In Vitro Inhibition of Fish Brain ATPase Activity by Cyclodiene Insecticides and Related Compounds[1]

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The reports on inhibition studies of ATPases from tissues of different organisms by chlorinated hydrocarbon pesticides have increased in recent years. Among these reports, some showed that Na^+-K^+ ATPase was sensitive to DDT and its analogs (MATSUMURA et al. 1969, AKERA et al. 1971). JANICKI and KINTER (1971) showed a general reduction of the total ATPase activity in marine teleosts. However, in our experiments, we have consistently found that the Mg^{2+} ATPase was sensitive to several organochlorine insecticides (KOCH 1969, 1969/1970) and more so the oligomycin-sensitive (mitochondrial) Mg^{2+} ATPase to be most sensitive to DDT both *in vitro* (KOCH et al. 1971, CUTKOMP et al. 1971, and DESAIAH et al. 1974a) and *in vivo* (DESAIAH et al. 1974b). Although several articles appeared on the effects of other major chlorinated hydrocarbon insecticides on the ATPase system (AKERA et al. 1971, DAVIS et al. 1972, WELLS et al. 1974), none of them attempted to study the differential inhibition of oligomycin-sensitive and insensitive Mg^{2+} ATPases. This report is to provide the reader with some values on inhibitory effects of chlorinated hydrocarbon insecticides on the ATPase system in fish brain.

The enzyme source was brain tissue from the blue gill fish, *Lepomis macrochirus*. The tissue homogenization, fractionation, and ATPase assay were essentially carried out as described by KOCH (1969). A comparison of inhibitory responses of three ATPases to several cyclodiene and related compounds at 20.8 µM concentration is made in Table I. In Table II a comparison of ID_{50} values of some insecticides is given.

The insecticidal isomers of chlordane and heptachlor, like DDT and its analogs (CUTKOMP et al. 1971), showed strong *in vitro* inhibition of oligomycin-sensitive (mitochondrial) Mg^{2+} ATPase from fish brain. However, the chlordane isomers and a related compound isobenzan (Telodrin^R) differed from DDT in having broader inhibition effectiveness against the ATPase enzyme system, in particular against Na^+-K^+ ATPase.

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Except for aldrin, the cyclodiene compounds known as chlorinated naphthalenes showed a stimulation of mitochondrial Mg^{2+} ATPase (Table I). The four naphthalene type cyclodienes were inhibitory toward the other ATPases, but showed considerable variation. These latter results appeared to indicate that other biochemical disruptions or perhaps a metabolite (or metabolites) may be responsible for the insecticidal potency of dieldrin, isodrin, and endrin. The same reasoning may apply to pentachlorophenol, lindane, and mirex. The other miscellaneous chlorinated hydrocarbon pesticides (Table I) showed reasonably strong inhibition of the ATPase system.

Recent in vitro studies by KOCH and DESAIAH (unpublished data) strongly suggest that mirex acts through a reduction product that may be formed as a metabolite of mirex in the imported fire ant [Solenopsis richteri (Forel)]. The reduction product, synthetically produced, showed greatest inhibition of the oligomycin-sensitive and -insensitive Mg^{2+} ATPase activity.

The in vitro results obviously cannot be translated into known toxicity values either to insects, fish, or warm-blooded (homeothermic) vertebrates. The mechanism of toxicity of several of these highly toxic cyclodienes, notably endrin, dieldrin, and isodrin, though basically neurotoxins (WANG and MATSUMURA 1970 and WANG et al. 1971), does not seem to prominently implicate the ATPases studied. However, there is the possibility that the insecticides, such as aldrin or dieldrin, are converted to a much more active metabolite (or metabolites) such as aldrin-transdiol which WANG et al. (1971) have shown to be capable of much more rapid stimulation of electrical discharges than dieldrin. This has not been tested on the ATPase system.

Our results on comparable compounds are in fairly close agreement with DAVIS et al. (1972). They showed a greater inhibition of Mg^{2+} ATPase than Na^{+}-K^{+} ATPase and with all compounds except chlordane and heptachlor the ranking of the compounds is very similar. We have, however, extended the effects to oligomycin-sensitive Mg^{2+} ATPase (mitochondrial Mg^{2+} ATPase); when this is done the two chlordane isomers and heptachlor give higher inhibition than on Na^{+}-K^{+} ATPase. Only two of our compounds, isobenzan and heptachlor epoxide, gave greater inhibition of Na^{+}-K^{+} ATPase. DAVIS et al. (1972) did not test them.

It is informative for future research to note that there is a strong correlation between certain measurements of neurotoxic effects and the ATPase inhibitors. To illustrate among the cyclodiene insecticides, nearly all of the chlordane analogs gave a more rapid response (shorter latent period) in nerve cord preparations from German cockroaches than did the aldrin group and mirex. If one selects those compounds listed (WANG and MATSUMURA 1970) as having the shortest latent periods, they are heptachlor epoxide, isobenzan, toxaphene, gamma chlordane, and heptachlor; Kepone also has a shorter latency period than mirex. These compounds are precisely the same compounds, along with endosulfan (not in the

TABLE I

Comparative inhibitory effects of chlorinated cyclodiene and related compounds on the ATPase enzyme of fish brain *in vitro* using a concentration of 20.8 μM in the reaction mixture at 37°C. Stimulation values are preceded by +. Each value is based upon a minimum of three replicates. Specific Activity = μmoles Pi/mg protein/hr.

	Percent Inhibition		
	Mg^{2+} ATPase		
	Oligomycin		
Compounds	Sensitive	Insensitive	Na^{+}-K^{+} ATPase
Indenes or Non-Naphthalenic			
alpha chlordane	93	69	53
gamma chlordane	85	61	61
heptachlor	63	40	43
heptachlor epoxide	35	42	55
isobenzan (Telodrin^{R})	66	36	68
Naphthalenes			
aldrin	55	50	48
dieldrin	+15	20	40
isodrin	+48	62	18
endrin	+37	15	36
Miscellaneous			
Kepone	95	60	62
toxaphene*	77	61	44
endosulfan	69	50	32
pentachlorophenol	+31	+7	37
lindane	3	10	14
mirex	+2	+3	0
Mean Specific Activity of Untreated	12.85	15.26	29.69

*Molecular Weight calculated as 413.85 based upon $C_{10}H_{10}Cl_8$.

neurotoxic study) which were the best inhibitors of the ATPases. The order is somewhat different, but in both studies the remaining cyclodienes are separable on the basis of longer latency periods for neurotoxic effects and rather unimpressive effects on the ATPase system.

The present study provides additional evidence for compounds effective on mitochondrial Mg^{2+} ATPase (also on other enzymes of

the ATPase system) and shows an interesting relationship with rapidity of electrical response in the nervous system. Further studies will be necessary, particularly on metabolites, not only to understand the reasons behind the results, but to relate overall toxicity to enzyme inhibition.

TABLE II

Inhibition--Dose response at 50 percent level (ID_{50}) for several chlorinated hydrocarbon insecticides on mitochondrial Mg^{2+} and Na^{+}-K^{+} ATPases in blue gill fish brain homogenate.

Compound	ID_{50} in μM*	
	Mitochondrial Mg^{2+} ATPase	Na^{+}-K^{+} ATPase
γ-chlordane	0.9	23.0
α-chlordane	3.5	20.0
Kepone	3.0	9.3
toxaphene	6.9	--
endosulfan	14.9	--
heptachlor	18.1	44.0
heptachlor epoxide	--	21.0
Telodrin^{R} (isobenzan)	--	7.8
aldrin	--	30.0

*The dosage-response relationships were analyzed according to Finney's probit analysis (FINNEY 1957, 1965), programmed following DAUM and KILLCREAS (1966) and calculated on an electronic computer. The regression lines were drawn and ID_{50} values were used.

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