COMMUNICATIONS

Preliminary Investigation on the Effects of Mirex and Its Derivatives on Adenosine Triphosphatase Activities from Fire Ants

The effects of Mirex and its derivatives on ATPase activities from fire ant, *Solenopsis richteri* (Forel), were studied. Derivatives (reduction products of mirex) with one and two α-chlorohydrogens were found to be more effective as inhibitors of the ATPase system than the other derivatives tested. Mirex had little or no effect on the ATPase activities in the fire ant preparations.

Mirex is inert to many common acids, bases, oxidizers, and reducers (McBee et al., 1956), and is highly stable in the environment (Eaton et al., 1960). However, Alley et al. (1973, 1974) showed that mirex does react in the presence of amines and copper salts to form monohydro and dihydro derivatives.

Although mirex has been shown to be highly toxic to certain aquatic organisms, particularly to crustaceans (Ludke et al., 1971), to fishes (Van Valin et al., 1968), and to certain insects (Lofgren et al., 1964; Plapp, 1973), no biochemical evidence is available on its mode of action. In previous studies we found that mirex did not show any effect on the ATPase system in fish (Yap et al., 1975) or *Escherichia coli* membranes (Koch and Desaiah, 1974). However, the ATPase system has been shown to be sensitive to several organochlorine pesticides (Koch 1969a,b; 1969/70; Cutkomp et al., 1971; Desaiah et al., 1972, 1974, 1975). The purpose of this present study was to determine the effects of derivatives of mirex on the ATPase system in fire ants and to compare their effects to those of mirex.

MATERIALS AND METHODS

Fire ants, *Solenopsis richteri* (Forel), were collected from the fields around Starkville, Miss., and kept in the laboratory until used. The heads of ants were removed by a scalpel, homogenized in a ground-glass homogenizer in 0.32 *M* sucrose solution, and fractionated as described by Koch (1969a,b). The sediment obtained at 13,000*g* was resuspended in 0.32 *M* sucrose solution, containing 10 m*M* imidazole-HCl (pH 7.5) and 1 m*M* EDTA, to contain about 20–25 μg of protein per 50 μl of homogenate. These samples were quick frozen in liquid nitrogen and stored at −20°.

ATPase activity was measured by a continuous method as reported by Koch (1971/72). ATP hydrolysis in this system is directly proportional to NADH oxidation, the latter being measured by the change in absorbance at 340 nm, using a Gilford 2400 recording spectrophotometer. The ATPase activity was calculated as the micromoles of P_i per milligram of protein per hour from a reaction time of 10 min at 37°. The protein concentration was determined by the method of Lowry et al. (1951).

A 3-ml reaction mixture consisted of: 4.5 m*M* ATP, 135 m*M* imidazole-HCl buffer (pH 7.5), 0.2 m*M* NADH, 0.5 m*M* phosphoenolpyruvate (PEP), 0.02% bovine serum albumin, approximately 9 units of pyruvate kinase, 12 units of lactic dehydrogenase (Sigma grades), 5 m*M* Mg^{2+}, 100 m*M* Na^+, 20 m*M* K^+ (all three as chlorides), and 50 μl of the homogenate fraction. Ouabain (1.0 m*M*) was used in the reaction mixture to differentiate Na^+–K^+ and Mg^{2+} ATPase activities from total ATPase activity. Oligomycin (2 μg/ml reaction mixture) was used to delineate oligomycin-sensitive (mitochondrial) and insensitive Mg^{2+} ATPase activities.

The structural formulas of the compounds used in this study are given in Figure 1. Mirex was obtained from the Pesticide Research Laboratory, Perrine, Fla. The mirex derivatives used in this study were kindly supplied by Drs. E. G. Alley and B. R. Layton of the Mississippi State Chemical Laboratory, Mississippi State, Miss.

The concentrated solutions of mirex (I) and its α-chloromonohydrogen derivative (II) were prepared in acetone, with further dilutions made in ethanol. The other derivatives (III, IV, and V) were dissolved in ethanol. One microliter of each solution at the required strength was injected below the surface of the reaction mixture while the latter was rapidly stirred on a Vortex Junior mixer.

RESULTS AND DISCUSSION

The results obtained are tabulated in Tables I–III. Mirex at the concentrations tested produced only a small apparent stimulation on Na^+–K^+ ATPase activity in the fire ant head homogenate (Table I). These results were in general agreement with the lack of effect of mirex observed in earlier studies on blue gill fish brain and *E. coli* membrane preparations [Yap et al. (1975) and Koch and Desaiah (1974), respectively]. However, the effects of reduction products of mirex were quite different from that of mirex. Derivatives II and III (one and two α-chlorohydrogens, respectively, Figure 1) showed the greatest inhibition of Na^+–K^+ ATPase activity among the compounds tested (Table I).

Mitochondrial Mg^{2+} ATPase activity was also inhibited by derivatives II and III (Table II). Derivative III gave the highest inhibition when compared to the other compounds tested. Mirex and derivatives IV and V had little or no effect on mitochondrial Mg^{2+} ATPase activity at least to 20 μM concentration (Table II). Oligomycin-insensitive Mg^{2+} ATPase activity was inhibited by all derivatives of mirex in the order II > III > IV > V (Table III). Mirex, on the other hand, did not show any effect on this enzyme activity (Table III).

These data show that the mirex derivatives having α-chlorohydrogens (II and III, Figure 1) were the most effective inhibitors of the ATPase system. Derivative III, which contains two α-chlorohydrogens and is the most polar of the derivatives, was the most effective inhibitor of the ATPase system (Tables I and III). Since mirex was ineffective as an ATPase inhibitor but was used in fire ant control (Lofgren et al., 1964), and exposed fire ants were killed by it, mirex was an apparent exception to our general theory that the mode of action of chlorinated hydrocarbon pesticides was through inhibition of ATPase activity (Desaiah et al., 1974, 1975). However, lethality by mirex for fire ants was time dependent (2 or 3 weeks in the field) which could indicate a possible conversion in vivo into the reduced derivatives of mirex. Layton (Mississippi State Chemical Lab., unpublished data) has obtained chromatographic (GLC) evidence for reduced mirex derivatives in fire ants killed by exposure to mirex. The derivatives found in the dead ants were identical with compounds II–V, Figure 1. Thus, under field exposure conditions mirex can be converted by the fire ant into derivatives that inhibit the ATPase system.

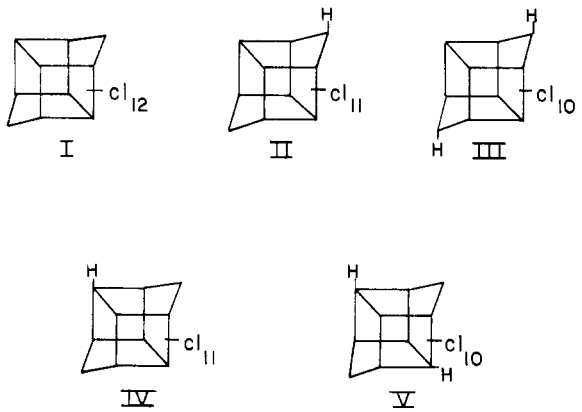

Figure 1. Structural formulas of mirex and its derivatives: I, dodecachloropentacyclo[5.3.0.0^{2,6}.0^{3,9}.0^{4,8}]decane (mirex); II, 1,2,3,4,5,6,7,8,9,10-undecachloropentacyclo[5.3.0.0^{2,6}.0^{3,9}.0^{4,8}]-decane; III, 1,2,3,4,5,6,7,8,9,10-decachloropentacyclo[5.3.0.0^{2,6}.-0^{3,9}.0^{4,8}]decane; IV, 1,2,3,4,5,6,7,8,9,10,10-undecachloropentacyclo[5.3.0.0^{2,6}.0^{3,9}.0^{4,8}]decane; V, 1,3,4,5,5,6,7,9,10,10-decachloropentacyclo[5.3.0.0^{2,6}.0^{3,9}.0^{4,8}]decane.

Table I. Effect of Mirex and Its Derivatives on Na^+-K^+ ATPase Activity from Fire Ant Homogenate

	% inhibition[a]				
Concn, μM	Mirex (I)	II[b]	III	IV	V
1.25		15.0	17.1		
2.5		19.2	32.2	9.5	
5.0		22.5	34.7	20.6	3.6
10.0	+12.3	26.2	34.4	16.9	8.0
20.0	+6.0	31.0	32.7	12.5	16.1
Control sp act.	8.1	14.1	14.1	14.1	14.1

[a] Percent inhibition represents the average value obtained from two separate homogenate preparations. Each preparation was tested 2-4 times and the variation in the replicates was less than 5% (in most tests 2%) of the average. Agreement between the two separate preparations was within 5% of the average. [b] Roman numerals same as listed in Figure 1.

If these findings prove to be correct, they appear to add an important new dimension to the mode of action of chlorinated hydrocarbon pesticides, i.e., the conversion of an inactive form of a pesticide by the insect into a form that is an inhibitor of the ATPase system. Further work on in vivo conversion of mirex to reduced derivatives and the toxicity of the derivatives is in progress. Results to be reported in a more complete form support the above findings.

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Table II. Effect of Mirex and Its Derivatives on Oligomycin-Sensitive (Mitochondrial) Mg^{2+} ATPase Activity from Fire Ant Head Homogenate

	% inhibition[a]				
Concn, μM	Mirex (I)	II	III	IV	V
1.25		7.3	36.5		
2.5		7.3	44.2	0	
5.0		13.6	52.0	+8.8	2.4
10.0	1.1	20.5	38.3	+7.3	4.6
20.0	12.2	31.7	46.6	1.5	17.6
Control sp act.	9.0	6.5	6.5	6.5	6.5

[a] See Table I.

Table III. Effect of Mirex and Its Derivatives on Oligomycin-Insensitive Mg^{2+} ATPase Activity from Fire Ant Head Homogenate

	% inhibition[a]				
Concn, μM	Mirex (I)	II	III	IV	V
1.25		32.3	30.8		
2.5		35.4	37.4	25.3	
5.0		45.0	47.0	32.2	2.3
10.0	+1.3	50.8	53.1	26.4	29.9
20.0	+2.7	54.1	50.0	32.2	16.0
Control sp act.	7.4	7.6	7.6	7.6	7.6

[a] See Table I.

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