Influence of Solvents on the Pesticide Inhibition of ATPase Activities in Fish and Insect Tissue Homogenates*

D. Desaiah[1] and R. B. Koch

Department of Biochemistry, Mississippi State University, Mississippi State, Miss. 39762

[1]Present address: Department of Pharmacology and Toxicology, The University of Mississippi Medical Center, Jackson, Miss. 39216

In earlier studies, we have shown that chlorinated hydrocarbon pesticides are inhibitors of the ATPase system in a number of tissues from several animal sources (KOCH, 1969a: CUTKOMP et al., 1971; DESAIAH et al., 1974). In these studies we have used ethanol and acetone as the solvent for the pesticides with no apparent differences in inhibition due to solvent. However, during the course of a study on the separation of the components of toxaphene by high pressure liquid chromatography, it was noted that none of the separated fractions showed inhibition of the ATPase activities from a catfish brain homogenate preparation. This was rather unusual since the starting material was a fairly strong inhibitor of the ATPase system (DESAIAH and KOCH, 1975). In considering the possible reason for the discrepancy, it was realized that the separated fractions were concentrated from the chromatographic solvent (cyclohexane), while the previous toxaphene sample was dissolved in 95% ethanol. Thus the solvent for the separated fractions (cyclohexane) was evaporated and the residues redissolved in ethanol. The resulting solutions showed the expected inhibitory effects on the ATPase system.

As a result of this finding, a number of other solvents were tested to determine their effects on the inhibition of ATPase activities from catfish brain and cockroach muscle and nerve cord by DDT, toxaphene, and plictran. The results

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* Paper No. 3246 Mississippi Agricultural and Forestry Experiment Station, Mississippi State University, Mississippi State, Mississippi 39762

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of these studies are being reported as a caution to those working in this area of research to indicate that the choice of solvent can have profound effects on the response of ATPase activity to chlorinated hydrocarbon pesticides.

Materials and Methods

Brain tissue of catfish, Ictalurus punctatus, and nerve cords and leg muscle tissues of American cockroach, Periplaneta americana, were used as enzyme sources in this study. The tissues were dissected and homogenized in ice cold 0.32 M sucrose solution containing 1 mM EDTA and 10 mM imidazole (pH 7.1). Fractionation of the homogenates was carried out as previously described by KOCH (1969b). The 'B' fraction obtained at 13,000 g centrifugation was resuspended in sucrose solution and divided into small aliquots to contain 20-30 µg protein per 100µl sample. These samples were quick frozen in liquid nitrogen and stored at -20° until used for ATPase determination.

The ATPase activities were measured by a continuous procedure. A 3.0 ml reaction mixture contained 4.3 mM ATP, 135 mM imidazole-HCl buffer (pH 7.5), 0.2 mM NADH, 0.5 mM phosphoenolpyruvate, 0.02 percent bovine serum albumin (BSA), 5 mM Mg^{2+}, 100 mM Na^{+}, 20 mM K^{+} (These three as chlorides), approximately 9 units of pyruvate kinase, and 12 units of lactic dehydrogenase. One hundred µl of the homogenate fraction was used per 3-ml reaction mixture. Absorbance was measured at 340 nm using a Gilford 2400 automatic recording spectrophotometer with temperature controlled at $37^{\circ}C$. Protein concentration was determined by the method of LOWRY et al. (1951). All chemicals (except the chlorides) used in the reaction mixture were obtained from Sigma Chemical Company. Ouabain, a specific inhibitor of Na^{+}-K^{+} ATPase (McILWAIN, 1963), at a concentration of 1.0 mM was used to differentiate Na^{+}-K^{+} ATPase from Mg^{2+} ATPase. Mg^{2+} ATPase was further delineated into oligomycin-sensitive (mitochondrial) and oligomycin-insensitive Mg^{2+} ATPases by adding 1 µl of oligomycin ethanol solution (0.5 mg/ml

95% ethanol) to the reaction mixture.

All of the solvents (chromatographic grade) used in this study had no effect on the ATPase activities when tested by themselves at levels between 1 to 10 µl per 3 ml reaction mixture.

Results and Discussion

Table I shows the results of a comparison of the influence of two solvents (cyclohexane and 95% ethanol) on the inhibition of total ATPase activity from a catfish brain homogenate fraction by three selected pesticides. It can be seen (Table I) that the inhibitory effects of the chlorinated hydro-

TABLE I

INFLUENCE OF SOLVENTS ON THE PESTICIDE INHIBITION OF TOTAL ATPase ACTIVITY IN CATFISH BRAIN HOMOGENATE*

Solvent	Insecticide (Dose)	Percent Inhibition
Cyclohexane	Toxaphene (20µM)	12.5
Ethanol	" "	53.5
Cyclohexane	DDT (10µM)	4.1
Ethanol	" "	47.6
Cyclohexane	Plictran (5µM)	82.3
Ethanol	" "	84.2

* All values are the average of two different assays of one brain preparation.

Total ATPase specific activity: 40.7 µmoles Pi mg^{-1} protein hr^{-1}.

TABLE II

INFLUENCE OF SOLVENTS* ON THE DDT (5μM) INHIBITION OF FISH BRAIN ATPase ACTIVITIES

Solvent	Percent Inhibition**		
	Na^+-K^+ ATPase	Mg^{2+} ATPase	
		Oligomycin	
		Sens.	Insens.
Ethanol	43.8	93.8	39.0
Cyclohexane	11.6	75.0	17.0
Hexane	7.6	54.1	8.5
Benzene	16.9	60.4	13.4
Pentane	4.6	62.5	15.8
Cyclopentane	7.0	50.0	7.3

* All of the solvents (1-10 μl/3 ml reaction mixture) had no effect on ATPase activity when tested by themselves.

** Percent inhibition was calculated on the average values of three different brain preparations.

Specific activities in μmoles Pi mg^{-1} protein hr^{-1}: Na^+-K^+ ATPase 17.1 ± 1.4, oligomycin sensitive Mg^{2+} ATPase 4.8 ± 0.2, oligomycin sensitive Mg^{2+} ATPase 8.2 ± 0.8.

TABLE III

INFLUENCE OF SOLVENTS ON THE DDT (5μM) INHIBITION OF COCKROACH NERVE CORDS ATPase ACTIVITIES

Solvent	Percent Inhibition*		
	Na^+-K^+ ATPase	Mg^{2+} ATPase	
		Oligomycin	
		Sens.	Insens.
Ethanol	42.4	100	23.5
Cyclohexane	3.2	21.0	5.8
Hexane	+1.5	64.0	5.8
Benzene	29.2	61.0	+5.8
Pentane	36.3	55.0	17.6
Cyclopentane	11.6	37.0	0

* Percent inhibition was calculated from the values of two different nerve cord preparations.

Specific activities in μmoles Pi mg^{-1} protein hr^{-1}: Na^+-K^+ ATPase 39.6, oligomycin sensitive Mg^{2+} ATPase 10.0, oligomycin insensitive 5.1.

TABLE IV

INFLUENCE OF SOLVENTS ON THE DDT (5μM) INHIBITION OF COCKROACH MUSCLE ATPase ACTIVITIES

Solvent	Percent Inhibition*	
	Mg^{2+} ATPase / Oligomycin	
	Sensitive	Insensitive
Ethanol	98.1	68.2
Cyclohexane	94.7	1.7
Hexane	87.1	8.9
Benzene	89.7	7.8
Pentane	75.1	1.4
Cyclopentane	37.4	4.6

* Percent inhibition was calculated on the average values of three different muscle preparations.

Specific activities in μmoles Pi mg^{-1} protein hr^{-1}: oligomycin sensitive Mg^{2+} ATPase 64.4 ± 1.1, oligomycin insensitive Mg^{2+} ATPase 28.0 ± 2.0.

carbon insecticides (toxaphene and DDT) were greatly reduced by cyclohexane as compared to ethanol when these compounds were used as solvents. However, ATPase inhibition by plictran (a non-chlorinated organotin hydroxide and a strong acaracide, DESAIAH et al., 1973) showed no difference due to solvent. These results indicate that possibly the solvent effect may be peculiar to chlorinated hydrocarbon pesticides. This point should merit further study. It was further noted that in exploratory experiments, the response due to the injection of an approximate LD50 dose of DDT in the abdomen of cockroaches, differed depending on the solvent used for the DDT. When dissolved in cyclohexane, DDT produced tremors which took much longer to appear compared to ethanol-DDT injections (approx. 24 hrs. compared to 1 hr, respectively).

Since DDT has been so widely studied by us and others, we decided to determine the effects of DDT dissolved in several different solvents on the ATPase activities from fish brain and cockroach nerve cord and muscle tissues. The results of this study are shown in Tables II-IV. Table II shows that all solvents tested reduced the inhibition of the ATPase activities compared to ethanol as a solvent. The amount of reduction was variable for the different solvents. However, in general, the Na^+-K^+ ATPase activity from the catfish brain preparation showed less inhibition by DDT dissolved in the non-polar solvents compared to the ethanol solvent, than did the Mg^{2+} ATPase activities (Table II). Table III shows the results of tests using a cockroach nerve cord preparation. The effects of solvent were quite variable and do not necessarily follow the same pattern as the other tissue preparations (Tables II and IV). Cyclohexane had the greatest effect on reducing the inhibition of the ATPase activities from the cockroach nerve cord (Table III). This was not true for a cockroach muscle preparation. Table IV shows that cyclopentane had the greatest effect on reducing the DDT inhibition of oligomycin-sensitive

Mg^{2+} ATPase, while cyclohexane had least affect. However, all solvents reduced the DDT inhibition of the oligomycin-insensitive Mg^{2+} ATPase when compared to ethanol.

It is obvious from the above studies that the type of solvent used for dissolving the chlorinated hydrocarbon pesticides, DDT and toxaphene (others should be tested as well), can have a great influence on their inhibitory action toward the ATPase system. Thus it is very important that the solvent used be clearly indicated in reporting research results. It appears that enthnol should be the solvent of choice for such studies.

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