EPA-660/2-73-027
May 1974

Environmental Protection Technology Series

A Tissue Enzyme Assay for Chlorinated Hydrocarbon Insecticides

Office of Research and Development
U.S. Environmental Protection Agency
Washington, D.C. 20460

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A TISSUE ENZYME ASSAY FOR CHLORINATED HYDROCARBON INSECTICIDES

By

Laurence K. Cutkomp
University of Minnesota
St. Paul, Minnesota 55101

Project 16030 ELZ
Program Element 1BA027

Project Officer

H. Page Nicholson
Southeast Environmental Research Laboratory
Environmental Protection Agency
Athens, Georgia 30601

Prepared for

OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

ABSTRACT

Certain chlorinated hydrocarbon insecticides, especially DDT and closely related chemicals, tested at low concentrations, adversely affect the ATPase enzyme system. DDT inhibited oligomycin-sensitive Mg^{2+}ATPase (mitochondrial) both *in vitro* and *in vivo*. About 1 µM (1×10^{-6} M) gave 50% inhibition in fish brain and 0.5 ppb of DDT in water inhibited about 50% of mitochondrial Mg^{2+}ATPase. Na^{+}-K^{+}ATPase was not inhibited in brain, but was inhibited *in vivo* in fish gills. Certain discriminating effects were found among chlorinated hydrocarbons, particularly with respect to inhibition of Mg^{2+}ATPase, but the ranking of compounds by enzymic effects does not always parallel toxicity values. Organophosphate and carbamate insecticides were ineffective. Further research is needed both *in vitro* and *in vivo* to determine how the adverse effects on the enzymes relate to practical interpretations of effects. The abnormally low ATPase activity in chronically treated fish is the first report of an adverse biochemical effect with sublethal doese of DDT. All effects appear to be primarily within the group of insecticides and acaricides which are persistent in parts of the environment and in organisms.

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ACKNOWLEDGEMENTS

We wish to acknowledge the research facilities of the University of Minnesota, Dept. of Entomology, Fisheries and Wildlife, St. Paul, Minnesota. In particular, we are indebted to Dr. Robert B. Koch, Honeywell Research Center, Bloomington, Minnesota, for his valuable active consultantship work. Research Fellows were Dr. Han Heng Yap and Dr. D. Desaiah. Graduate Research Assistants were Dr. Elymar V. Vea and Mr. E.Y. Cheng and initially graduate students F. M. Do and Y. C. Chu. Laboratory assistance was carried out by Misses Jennifer Mather and Jean Jahr and Mr. Thomas Lorsbach.

SECTION I

CONCLUSIONS

DDT and related insecticides and acaricides cause a significant inhibition or depression of mitochondrial Mg^{2+}ATPase from fish brain when tested in vitro or when fish are continuously exposed to ½ to 2 ppb of DDT. ATPases from fish gills of treated fish, in contrast, showed some inhibition of Na^{+}-K^{+}ATPase and also Mg^{2+}ATPases. Organophosphate and carbamate insecticides do not produce an inhibition of the ATPases. Therefore the enzyme sensitivity to most chlorinated hydrocarbons, particularly DDT and PCB compounds is a discriminating characteristic and may be useful in identifying the causative agents and in giving an indication of exposure time in contaminated water. These studies are the first to show a significant quantitative reduction of enzyme activity in any vertebrate exposed chronically to DDT.

SECTION II

RECOMMENDATIONS

It is recommended that chronic toxicity studies on fish be made with other persistent materials, notably PCBs and mercury, to relate any adverse effects in the ATPase system to the residue burden in selected tissues, e.g., brain and gills. Some additional experiments should include detectable, but less persistent, pesticides. Of great importance is the need to determine whether the abnormal ATPase enzymic condition is a critical factor in survival of young fish or whether the residue burden is the most important, or whether the combination may be a limiting factor in reproduction.

SECTION III

INTRODUCTION

The ATPase enzyme system from several animal species had been shown to be sensitive (usually inhibited) to chlorinated hydrocarbon insecticides. However, there were many unknown aspects. The plan of study was to examine homogenates from numerous tissues, brain, muscle, liver and kidney - possibly gills - and determine their relative usefulness for determining the effect on the enzyme system. The ATPases could be obtained from any of the tissues but selective sensitivity was not known. Furthermore, a number of chemically related pesticides needed to be tested for their effectiveness, along with unrelated components to determine discriminatory effects on ATPases. The ATPase system itself required experimentation into the important components, Na^{+}-K^{+}ATPase, and two forms of Mg^{2+}ATPase.

The above considerations required study by in vitro techniques primarily, i.e. with tissue homogenates centrifuged at a speed (13,000 g) which retained most of the mitochondria and nerve endings. A second phase of the study was to relate the enzyme assay to insecticide toxicity. Both acute and chronic toxicity needed to be considered. Some of the comparisons were made on cockroaches which could be used in greater numbers than fishes. There were established insecticide doses with both species. Many similarities were evident when comparing responses of ATPases in fish and insects.

Objectives

To determine whether an enzyme assay, using the ATPases, can be used as a detecting system in fish or related organisms for insecticide-polluted water.

To utilize ATPase enzymes from fish tissues as sensitive detectors of contamination due to chlorinated hydrocarbon insecticides and related compounds.

To determine the most suitable fish tissues for analyzing inhibition of ATPases by chlorinated hydrocarbons.

To determine the usefulness of selectivity of pesticide effects on components of the ATPase system.

To determine whether *in vitro* and *in vivo* effects on ATPase can be related to overall toxicity to fish.

SECTION IV

EXPERIMENTAL

MATERIALS AND METHODS

The enzyme source for *in vitro* studies and *in vivo* was brain tissue from blue gill fish, *Lepomis machrochirus*. For a study of chronic effects of DDT brain and gill tissues were used from the fat head minnow, *Pimephales promelas*. The tissue was dissected and homogenized under iced conditions using 0.32 M sucrose, 1 mM EDTA and 10 mM imidazole. The homogenate fraction (B) used was the sediment obtained by centrifuging at 13,000 x g for 20 minutes, and prepared as described [1, 2, 3, 4, 5]. ATPase activities were determined using a continuous enzymatic procedure essentially described by Pullman et al. [6] and Fritz and Hamrick[7] and reported by Yap and Cutkomp[8]. Protein determinations were by the Lowry method[9].

Total ATPase activity was measured with Mg^{2+}, Na^{+}, K^{+} in the reaction mixture. Mg^{2+}ATPase activity was measured when 1mM ouabain was in the mixture. Ouabain is a specific inhibitor of Na^{+}-K^{+}ATPase[10]. Na^{+}-K^{+} ATPase activity is total activity minus the Mg^{2+}ATPase activity. Mg^{2+}ATPase activity was further delineated by adding one µl of oligomycin in ethanol (0.03 µg per ml reaction mixture); the oligomycin-sensitive portion is designated mitochondrial Mg^{2+}ATPase activity in this study. The oligomycin contained approximately 15% oligomycin A and 85% oligomycin B as obtained from Sigma Chem. Co., St. Louis, Missouri.

A 3 ml reaction mixture contained: 4.3 mM ATP, 5 mM Mg^{++}, 100 mM Na^{+}, 20 mM K^{+} (all as chlorides), 120 mM imidazole buffer pH 7.5, 0.19 mM NADH 0.5 mM PEP (phosphoenol pyruvate), 0.02% BSA (bovine serum albumin), approximately 9 units pyruvate kinase, and 12 units lactic dehydrogenase, and 100 µl homogenate fraction. Absorbance changes were measured at 340 nm using a Beckman DU spectrophotometer with temperature controlled

for 37° in the reaction mixture. This temperature was used to insure comparable results with earlier extensive studies with mammals (rats and rabbits).

Analytical grades of insecticides and designated PCBs (Aroclors) were used. Each insecticide was dissolved in ethanol and 1 to 5 µl were added to a rapidly stirred reaction mixture using a Hamilton microsyringe. Ethanol had no effect at the amounts used.

The dosage-response relationships suited to statistical treatment were analyzed according to Finney's probit analysis [11, 12] programmed following Daum[13] and calculated on an electronic computer. The regression lines and 50% inhibitory values (I_{50}) are given in each figure.

The blue gill fish used in the study were collected as young in an isolated lake believed to be free of any possible pesticide contamination. They were maintained in deionized water for several weeks before experimental use. Fat head minnows used for pesticide treatments were reared throughout their lifetime in uncontaminated water at the Duluth Water Quality Laboratory, Environmental Protection Agency.

SECTION V

RESULTS

The research has clarified numerous points regarding the sensitivity of the ATPases by chlorinated hydrocarbon pesticides and polychlorinated biphenyl compounds. Twelve research publications give this information in great detail.

The major points determined are as follows, with the first eight points referring to in vitro studies:

(1) The oligomycin-sensitive Mg^{2+}ATPase (known as mitochondrial Mg^{2+}ATPase) is the most sensitive of the ATPase systems to DDT and closely related compounds [14], followed by Na^{+}-K^{+}ATPase and oligomycin-insensitive Mg^{2+}ATPase [16]. Fig. 1 shows DDT effects on Mg^{2+}ATPases in cockroach muscle and Fig. 2 the effects in blue gill fish brain. Fig. 3 gives a comparison of 2 analogs and DDE, the chief metabolite of DDT. Table 1 also shows DDT to be more effective than TDE, Perthane and methoxychlor and from 7 to 8x as effective as DDE. Dicofol (Kelthane) is somewhat exceptional and Fig. 4 shows the effect resulting from this acaricide which is closely related to DDT.

(2) The inhibition of ATPases occurs in all tissues studied, including brain, muscle, liver, kidney and gill, but generally is somewhat greater in muscle [1, 2, 3, 14, 15]. Fig. 2 shows the DDT inhibition of fish brain Mg^{2+}ATPase to be about 8x more DDT than within muscle (Fig. 1). However, consistency of inhibition is somewhat greater in brain homogenates.

A tabular comparison of the inhibition of ATPases by DDT using different tissues is given in Table 2. It will be noted, not only that Mg^{2+}ATPase from muscle is affected more prominently, but that Mg^{2+}ATPase is inhibited to a greater extent than Na^{+}-K^{+}ATPase. Comparisons of this enzyme were possible in brain and kidney.

(3) The toxicity to fish by DDT and closely related chemicals show a certain parallelism to ATPase enzyme inhibition; however, several

acaricides (miticides) such as chlorobenzilate, tetradifon and other chlorinated bridged biphenyl compounds also are good inhibitors of mitochondrial Mg^{2+}ATPase and relatively ineffective on Na^{+}-K^{+}ATPase[17, 18]. Fig. 5 gives the results with tetradifon and Figs. 6 and 7 with 5 additional compounds having chemical structures given in Fig. 8. Tetradifon was highly effective, inhibiting 50% of mitochondrial Mg^{2+}ATPase at 4.9×10^{-8}M concentration.

(4) The inhibition of ATPases by chlordane-type compounds also shows some distinctive characteristics consistent with toxicity[20, 21]; a greater inhibition of Na^{+}-K^{+}ATPase occurs than with DDT-type compounds as shown in Table 2. The remainder of the Cyclodiene compounds, such as aldrin and dieldrin, do not show a good correlation between enzyme inhibition and toxicity (Table 2). The manner of biodegradation and penetration of the cyclodiene compounds to the site of action within the fish undoubtedly affects the results.

(5) The organophosphates and carbamates insecticides tested (cholinesterase inhibitors) are <u>not</u> ATPase inhibitors[21].

(6) The inhibitory effects of chlorinated hydrocarbons in insect homogenates are similar to fish homogenates of the same type of tissue with minor differences in sensitivity[14] (also see Figs. 1 and 2).

(7) At least one chemically distinct compound, Plictran, an acaricide, was found to be an outstanding inhibitor of mitochondrial Mg^{2+}ATPase[22]. The compound was also a better inhibitor of Na^{+}-K^{+}ATPase and oligomycin-insensitive Mg^{2+}ATPase than DDT (see Fig. 9). In these respects its broad inhibiting effects are readily distinguished from DDT[14] which inhibits mitochondrial Mg^{2+}ATPase to a much greater extent than either Na^{+}-K^{+}ATPase or oligomycin-insensitive Mg^{2+}ATPase.

<u>In vivo</u> results, involving the treatment of fish or insects, followed by an analysis for enzyme activity, gave the following results:

(8) Fish or insects treated with sublethal doses of DDT and closely related chemicals had the mitochondrial Mg^{2+}ATPase reduced or inhibited when compared with untreated[21]; also see Table 4.

(9) Brain tissues taken from fat head minnows continuously exposed to DDT showed a reduction of mitochondrial Mg^{2+}ATPase amounting to 36, 46.5, 52.5 and 45% when examined 56, 118, 225 and 266 days, respectively, after treatment started (see Table 4). The values given above referred to fish treated with a combination of 0.5 ppb DDT in water and 57 ppm in food. The maximum reduction of the enzyme activity when 0.5 ppb DDT was in the water alone was 41.9% after 225 days and 56.6% after 266 days continuous exposure. The effects on Na^{+}-K^{+}ATPase and oligomycin-insensitive Mg^{2+}ATPase from brain were a modest stimulation (never any inhibition) which gradually increased through the 266th day of exposure. A summary is given in Tables 5 and 6.

(10) Gill tissue from chronically exposed minnows gave contrasting results with respect to Na^{+}-K^{+}ATPase. Using the same specimens as examined for brain sensitivity, the activity of Na^{+}-K^{+}ATPase was reduced by over 30% (see Table 7). The Mg^{2+}ATPases from gills were also reduced, the greatest reduction being 49.7%. Thus, the effect on the Mg^{2+}ATPases in gills was similar, but less pronounced than in brain, but contrasted sharply in having the additional significant reduction in Na^{+}-K^{+}ATPase activity.

(11) Injection of several small doses of DDT in cockroaches (chronic treatments) resulted in a maximum of 35% inhibition of mitochondrial Mg^{2+}ATPase in nerve cords 25 days after the initial treatment, indicating a similar sensitivity to that of brain in fish, and also corresponding to the effects obtained *in vitro*[21]. However, it was found that moderately high levels of DDT (acute LD_{50} doses) resulted in a stimulation of mitochondrial Mg^{2+}ATPase, amounting to 50% in muscle and 30% in nerve cord.

(12) The polychlorinated biphenyl compounds (PCBs), like DDT, inhibited total Mg^{2+}ATPase both *in vitro* and *in vivo*. They differed in that the *in vitro* effect was greater on the oligomycin-insensitive Mg^{2+}ATPase[23, 24]. The *in vivo* effects were rather similar to those of DDT[24, 25, 26]. Aroclor 1242 appeared to be quite effective with Aroclor 1254 giving a somewhat smaller effect.

SECTION VI

DISCUSSION

The consistent inhibition of the ATPase enzyme system *in vitro* by chlorinated hydrocarbon insecticides and acaricides has been demonstrated. The DDT-group of compounds give a more predictable pattern, primarily inhibiting mitochondrial Mg^{2+}ATPase. We must emphasize that *only* with mitochondrial Mg^{2+}ATPase (oligomycin-sensitive) can one show inhibition values which can be illustrated as regression lines which parallel toxicity. DDE, for example, is a much poorer inhibitor than DDT and it was not possible to obtain 70% inhibition of mitochondrial Mg^{2+}ATPase. The criticism of Jackson and Gardner (1973) is invalid because they compared insecticidal effects on *total* ATPases instead of discrete ATPases as we have done. Further, in their techniques of adding the insecticide in ethanol to the reaction mixture they used a 25 µl aliquot while we never exceeded 5 µl, and usually used 1 µl with constant stirring conditions. The larger volume which they used would be conducive to precipitation, a feature which we avoided. We could detect such an occurrence because we were continuously monitoring the reaction in a spectrophotometer and only used results which showed the same rate of reaction to be consistent over a total of 15 or 20 minutes.

Results with cyclodiene compounds are not comparable to the DDT analogs because of greater variability in their inhibitory characteristics; lindane and dieldrin are poor inhibitors of all the ATPases tested under our conditions. The compound Kepone was an excellent inhibitor of mitochondrial, yet its close chemical relative, Mirex, was almost without effect. Thus, the usefulness of the bioassay lies primarily with the DDT analogs.

Response differences between ATPases from different tissues are not very great; most comparisons show muscle homogenates to be slightly more sensitive than brain; a lesser number of comparisons were made with kidney, liver and gills. Considerable detailed information is

given in the research papers, and the figures and tables present the important findings.

In general, the inhibition of mitochondrial Mg^{2+}ATPase by DDT and related compounds has some parallels to acetylcholinesterase inhibition by organophosphates and carbamates. In both cases the *in vitro* inhibition is readily established, but for various reasons, notably metabolic changes in the organisms, varying degradation patterns of the pesticide and penetration to the site of action cause some compounds to show a poor effect *in vivo*. We have principally studied the *in vivo* effects of DDT. The effects obtained, however, indicate the value of a follow-up study, along with the further development of a gill assay.

One relevant feature of the findings is the fact that only the pesticides which are persistent in the environment and in biological systems, and are capable of bioconcentration are effective inhibitors of the ATPase system. We do not find the inhibitory characteristic among the organophosphates and carbamates, which are relatively non-persistent, and although pyrethrins have some inhibitory effect on Na^{+}-K^{+}ATPase,[26] the concentration required is one or 2 magnitudes higher than DDT and related compounds. It is of course, this group of compounds (chlorinated hydrocarbons) which cause the greatest concern because residues continue to be found in soils, in certain predator birds, in certain fish, and in some bodies of water.

Based on this survey of compounds capable of ATPase inhibition, further research should investigate ways of utilizing this type of information for studying prolonged effects in various key organisms.

Chronically treated fish deserve further discussion. Fat head minnows treated with DDT showed a 50% average depression of mitochondrial Mg^{2+}ATPase from brain after 266 days exposure. The first sampling, at 56 days, showed about a 30-40% depression, thus there appeared to be a slow, but progressive adverse effect on the enzyme. There was no marked difference attributable to the method of DDT treatment, whether in water alone, or a combination with food. There is, of course, the possibility of small amounts of DDT getting into the water from food. Earlier in

the experiment (56 days) the greatest effects of brain ATPases seemed to result from mixed treatments of both food and water. Gill tissue ATPases reacted differently in two respects. First, Na^+-K^+ATPase from gills was 30% less (presumably inhibited) than the control, yet this enzyme had increased activity in brain tissue (presumably stimulated). Second, in the earlier treatments, the greatest effect on gills occurred in DDT-treated water, rather than in treated food. Furthermore, the combined ATPase inhibition was greatest in gill tissue at the last determination of 266 days.

The results show that chronically treated fish do have an impressive reduction of the energy-related mitochondrial Mg^{2+}ATPase with DDT-contaminated water, and the ATPase affected included a depression of Na^+-K^+ATPase in gills. Thus the exposure of the fish is very important in this differential effect because in vitro effects show no significant differences in sensitivity between brain and gill enzymes.

We believe this is the first report of significant abnormal biochemical or physiological conditions in a vertebrate exposed to controlled sublethal chronic doses of DDT.

Table 1. IN VITRO INHIBITION OF FISH BRAIN MITOCHONDRIAL Mg^{2+}ATPase BY DDT AND SOME ANALOGUES[a,b]

	uM for Inhibition		
	I_{50}	I_{70}	I_{90}
DDT	1.3	3.5	14.1
Dicofol	0.8	3.7	37.4
TDE	2.7	7.6	34.3
Perthane	3.8	13.4	-
Methoxychlor	4.9	27.3	-
DDE	8.8	30.8	-

[a] Reaction mixtures given under Materials and Methods.

[b] Specific activity of untreated mitochondrial Mg^{2+}ATPase (μ moles $P_i \cdot mg^{-1}$ protein hr^{-1}) was 10.91 ± 0.86 (mean value of 6 determinations).

Table 2. INHIBITION OF ATPases BY DDT USING 4 DIFFERENT TISSUES FROM BLUE GILL FISH

DDT Conc. x 10^{-6} M	% Inhibition: Total Mg^{2+} ATPase	% Inhibition: Na^{+} -K^{+} ATPase
(A) Muscle		
Specific activity =	49.9 ± 5.3	6.2* ± 1.6
1.3	15	--
5.2	34	--
10.4	51	--
20.8	63	--
(B) Brain		
Specific activity =	22.1 ± 1.0	33.0 ± 1.4
1.3	16	16
5.2	32	22
10.4	41	23
20.8	44	31
(C) Kidney		
Specific activity =	40.5 ± 3.0	46.7 ± 1.0
0.8	22	6
1.5	32	9
6.1	46	26
12.2	53	31
(D) Liver		
Specific activity =	28.2 ± 3.7	4.0*± 1.8
0.8	25	--
1.5	33	--
6.1	48	--
12.2	51	--

*Specific activity too low for accurate inhibition determinations.

Specific activity expressed as μmoles P_i mg^{-1} protein hr^{-1}.

Table 3. INHIBITION OF ATPases FROM FISH BRAIN BY DDT AND SEVERAL CYCLODIENE INSECTICIDES

Compounds[c]	Per cent inhibition		
	Mg^{2+} ATPase / Oligomycin		
	Sensitive	Insensitive	Na^+ -K^+ ATPase
DDT	95	20	31[b]
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^R	95	60	62
toxaphene[a]	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

[a] Mol. Wt. calculated as 413.85 based upon $C_{10}H_{10}Cl_8$.

[b] DDT inhibited 63.9% of Na^+ -K^+ ATPase from cockroach nerve cord.

[c] All compounds compared at a concentration of 20.8 μm

Table 4. REDUCTION OF MITOCHONDRIAL Mg^{2+} ATPase OF BRAIN FROM FISH[a] CONTINUOUSLY EXPOSED TO DDT

DDT Concentration	Per cent reduction from untreated			
	56 days	118 days	225 days	266 days
57 ppm in food	23.4	36.2	22.3	55.2
0.5 ppb in water	29.5	36.6	41.9	56.6
2 ppb in water	39.8	54.3	36.0	57.2
0.5 ppb in water + 57 ppm in food	36.0	46.5	52.5	45.0
2 ppb in water + 57 ppm in food	42.8	50.1	----	----
Untreated sp. act.[b]	9.60	7.90	7.27	7.55
±S.E.	±0.20	±0.45	±0.34	±0.15

[a] Fat head minnow, Pimephales promelas.

[b] Specific activity expressed as μmoles P_i mg^{-1} protein hr^{-1}

Table 5. STIMULATION (INCREASE) IN Na^+-K^+ATPase OF BRAIN FROM FISH[a] CONTINUOUSLY EXPOSED TO DDT

DDT concentration	Per cent increase over untreated			
	56 days	118 days	225 days	266 days
57 ppm in food	+ 7.6	+12.5	+ 1.9	+27.7
0.5 ppb in water	+ 0.2	+ 9.9	+14.5	+18.9
2 ppb in water	+ 7.5	+10.2	+ 5.7	+21.7
0.5 ppb in water + 57 ppm in food	+10.1	+ 6.4	+13.5	+ 6.3
2 ppb in water + 57 ppm in food	+18.4	+ 9.1	----	----
Untreated sp. act.[b]	27.28	22.62	24.03	18.38
±S.E.	±1.84	±0.64	±0.69	±1.70

[a] Fat head minnow, Pimephales promelas

[b] Specific activity expressed as µmoles P_i mg^{-1} protein hr^{-1}

Table 6. STIMULATION (INCREASE) OF OLIGOMYCIN-INSENSITIVE Mg^{2+}ATPase OF BRAIN FROM FISH[a] CONTINUOUSLY EXPOSED TO DDT

DDT concentration	Per cent increase over untreated			
	56 days	118 days	225 days	266 days
57 ppm in food	+19.3	+13.9	+16.9	+33.6
0.5 ppb in water	+16.7	+21.2	+14.7	+39.6
2 ppb in water	+27.5	+ 8.3	+11.6	+38.5
0.5 ppb in water + 57 ppm in food	+26.9	+ 5.5	+23.5	+36.4
2 ppb in water + 57 ppm in food	+29.2	+14.7	----	----
Untreated sp. act.[b]	12.04	12.35	10.48	8.96
±S.E.	±0.85	±0.79	±0.37	±0.55

[a] Fat head minnow, Pimephales promelas

[b] Specific activity expressed as μmoles P_i mg^{-1} protein hr^{-1}

Table 7. PER CENT CHANGES IN ATPases OF GILL TISSUE FROM FISH[a] CONTINUOUSLY EXPOSED TO DDT

DDT concent.	Per cent reduction from untreated					
			OLIGOMYCIN			
	Na^+ -K^+		Sensitive		Insensitive	
	225 days	266 days	225 days	266 days	225 days	266 days
57 ppm in food	+0.4*	35.3	3.2	41.3	+ 4.5*	28.6
0.5 ppb in water	31.2	30.3	27.2	49.7	24.3	14.2
2 ppb in water	27.9	12.8	+7.2*	26.4	3.5	8.7
0.5 ppb in water + 57 ppm in food	18.1	13.7	33.8	23.7	20.5	3.2
Untreated sp. act.[b]	13.7	10.6	5.50	4.55	24.15	24.36
±S.E.	±0.93	±1.26	±0.32	±0.25	±1.14	±2.33

* (+) Values represent the enzyme activation

[a] Fat head minnow, Pimephales promelas

[b] Specific activity expressed as umoles P_i mg^{-1} protein hr^{-1}

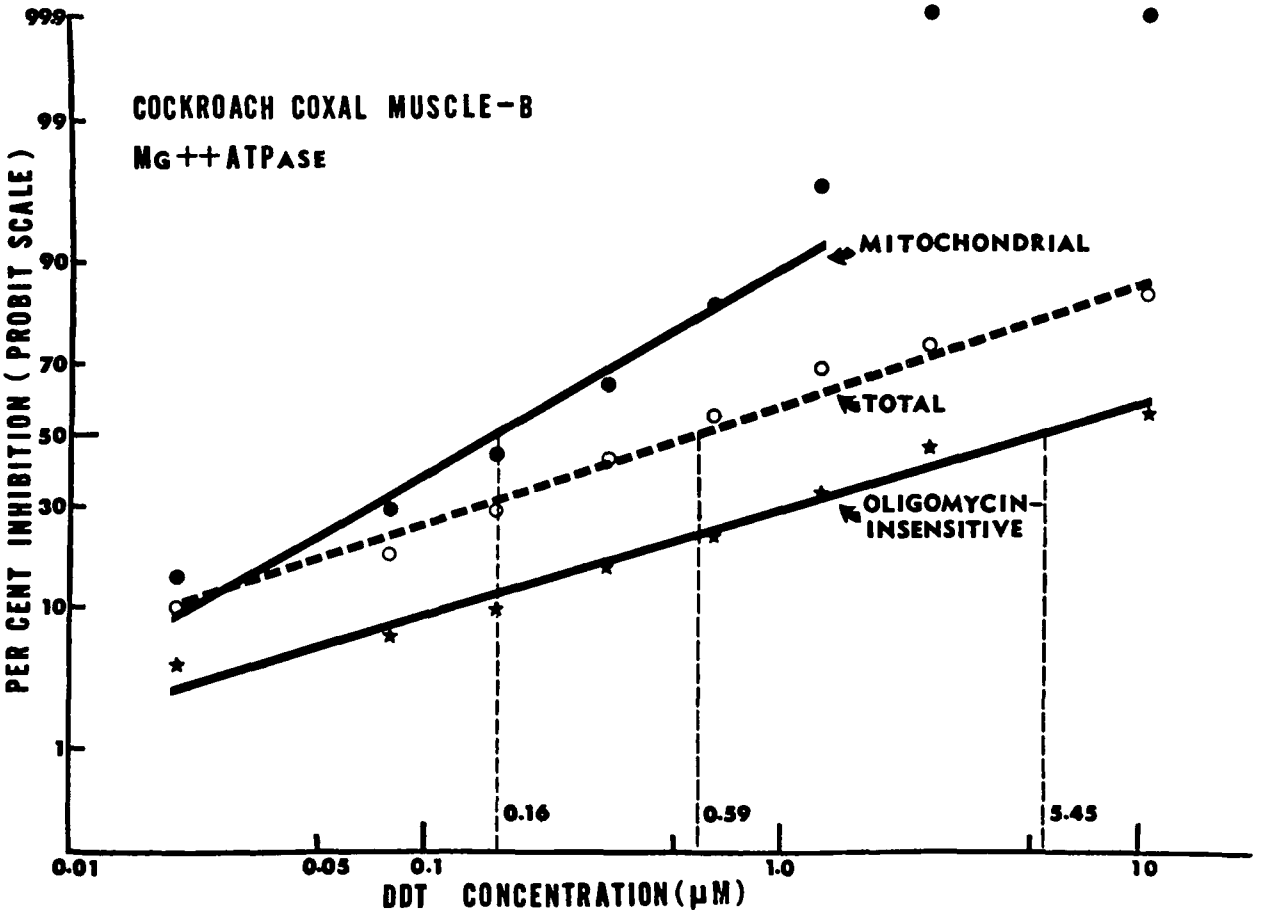

Figure 1. In vitro inhibition of Mg^{2+}ATPase from cockroach muscle homogenates treated with DDT

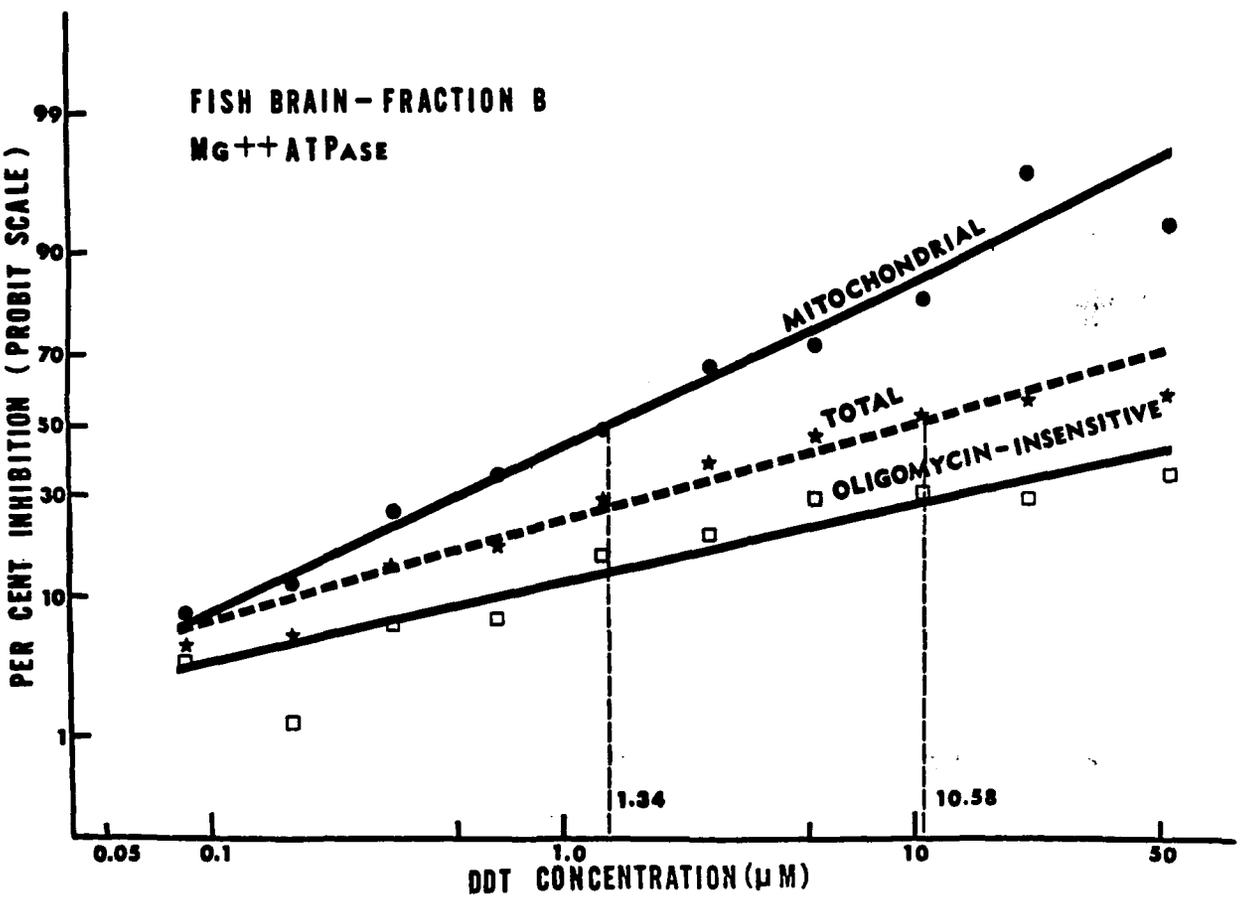

Figure 2. <u>In vitro</u> inhibition of Mg^{2+}ATPase from fish brain homogenates treated with DDT

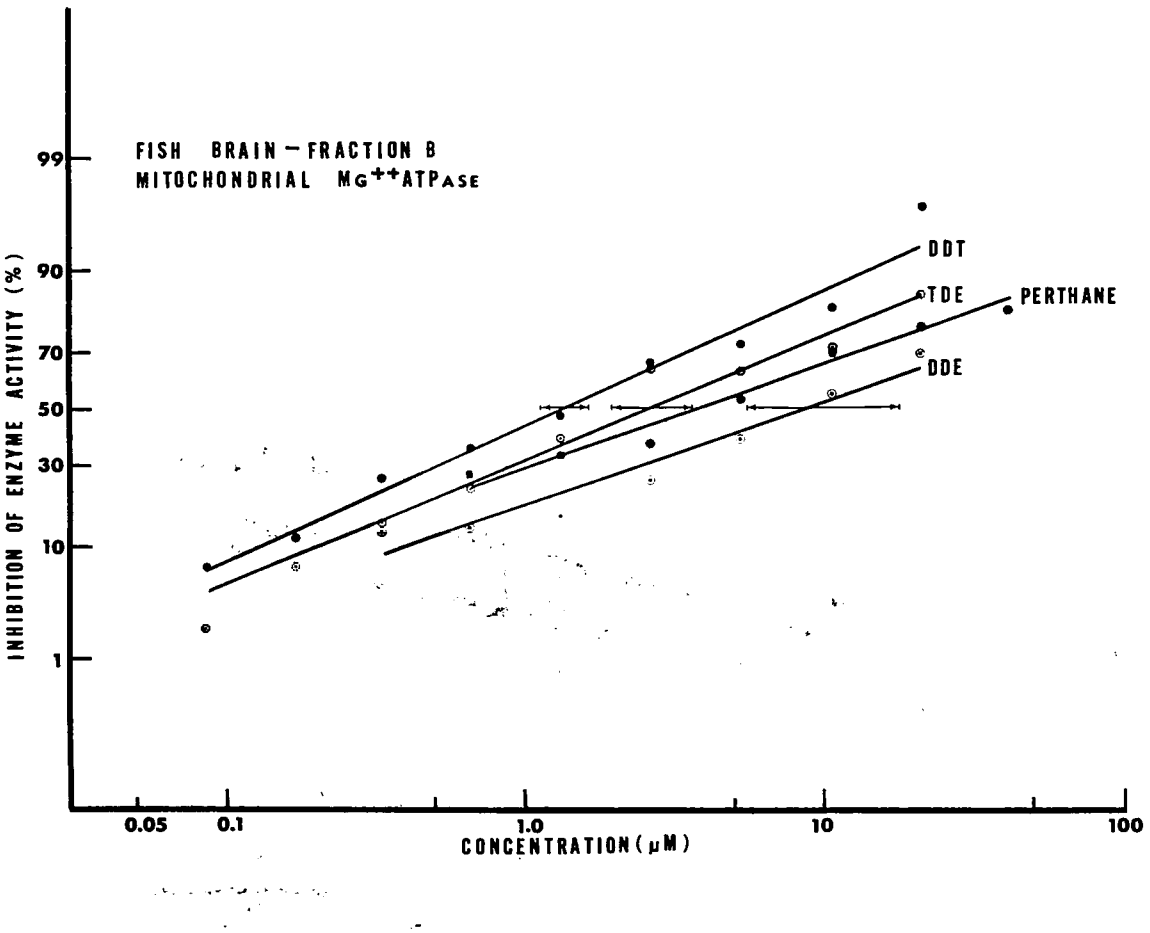

Figure 3. <u>In vitro</u> inhibition of mitochondrial Mg^{2+}ATPase from fish brain homogenates with DDT, 2 analogs of DDT, and DDE, the principal metabolite

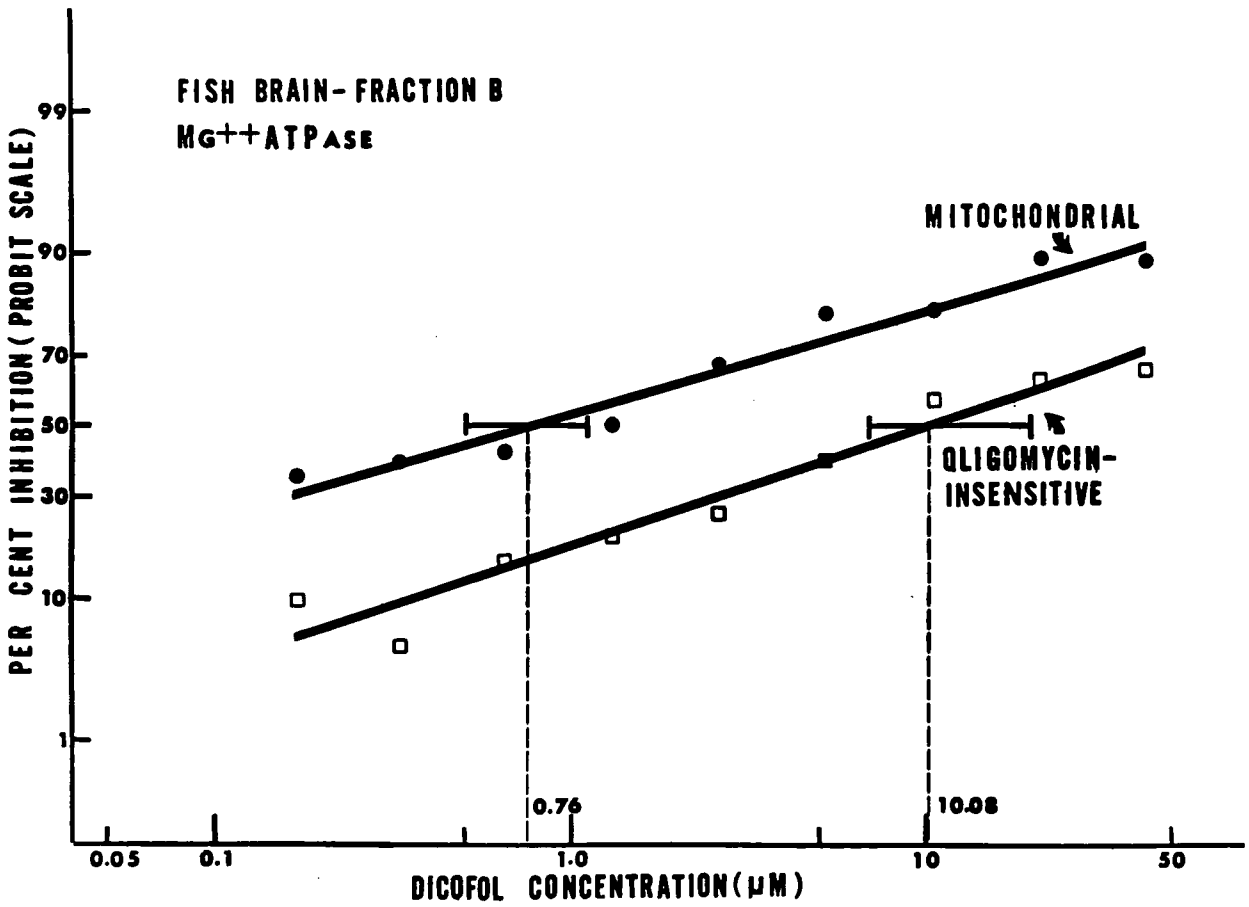

Figure 4. In vitro inhibition of Mg^{2+}ATPase from fish brain homogenates treated with dicofol (Kelthane)

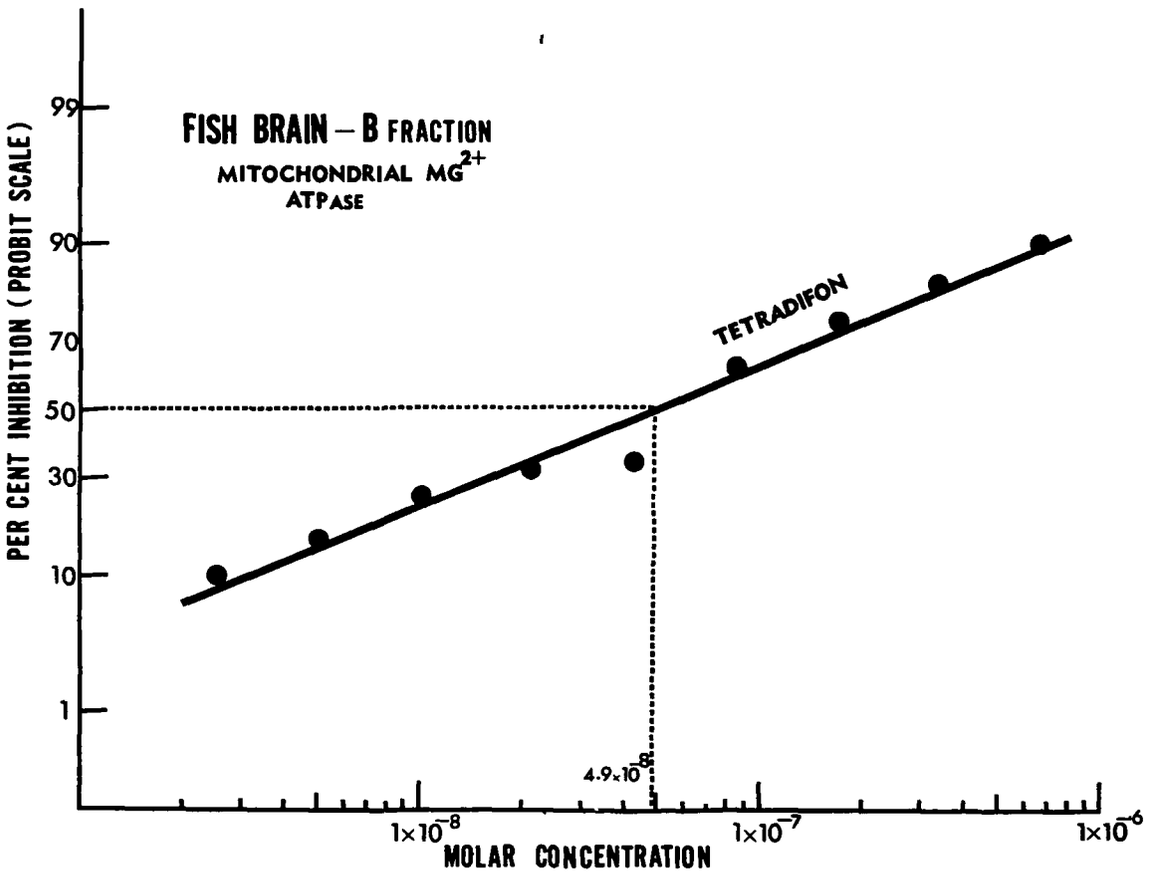

Figure 5. In vitro inhibition of Mg^{2+}ATPase from fish brain homogenates treated with tetradifon (Tedion)

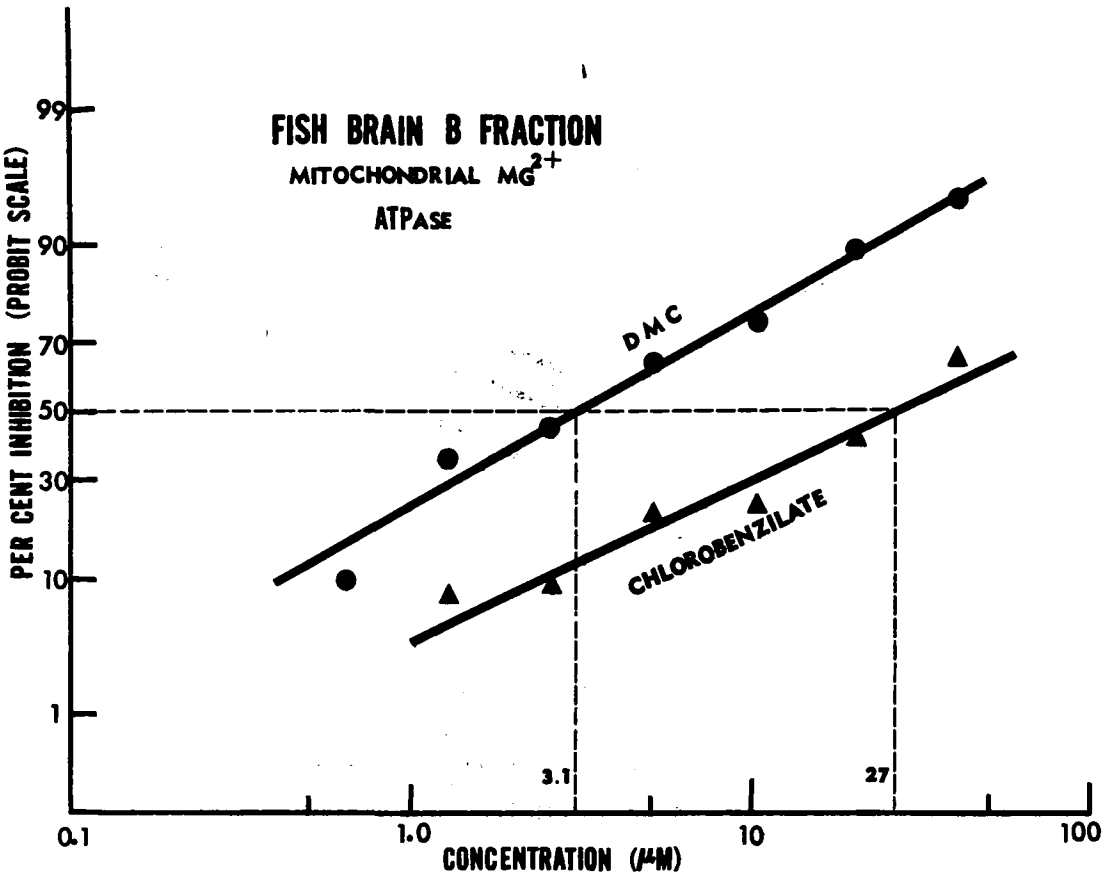

Figure 6. In vitro inhibition of Mg^{2+}ATPase from fish brain homogenates treated with dichlorodiphenylethanol (DMC of Dimite) and chlorobenzilate

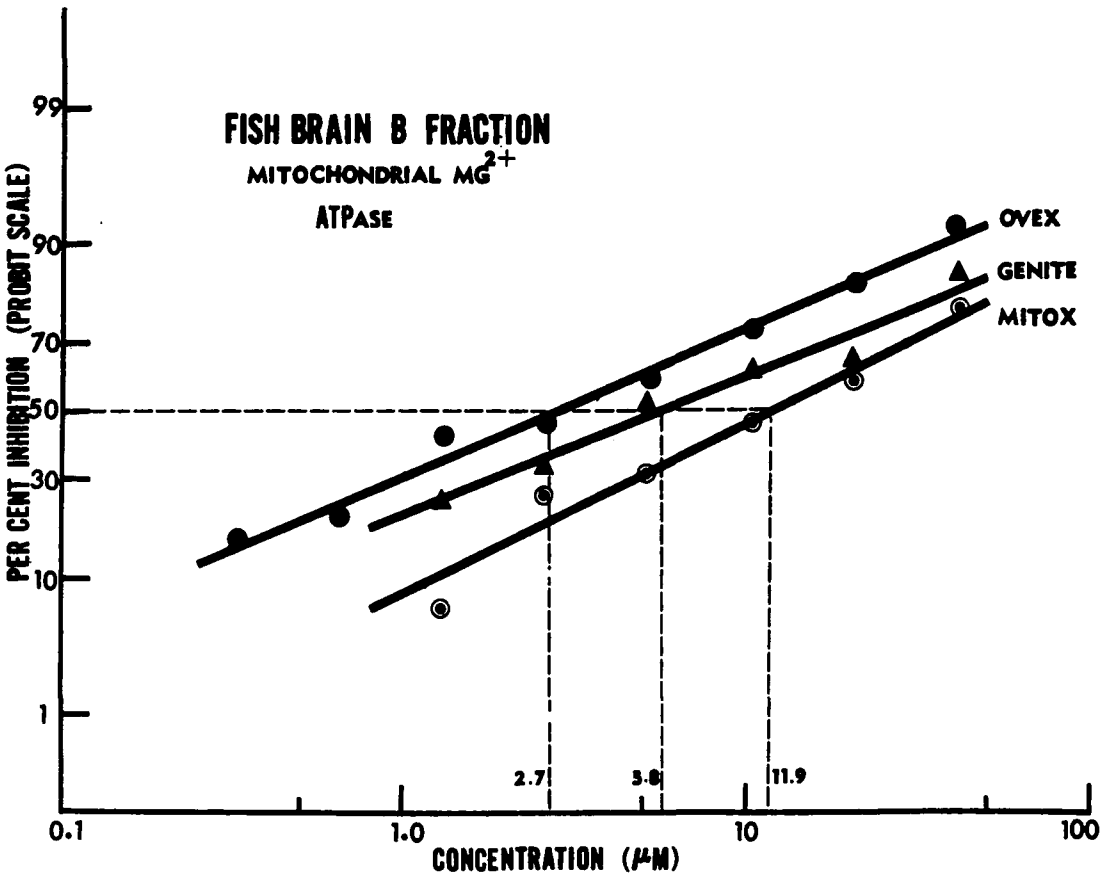

Figure 7. In vitro inhibition of mitochondrial Mg^{2+}ATPase from fish brain homogenates treated with ovex, Genite and chlorbenside (Mitox)

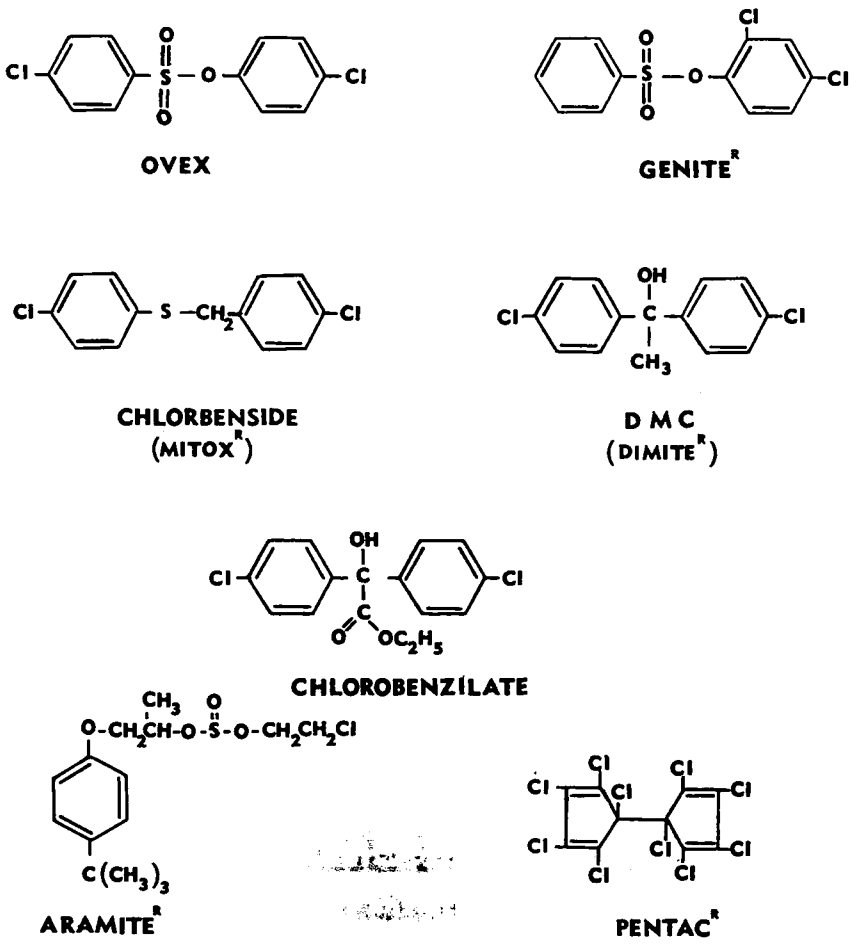

Figure 8. Structural formulas of chlorinated hydrocarbon acaricides tested on ATPase system of blue gill fish

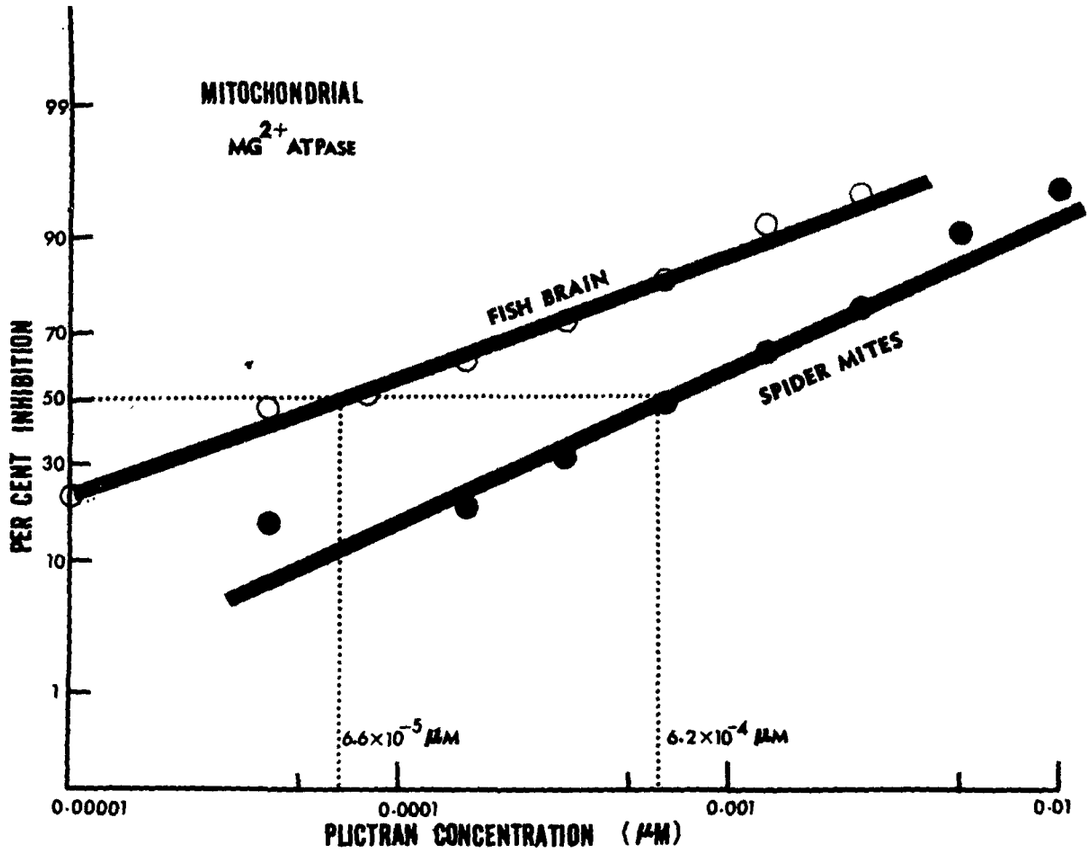

Figure 9. In vitro inhibition of mitochondrial Mg^{2+}ATPase from brain homogenates of fish and homogenates of two-spotted spider mites

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SECTION IX

GLOSSARY

ADP, adenosine diphosphate

ATP, adenosine triphosphate

ATPase, ATP phosphohydrolase, E.C.3.6.1.3

BSA, bovine serum albumin

EDTA, ethylenediamine tetraacetic acid

LDH, lactic dehydrogenase

NAD, nicotinamide adenine dinucleotide phosphate

NADH, reduced nicotinamide adenine dinucleotide phosphate

PEP, phospho (enol) pyruvate

Mg^{2+}ATPase, Mg^{++}ATP phosphohydrolase

Na^{+} -K^{+}ATPase, Na^{+} and K^{+} dependent, Mg^{++}ATP phosphohydrolase

PK, pyruvate kinase

S.E., Standard Error

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1. Report No. | 2. | W

4. Title: A Tissue Enzyme Assay for Chlorinated Hydrocarbon Insecticides

5. Report Date

6.

8. Performing Organization Report No.

10. Author(s): Laurence K. Cutkomp

Project No: 16030 ELZ

9. Organization: Department of Entomology, Fisheries, & Wildlife, University of Minnesota, St. Paul, Minn.

Contract/Grant No: R 801029

12. Sponsoring Organization: U.S. Environmental Protection Agency

13. Type of Report and Period Covered: Final-7/1/69-6/30/72

15. Supplementary Notes:

Environmental Protection Agency report number, EPA-660/2-73-027, May 1974.

16. Abstract: Certain chlorinated hydrocarbon insecticides, especially DDT and closely related chemicals, tested at low concentrations, adversely affect the ATPase enzyme system. DDT inhibited oligomycin-sensitive Mg^{2+}ATPase (mitochondrial) both in vitro and in vivo. About 1 µM (1 x 10^{-6} M) gave 50% inhibition in fish brain and 0.5 ppb of DDT in water inhibited about 50% of mitochondrial Mg^{2+}ATPase. Na^{+}-K^{+}ATPase was not inhibited in brain, but was inhibited in vivo in fish gills. Certain discriminating effects were found among chlorinated hydrocarbons, particularly with respect to inhibition of Mg^{2+} ATPase, but the ranking of compounds by enzymic effects does not always parallel toxicity values. Organophosphate and carbamate insecticides were ineffective. Further research is needed both in vitro and in vivo to determine how the adverse effects on the enzymes relate to practical interpretations of effects. The abnormally low ATPase activity in chronically treated fish is the first report of an adverse biochemical effect with sublethal doses of DDT. All effects appear to be primarily within the group of insecticides and acaricides which are persistent in parts of the environment and in organisms.

17a. Descriptors: Chlorinated hydrocarbon insecticides, enzyme assay.

17b. Identifiers

17c. COWRR Field & Group

18. Availability

19. Security Class. (Report)

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