohondrijalne enzime ali je inhibirao mitohondrijalne.

TABLES

Table 1. Kinetic parameters for *Boops boops* muscle lactate dehydrogenase in presence of pesticides.

		10^4 K_m* M	10^8 V_{max}* moles/min/mg
White Muscle			
Control	—	2.05±0.24	2.86±0.10
Aldrin	1 ppm	3.16±0.21	3.41±0.08
	5 ppm	3.32±0.30	3.34±0.11
Dimethoate	1 ppm	2.94±0.34	3.29±0.15
	5 ppm	1.84±0.41	2.53±0.19
Control	—	8.54±1.91	4.96±0.64
Permethrin	1 ppm	8.80±2.34	5.02±0.78
	5 ppm	8.00±2.91	5.07±0.91
Red Muscle			
Control	—	5.33±0.70	0.17±0.01
Aldrin	1 ppm	5.73±0.9	0.17±0.01
	5 ppm	4.73±0.67	0.15±0.01
Control	—	4.74±0.72	0.13±0.01
Dimethoate	1 ppm	4.32±0.46	0.12±0.01
	5 ppm	4.19±0.61	0.11±0.01
Permethrin	1 ppm	4.03±0.21	0.12±0.003
	5 ppm	3.5 ±0.34	0.12±0.004

* Values include computed S. E.

Table 2. Kinetic parameters for *Coryphaena hippurus* muscle lactate dehydrogenase in presence of pesticides.

		10^4 K_m* M	10^8 V_{max}* moles/min/mg
White Muscle			
Control	—	1.23±0.11	3.85±0.09
Aldrin	1 ppm	2.02±0.27	4.39±0.17
	5 ppm	1.61±0.22	4.28±0.16
Dimethoate	1 ppm	2.50±0.23	5.12±0.15
	5 ppm	2.21±0.18	4.84±0.12
Control	—	6.53±2.14	2.63±0.42
Permethrin	1 ppm	8.84±2.89	3.09±0.59
	5 ppm	8.01±1.51	2.96±0.30
Red Muscle			
Control	—	6.76±1.10	2.46±0.21
Aldrin	1 ppm	7.36±1.52	2.57±0.29
	5 ppm	7.92±1.30	2.81±0.26
Control	—	7.42±0.76	2.62±0.14
Dimethoate	1 ppm	6.17±0.97	2.38±0.19
	5 ppm	7.68±1.07	2.81±0.21
Permethrin	1 ppm	6.43±1.05	2.5 ±0.21
	5 ppm	7.90±1.98	2.77±0.38

* Values include computed S. E.

Table 3. Kinetic parameters of *Mugil cephalus* muscle lactate dehydrogenase in presence of pesticides.

		$10^4 K_m$* M	$10^8 V_{max}$* moles/min/mg
White Muscle			
Control	—	2.75±0.48	1.87±0.13
Aldrin	1 ppm	3.72±0.86	2.08±0.17
	5 ppm	4.36±0.13	2.71±0.03
Dimethoate	1 ppm	3.18±0.27	2.3 ±0.07
	5 ppm	3.29±0.47	2.41±0.12
Control	—	9.02±1.73	5.59±0.62
Permethrin	1 ppm	8.42±0.39	5.41±0.14
	5 ppm	9.41±0.55	5.78±0.19
Red Muscle			
Control	—	6.98±0.85	2.49±0.12
Aldrin	1 ppm	5.75±0.28	2.41±0.06
	5 ppm	7.45±0.61	2.89±0.13
Dimethoate	1 ppm	7.59±1.09	2.58±0.2
	5 ppm	5.99±0.39	2.41±0.08
Permethrin	1 ppm	8.69±1.3	3.22±0.26
	5 ppm	8.37±0.63	3.08±0.13

* Values include computed S. E.

Table 4. Kinetic parameters for *Boops boops* muscle malate dehydrogenase in presence of pesticides.

		$10^4 K_m$* M	$10^9 V_{max}$* moles/min/mg
White Muscle			
Control	—	0.26±0.06	1.35±0.09
Aldrin	1 ppm	0.36±0.07	1.43±0.09
	5 ppm	0.50±0.14	1.51±0.18
Dimethoate	1 ppm	0.29±0.08	1.31±0.11
	5 ppm	0.37±0.09	1.32±0.13
Control	—	2.24±0.94	0.83±0.22
Permethrin	1 ppm	3.19±0.93	1.06±0.21
	5 ppm	3.43±1.08	1.11±0.27
Red Muscle			
Control	—	0.31±0.05	0.32±0.02
Aldrin	1 ppm	0.34±0.09	0.35±0.03
	5 ppm	0.38±0.08	0.34±0.03
Dimethoate	1 ppm	0.30±0.05	0.35±0.02
	5 ppm	0.35±0.06	0.31±0.02
Control	—	0.98±0.44	0.69±0.16
Permethrin	1 ppm	0.87±0.31	0.74±0.13
	5 ppm	0.86±0.15	0.81±0.06

* Values include computed S. E.

Table 5. Kinetic parameters for *Coryphaena hippurus* muscle malate dehydrogenase in presence of pesticides.

		10^4 K_m* M	10^9 V_{max}* moles/min/mg
White Muscle			
Control	—	2.29±0.56	1.24±0.22
Aldrin	1 ppm	1.23±0.41	0.96±0.2
	5 ppm	1.68±0.72	1.48±0.35
Control	—	0.57±0.21	3.02±0.54
Dimethoate	1 ppm	0.61±0.09	2.50±0.16
	5 ppm	1.10±0.26	2.52±0.48
Control	—	2.21±0.62	1.44±0.25
Permethrin	1 ppm	1.84±0.71	1.65±0.4
	5 ppm	1.70±0.4	1.33±0.23
Red Muscle			
Control	—	0.45±0.04	6.16±0.24
Aldrin	1 ppm	0.47±0.11	6.51±0.64
	5 ppm	0.37±0.04	5.53±0.19
Dimethoate	1 ppm	0.36±0.07	6.09±0.39
	5 ppm	0.35±0.07	5.71±0.38
Control	—	1.89±0.49	0.46±0.1
Permethrin	1 ppm	2.21±0.53	0.56±0.13
	5 ppm	1.54±0.52	0.45±0.10

* Values include computed S. E.

Table 6. Kinetic parameters for *Mugil cephalus* muscle malate dehydrogenase in presence of pesticides.

		10^4 K_m* M	10^9 V_{max}* moles/min/mg
White Muscle			
Control	—	1.68±047	0.51±0.09
Aldrin	1 ppm	1.85±0.73	0.61±0.12
	5 ppm	2.82±0.36	0.65±0.05
Dimethoate	1 ppm	1.52±0.36	0.5 ±0.07
Control	—	2.11±0.76	1.63±0.37
Dimethoate	5 ppm	1.57±0.70	1.18±0.31
Control	—	1.68 (as above)	0.51 (as above)
Permethrin	1 ppm	1.83±0.30	0.6 ±0.06
	5 ppm	2.74±0.40	0.62±0.06
Red Muscle			
Control	—	0.57±0.18	2.62±0.30
Aldrin	1 ppm	0.62±0.07	2.69±0.14
	5 ppm	0.61±0.10	2.18±0.16
Control	—	0.42±0.07	2.17±0.14
Dimethoate	1 ppm	0.45±0.17	2.39±0.33
	5 ppm	0.69±0.06	2.91±0.10
Permethrin	1 ppm	0.47±0.06	2.45±0.11
	5 ppm	0.38±0.14	2.28±0.31

* Values include computed S. E.

Table 7. Kinetic parameters for *Boops boops* muscle succinate dehydrogenase in presence of pesticides.

		$10^3 K_m$* M	$10^5 V_{max}$* units/min/mg
White Muscle			
Control	—	1.23±0.39	3.86±0.20
Aldrin	1 ppm	1.14±0.24	3.97±0.13
	5 ppm	0.88±0.23	3.92±0.15
Dimethoate	1 ppm	0.85±0.10	3.11±0.06
	5 ppm	1.17±0.65	3.22±0.29
Control	—	0.26±0.06	1.43±0.06
Permethrin	1 ppm	0.33±0.04	1.62±0.04
	5 ppm	0.25±0.01	1.42±0.01
Red Muscle			
Control	—	0.71±0.24	3.19±0.28
Aldrin	1 ppm	0.19±0.06	3.37±0.12
	5 ppm	0.13±0.03	3.29±0.07
Control	—	0.15±0.02	2.93±0.04
Dimethoate	1 ppm	0.33±0.06	3.01±0.1
	5 ppm	0.16±0.02	2.90±0.05
Control	—	0.71 (as above)	3.19 (as above)
Permethrin	1 ppm	0.57±0.18	3.04±0.23
	5 ppm	0.32±0.13	2.97±0.25

* Values include computed S. E.

Table 8. Kinetic parameters for *Coryphaena hippurus* muscle succinate dehydrogenase in presence of pesticides.

		$10^3 K_m$* M	$10^5 V_{max}$* units/min/mg
White Muscle			
Control	—	0.12±0.02	2.06±0.03
Aldrin	1 ppm	0.16±0.03	2.1 ±0.04
	5 ppm	0.17±0.02	2.07±0.04
Dimethoate	1 ppm	0.14±0.02	2.04±0.02
	5 ppm	0.24±0.05	2.04±0.06
Control	—	0.25±0.03	2.30±0.04
Permethrin	1 ppm	0.16±0.02	2.08±0.03
	5 ppm	0.2 ±0.03	2.17±0.04
Red Muscle			
Control	—	0.28±0.07	8.86±0.38
Aldrin	1 ppm	0.39±0.15	8.71±0.67
	5 ppm	0.47±0.11	8.86±0.51
Dimethoate	1 ppm	0.25±0.04	8.33±0.22
	5 ppm	0.26±0.05	8.31±0.28
Control	—	0.38±0.08	5.91±0.28
Permethrin	1 ppm	0.34±0.06	5.29±0.19
	5 ppm	0.35±0.05	5.51±0.17

* Values include computed S. E.

Table 9. Kinetic parameters for *Mugil cephalus* muscle succinate dehydrogenase in presence of pesticides.

		10^3 K_m* M	10^5 V_{max}* units/min/mg
White Muscle			
Control	—	0.72±0.15	4.32±0.11
Aldrin	1 ppm	0.97±0.21	4.57±0.15
	5 ppm	1.09±0.18	3.41±0.09
Dimethoate	1 ppm	0.41±0.13	3.67±0.09
	5 ppm	0.41±0.2	3.8 ±0.15
Control	—	0.32±0.07	1.97±0.08
Permethrin	1 ppm	0.34±0.04	1.97±0.04
	5 ppm	0.28±0.08	2.02±0.10
Red Muscle			
Control	—	0.29±0.06	4.00±0.15
Aldrin	1 ppm	0.17±0.08	3.71±0.21
	5 ppm	0.25±0.06	3.53±0.13
Dimethoate	1 ppm	0.30±0.08	3.70±0.18
	5 ppm	0.33±0.09	3.37±0.19
Permethrin	1 ppm	0.28±0.05	3.66±0.12
	5 ppm	0.24±0.06	3.5 ±0.14

* Values include computed S. E.

Table 10. Kinetic parameters for *Boops boops* muscle cytochrome oxidase in presence of pesticides.

		10^6 K_m* M	10^8 V_{max}* moles/min/mg
White Muscle			
Control	—	2.13±0.17	0.20±0.01
Aldrin	1 ppm	3.06±0.35	0.19±0.01
	5 ppm	2.34±0.35	0.15±0.01
Dimethoate	1 ppm	1.42±0.29	0.16±0.01
	5 ppm	1.45±0.32	0.16±0.01
Control	—	9.20±1.36	1.06±0.08
Permethrin	1 ppm	7.34±2.17	1.01±0.13
	5 ppm	9.10±3.23	1.07±0.19
Red Muscle			
Control	—	8.80±2.42	1.89±0.27
Aldrin	1 ppm	10.86±2.21	1.87±0.21
	5 ppm	5.69±0.17	1.44±0.2
Dimethoate	1 ppm	6.65±0.92	1.62±0.11
	5 ppm	8.48±0.9	1.86±0.10
Permethrin	1 ppm	7.8 ±1.73	1.62±0.19
	5 ppm	10.2 ±0.79	2.13±0.09

* Values include computed S. E.

Table 11. Kinetic parameters for *Coryphaena hippurus* muscle cytochrome oxidase in presence of pesticides.

		10^6 K_m* M	10^8 V_{max}* moles/min/mg
White Muscle			
Control	—	2.25±0.82	0.16±0.02
Aldrin	1 ppm	3.28±0.41	0.12±0.01
	5 ppm	4.67±1.04	0.13±0.01
Dimethoate	1 ppm	2.96±0.87	0.11±0.01
	5 ppm	2.05±0.64	0.12±0.01
Control	—	9.75±0.81	1.25±0.05
Permethrin	1 ppm	13.45±1.36	1.58±0.09
	5 ppm	13.45±1.36	1.58±0.09
Red Muscle			
Control	—	26.47±3.37	20.12±1.88
Aldrin	1 ppm	16.12±2.18	13.62±1.15
	5 ppm	23.25±2.02	25.60±1.51
Dimethoate	1 ppm	12.13±1.64	11.83±0.87
	5 ppm	20.76±3.40	17.34±3.40
Permethrin	1 ppm	18.08±2.5	14.15±1.3
	5 ppm	12.29±2.17	11.90±1.24

* Values include computed S. E.

Table 12. Kinetic parameters for *Mugil cephalus* muscle cytochrome oxidase in presence of pesticides.

		10^6 K_m* M	10^8 V_{max}* moles/min/mg
White Muscle			
Control	—	2.41±0.48	0.37±0.02
Aldrin	1 ppm	1.06±0.45	0.3 ±0.03
	5 ppm	1.7 ±0.36	0.26±0.01
Dimethoate	1 ppm	1.21±0.16	0.31±0.01
	5 ppm	1.9 ±0.41	0.32±0.02
Control	—	7.13±1.38	0.29±0.03
Permethrin	1 ppm	6.43±1.57	0.29±0.04
	5 ppm	3.39±0.82	0.21±0.02
Red Muscle			
Control	—	18.44±1.25	5.95±0.25
Aldrin	1 ppm	14.07±2.04	4.72±0.41
	5 ppm	10.04±2.36	3.69±0.49
Dimethoate	1 ppm	8.25±2.33	3.63±0.46
	5 ppm	6.63±0.49	3.08±0.11
Permethrin	1 ppm	10.04±2.36	3.69±0.49
	5 ppm	12.55±3.25	4.19±0.58

* Values include computed S. E.

Table 13. Kinetic parameters for *Boops boops* white muscle pyruvate kinase in presence of pesticides.

		10^3 $K_{0.5}$* M	10^{10} V_{max}* moles/min/mg
*Without FDP***			
Control	—	1.62±0.39	0.7 ±0.08
Aldrin	1 ppm	0.97±0.17	0.57±0.04
	5 ppm	1.70±0.24	0.75±0.06
Control	—	1.47±0.41	0.52±0.08
Dimethoate	1 ppm	0.79±0.07	0.39±0.02
	5 ppm	1.15±0.16	0.51±0.03
Control	—	1.62 (as above)	0.7 (as above)
Permethrin	1 ppm	1.53±0.49	0.73±0.12
	5 ppm	1.25±0.18	0.67±0.05

* Values include computed S. E.
** Data were hyperbolic ($n_H = 1$).

Table 14. Kinetic parameters for *Coryphaena hippurus* white muscle pyruvate kinase in presence of pesticides.

		10^4 $K_{0.5}$* M	10^{10} V_{max}* moles/min/mg
*Without FDP***			
Control	—	4.07±0.71	2.31±0.12
Aldrin	1 ppm	6.3 ±0.68	3.36±0.12
	5 ppm	6.69±0.95	3.40±0.17
Dimethoate	1 ppm	6.76±1.02	3.14±0.16
Control	—	7.02±0.55	1.88±0.05
Dimethoate	5 ppm	7.49±0.85	2.2 ±0.09
Control	—	4.07 (as above)	2.31 (as above)
Permethrin	1 ppm	6.06±0.72	3.32±0.13
	5 ppm	7.8 ±1.13	3.75±0.23
		10^4 $K_{0.5}$* M	10^9 V_{max}* moles/min/mg
*With FDP***			
Control	—	2.97±1.22	5.46±0.91
Aldrin	1 ppm	2.71±1.11	5.84±0.79
	5 ppm	3.69±0.97	6.29±0.74
Control	—	5.14±0.93	5.70±0.5
Dimethoate	1 ppm	4.95±0.55	6.03±0.31
	5 ppm	4.87±1.26	5.84±0.72
Permethrin	1 ppm	4.32±0.5	5.50±0.28
	5 ppm	5.33±0.91	6.31±0.52

* Values include computed S. E.
** Data were hyperbolic ($n_H = 1$).

Table 15. Kinetic parameters for *Mugil cephalus* white muscle pyruvate kinase in presence of pesticides.

		n_H*	$10^3 K_{0.5}$* M	$10^9 V_{max}$* moles/min/mg
Without FDP				
Control	—	2.82±0.24	1.13±0.1	1.90±0.04
Aldrin	1 ppm	2.60±0.24	1.18±0.11	2.01±0.04
	5 ppm	2.59±0.23	1.13±0.10	2.06±0.05
Dimethoate	1 ppm	2.36±0.13	1.02±0.06	2.11±0.05
	5 ppm	2.28±0.16	1.10±0.09	2.58±0.12
Permethrin	1 ppm	2.50±0.17	1.09±0.08	1.96±0.04
	5 ppm	2.52±0.17	1.07±0.07	2.26±0.05
			$10^4 K_{0.5}$* M	$10^9 V_{max}$* moles/min/mg
*With FDP***				
Control	—		3.03±1.27	7.69±1.22
Aldrin	1 ppm		3.23±1.06	8.37±1.14
	5 ppm		3 ±0.52	8.43±0.58
Control	—		4.45±0.88	7.08±0.63
Dimethoate	1 ppm		2.79±0.91	6.23±0.69
	5 ppm		3.67±0.74	6.87±0.55
Control	—		3.03 (as above)	7.69 (as above)
Permethrin	1 ppm		2.46±0.66	7.55±0.79
	5 ppm		3.62±0.92	9.25±1.06

* Values include computed S. E.

** Data were hyperbolic ($n_H = 1$).