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INHIBITION OF OLIGOMYCIN-SENSITIVE AND -INSENSITIVE MAGNESIUM ADENOSINE TRIPHOSPHATASE ACTIVITY IN FISH BY POLYCHLORINATED BIPHENYLS*†

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Abstract—Tests *in vitro* with four polychlorinated biphenyls (PCBs) on four tissues of fish showed prominent inhibitory effects on oligomycin-insensitive Mg^{2+} ATPase, with muscle homogenate being most sensitive. Aroclors 1242 and 1254, in the intermediate range of chlorination, were more effective than 1221 and 1268. Mg^{2+} ATPase from mitochondria was not as sensitive to the PCBs when compared with DDT-type compounds which were more effective on mitochondrial Mg^{2+} ATPase than on oligomycin-insensitive Mg^{2+} ATPase.

Some stimulation of Mg^{2+} ATPase was evident from the poorest inhibitors, Aroclors 1221 and 1268. $Na^+ - K^+$ ATPase from fish brain homogenate was inhibited by Aroclor 1242 but the dose required was several times that for Mg^{2+} ATPase.

POLYCHLORINATED biphenyls (PCBs) are being used widely in industry as plasticizers in paints, resins and plastics, as well as insulators and heat exchange fluids.[1] Some of these chemicals also are being released into the environment as industrial wastes. The residues of PCBs have been found in many living organisms in many parts of the world both in water and on land, thus causing concern about the hazard to wildlife and fish.[2–14] As early as 1955 Von Oettingen[15] reviewed the limited literature available on the toxicity of these compounds; more recently it was reviewed by Peakall and Lincer.[16] Though general information on PCBs is being accumulated, relatively little data are available on the biochemical action of these compounds. The PCBs are structurally related to DDT-type chlorinated hydrocarbons, and it has been shown that the ATPase system is sensitive to DDT and its analogues.[17–23]§ Recently we have shown that PCBs inhibit Na^+–K^+ and Mg^{2+} ATPase activity *in vitro* in fish tissue homogenate fractions.[24]

We have designed this present research to investigate *in vitro* the specific inhibitory action of PCBs on Mg^{2+}-dependent, oligomycin-sensitive and insensitive ATPase activity in fish homogenate fractions.

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MATERIALS AND METHODS

Tissues from blue gill fish, *Lepomis machrochirus*, were dissected, homogenized, and fractionated by centrifuging at 13,000 *g* for 20 min, and the sediments were resuspended in 0·32 M sucrose, 1 mM EDTA and 10 mM imidazole according to the procedure reported by Koch.[25] The fraction obtained (B) contained mitochondria and nerve ending particles. Each preparation was appropriately diluted, and the samples were quick frozen in liquid nitrogen and stored at −20° until the ATPase assay. ATPase activity was determined using the enzymatic or continuous procedure described by Pullman *et al.*[26] and Fritz and Hamrick[27] and as reported by Yap and Cutkomp.[28] Mg^{2+} ATPase activity was measured when 1 mM ouabain was in the reaction mixture. Ouabain is a cardiac glycoside which specifically inhibits the Na^+–K^+ ATPase.[29] Mg^{2+} ATPase was further separated into oligomycin-sensitive (mitochondrial) and oligomycin-insensitive portions by adding 0·03 μg oligomycin (oligomycin A 15%, B 85%)/ml of reaction mixture.[30,31,*]

The assay for protein determination was carried out according to the method of Lowry *et al.*[32] with absorbance measured at 660 nm using a Spectronic 20 Colorimeter.

Four PCB preparations (Aroclor 1221, Aroclor 1242, and Aroclor 1254 and Aroclor 1268) were used in this investigation.[1] Aroclors 1221, 1242 and 1254 were dissolved in ethanol and Aroclor 1268 in acetone and added to the reaction mixture by slowly releasing the solution from Hamilton micro-syringe under the liquid surface of vortex of a rapidly stirred reaction mixture. Reaction temperature was 37°, except for muscle where the temperature was maintained at 27°. The amount of solvent added with the PCBs had no effect on the ATPase activities. The 3-ml reaction mixture used contained: 4·3 mM ATP, 5 mM Mg^{2+}, 100 mM Na^+, 20 mM K^+, 135 mM imidazole buffer (pH 7·5) 0·2 mM NADH, 0·5 mM PEP (phospho-enol-pyruvate), 0·02% BSA (bovine serum albumin), approximately 9 units of pyruvate kinase and 12 units of lactic dehydrogenase and 100 μl homogenate fraction. Absorbance changes were measured at 340 nm over a period of 15 min using a Beckman DU Spectrophotometer with temperature control.

The present investigation was designed to determine and differentiate effects of the PCBs on a mitochondrial Mg^{2+} ATPase (determined by inhibiting its effect using oligomycin) and oligomycin-insensitive Mg^{2+} ATPase. Oligomycin-insensitive Mg^{2+} ATPase apparently occurs in microsomes (endoplasmic reticulum and plasma membrane) and to a small extent in mitochondria. Determinations were made from fish brain, kidney, liver and muscle tissues. These tissues, in particular, have provided relevant information for studying sensitivity of ATPases to chorinated hydrocarbons. Brain has a high concentration of Na^+–K^+ ATPase and is a much-studied key tissue for living animals; kidney has high concentrations of Mg^{2+} ATPases and Na^+–K^+ ATPase; liver has distinctive metabolic activity, and muscle high activity of Mg^{2+} ATPases plus a high sensitivity to numerous chlorinated hydrocarbons.

Where data permitted (dosage–effect relationship may be transformed for reliable statistical comparisons), they were subjected to probit analysis,[33–35] a widely accepted procedure in toxicological research. The data were transformed using the procedure of Finney,[33–34] programmed according to Daum and Killcreas,[35] analyzed on an IBM computer and plotted as regression lines using the probit scale. Each point on Figs.1–5 represents three replicates, and a minimum of four different concentrations

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was used to establish 50 per cent inhibition values (I_{50}). Probit plots were not possible for some data (see Tables 1–4) either because of inconsistent effects or because stimulation of enzyme activity occurred at low PCB concentrations or inhibition at higher concentrations.

RESULTS

We had previously reported[24] the inhibition *in vitro* of Mg^{2+} ATPase in four fish tissue homogenates by Aroclors 1221 and 1254, but Aroclor 1268 inhibited total Mg^{2+} ATPase only in muscle, causing stimulation in brain, kidney and liver homogenates.

The present results show, in general, that the PCBs tested under conditions *in vitro* were most effective inhibitors of oligomycin-insensitive Mg^{2+} ATPase and that muscle was the most sensitive tissue.

Aroclors 1242 and 1254 were the most effective inhibitors. The greatest inhibition was on oligomycin-insensitive Mg^{2+} ATPase in muscle. Aroclor 1242 showed an I_{50} value of 0·6 ppm, compared to 4·3 for kidney, 6·5 for liver and 10·8 for brain (Fig. 1). Values for Aroclor 1254 were slightly higher in muscle being 1·2 ppm for oligomycin-insensitive Mg^{2+} ATPase, but I_{50} values were slightly lower for other tissues, being 4 ppm for kidney, 5·2 ppm for brain and 7·2 ppm for liver (Fig. 2).

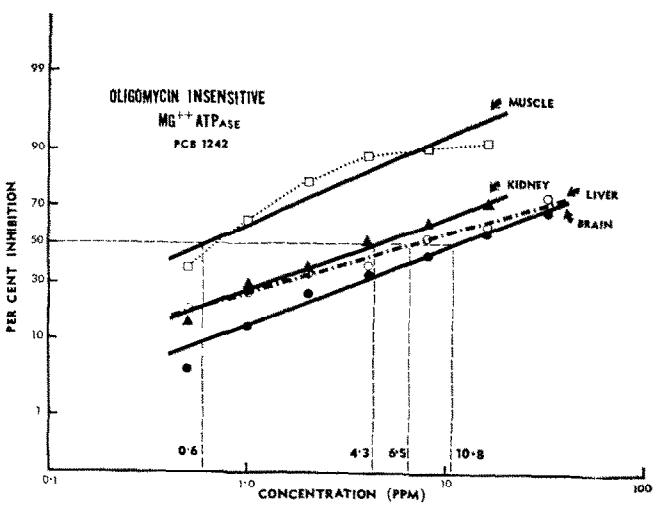

FIG. 1. Aroclor 1242 inhibition (probit units) of oligomycin-insensitive Mg^{2+} ATPase from fish homogenates. ATPase activity of untreated tissue in micromoles of P_i mg^{-1} protein hr^{-1} for brain 14·5 ± 0·03; kidney 16·1 ± 0·02; liver 10·4 ± 0·5; muscle 46·2 ± 9·6. Actual muscle values connected with a dotted line. Straight line statistically computed.

These two PCBs, Aroclors 1242 and 1254, were also inhibitors of mitochondrial Mg^{2+} ATPase, but required somewhat higher concentrations. The I_{50} values, in ppm, for Aroclor 1242 were: 3·5, 11·5, 4·0 and 2·0 for brain, liver, kidney and muscle respectively (Fig. 3, Table 1). The I_{50} values, in ppm, for Aroclor 1254 were: 6·0, 5·3, 4·0 and 5·10 for brain, liver, kidney and muscle respectively (Fig. 4, Table 2).

Aroclor 1221 was a less effective inhibitor than 1242 and 1254, but the sensitivity of the tissues followed a similar pattern. The oligomycin-insensitive Mg^{2+} ATPase was inhibited to the greatest extent. The I_{50} values were: 14·6, 35·0, 9·0 and 2·25 ppm for

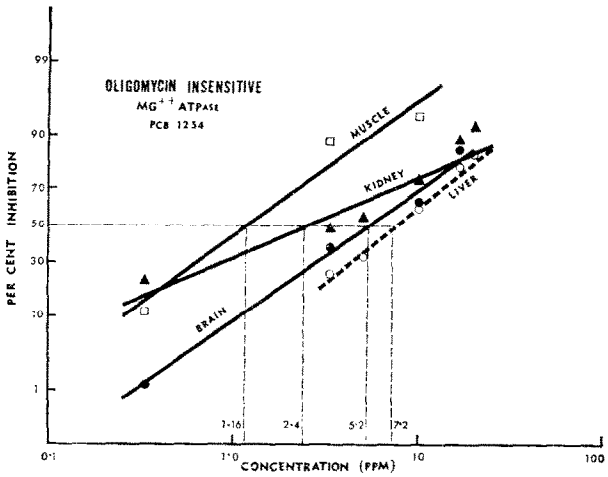

FIG. 2. Aroclor 1254 inhibition (probit units) of oligomycin-insensitive Mg^{2+} ATPase from fish homogenates. ATPase activity of untreated tissue in micromoles of P_i mg^{-1} protein hr^{-1} for brain 14·2 ± 0·4; kidney 17·3 ± 3·0; liver 10·0 ± 0·05; muscle 63·7 ± 5·5.

brain, liver, kidney and muscle respectively (Fig. 5). Tests on mitochondrial Mg^{2+} ATPase showed greater variability with stimulation occurring at low concentrations in three tissues: kidney, liver and muscle. About 10 ppm was required for 50 per cent inhibition of mitochondrial Mg^{2+} ATPases in brain and kidney (Table 2).

Aroclor 1268 was the poorest inhibitor of Mg^{2+} ATPase of the four PCBs tested. Results presented in Table 3 show that in only one tissue, muscle, was 50 per cent inhibition achieved; that was on oligomycin-insensitive Mg^{2+} ATPase. Aroclor 1268

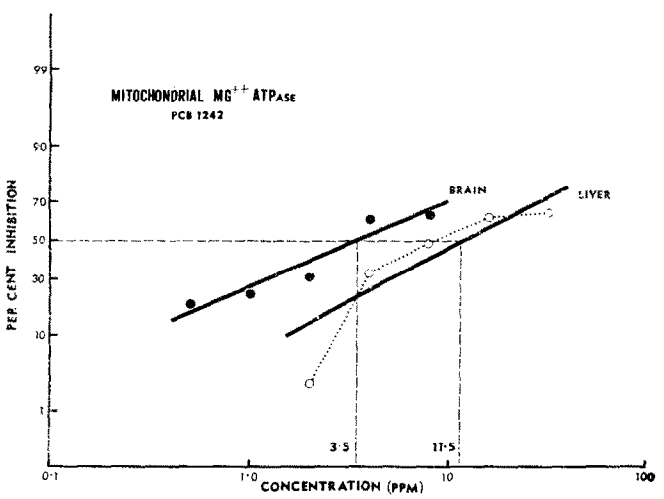

FIG. 3. Aroclor 1242 inhibition (probit units) of mitochondrial Mg^{2+} ATPase from fish homogenates. ATPase activity of untreated tissue in micromoles of P_i mg^{-1} protein hr^{-1} for brain 13·2 ± 1·1; liver 29·1 ± 0·04. Actual liver values connected with a dotted line. Straight line statistically computed.

TABLE 1. PER CENT INHIBITION OF MITOCHONDRIAL Mg^{2+} ATPase ACTIVITY BY AROCLOR 1242 IN FISH KIDNEY AND MUSCLE HOMOGENATES

Concn. (ppm)	Per cent inhibition*	
	Kidney	Muscle
0·5	+15·8	+35·9
1·0	+11·6	25·9
2·0	17·4	67·8
4·0	62·0	69·4
8·0	66·2	77·9
16·0	66·0	76·6
Untreated sp. act. ± S.E.	27·2 ±2·5	5·6 ±1·1

* Values marked with a plus sign represent the per cent of enzyme activation.

actually stimulated mitochondrial Mg^{2+} ATPase activity in all four tissue homogenates. In most cases the stimulatory effects were inconsistent, in that the dosage-effect did not follow a predictable relationship, with an exception of kidney homogenate (Table 3) where mitochondrial Mg^{2+} ATPase was stimulated from 27 to 88 per cent by progressively higher concentrations. This value represents the greatest stimulation by any PCB on any tissue.

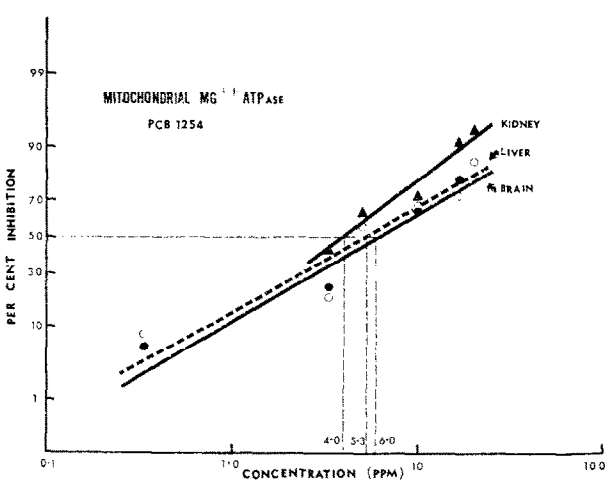

FIG. 4. Aroclor 1254 inhibition (probit units) of mitochondrial Mg^{2+} ATPase from fish homogenates. ATPase activity of untreated tissue in micromoles of P_i mg^{-1} protein hr^{-1} for brain 13·5 ± 0·35; kidney 28·6 ± 0·85; liver 39·0 ± 1·25.

TABLE 2. PERCENT INHIBITION OF MITOCHONDRIAL Mg^{2+} ATPase ACTIVITY BY AROCLORS 1221 AND 1254 IN FISH HOMOGENATES

Concn. (ppm)	Per cent inhibition*				
	1221				1254
	Brain	Kidney	Liver	Muscle	Muscle
0·03	12·3			+43·6	29·5
0·33	1·7	+20·8	+4·5	8·3	+6·8
3·30	5·2	+20·0	+2·4	+61·8	31·1
5·00		+ 0·1			
10·00	53·0	48·4	31·4	+37·5	55·9
16·70	57·0	50·3	41·8		
20·00		57·7	44·3		
Untreated sp. act. ±S.E.	13·5 ±0·35	28·6 ±0·85	32·3 ±3·2	4·3 ±0·89	4·3 ±0·89

* Values marked with a plus sign represent the per cent of enzyme activation.

Results of testing *in vitro* of Na^+–K^+ ATPase from brain and kidney by Aroclor 1242 are given in Table 4. They show a greater sensitivity in brain homogenate, a 51 per cent inhibition occurring with 32 ppm of Aroclor 1242. The inhibition, although significant, is considerably less than that on Mg^{2+} ATPase. About nine times the concentration of 1242 which inhibits Mg^{2+} ATPase is required for the equivalent inhibition of Na^+–K^+ ATPase.

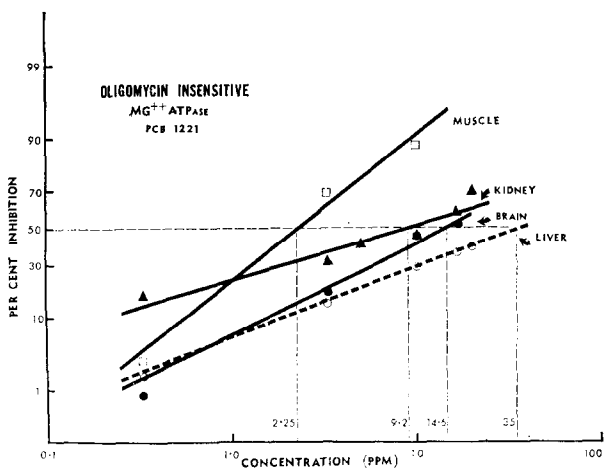

FIG. 5. Aroclor 1221 inhibition (probit units) of oligomycin-insensitive Mg^{2+} ATPase from fish homogenates. ATPase activity of untreated tissue in micromoles of P_i mg^{-1} protein hr^{-1} for brain 14·2 ± 0·4; kidney 17·3 ± 3·0; liver 10·7 ± 0·07; muscle 63·7 ± 5·5.

TABLE 3. PER CENT INHIBITION OF MITOCHONDRIAL AND OLIGOMYCIN-INSENSITIVE Mg^{2+} ATPase ACTIVITY BY AROCLOR 1268 IN FISH HOMOGENATES

	Per cent inhibition*							
	Brain		Kidney		Liver		Muscle	
Concn. (ppm)	Mito-chondr.	Oligo-insens.	Mito-chondr.	Oligo-insens.	Mito-chondr.	Oligo-insens.	Mito-chondr.	Oligo-insens.
0·03	2·8	6·8	+1·7	6·3			18·9	13·6
0·33	+0·7	7·0	+27·3	11·2	+6·0	15·7	+43·6	24·9
3·30	+12·9	19·9	+37·5	21·2	+6·6	19·5	+89·4	40·1
10·00	+ 4·0	27·1	+44·2	24·6	+2·1	20·5	+43·6	63·7
16·70	1·0	36·7	+87·5	33·5	+5·6	31·2		
20·00					4·6	38·0		
Untreated sp. act. ± S. E.	13·5 ± 0·35	14·2 ± 0·4	19·7 ±7·9	13·4 ±0·82	32·3 ±3·2	10·7 ±0·07	4·2 ±0·99	63·7 ±5·5

* Values marked with a plus sign represent the per cent of enzyme activation.

TABLE 4. PER CENT INHIBITION OF Na^+–K^+ ATPase ACTIVITY BY AROCLOR 1242 IN FISH BRAIN AND KIDNEY HOMOGENATES

Concn (ppm)	Per cent inhibition	
	Brain	Kidney
0·5	3·1	13·9
1·0	11·0	11·0
2·0	21·6	22·7
4·0	37·7	18·0
8·0	43·0	25·4
16·0	45·0	28·4
32·0	51·2	
Untreated sp. act. ±S.E.	37·1 ± 2·3	50·0 ± 4·2

DISCUSSION

The biochemical effects of PCBs, as measured by inhibition of Mg^{2+} ATPase, show decided selectivity among the compounds. Those intermediate in chlorination, Aroclors 1242 and 1254, have the greatest inhibitory effect on oligomycin-insensitive Mg^{2+} ATPase. The effect correlates with fish toxicity data of Nebeker and Puglisi,* in which Aroclors 1242, 1248, 1254 and 1260 (intermediate in chlorination) were most toxic, while chemicals out of this range, such as Aroclors 1221 and 1268, show the least toxicity. Earlier studies, which were much less detailed,[16] indicated higher toxicity with less chlorination as with Aroclor 1221, and Lichtenstein *et al.*,[36] testing

* A. V. NEBEKER and F. A. PUGLISI, in preparation.

the PCBs in combination with DDT and dieldrin, concluded that the combined toxicity increased with a decrease in chlorine content of PCBs. Our results do not support this concept.

DDT, when tested *in vitro*, is also a strong inhibitor of Mg^{2+} ATPase, especially in muscle tissues† but the inhibition from DDT is greater on mitochondrial Mg^{2+} ATPase. In contrast, all PCBs tested were much more effective inhibitors of oligomycin-insensitive Mg^{2+} ATPase. The I_{50} for DDT on mitochondrial Mg^{2+} ATPases in fish brain and the oligomycin-insensitive Mg^{2+} ATPase from muscle was about 0·5 ppm.‡ By comparison Aroclor 1242, the most effective of the PCB compounds tested, had I_{50} values of about 2·0 ppm for mitochondrial Mg^{2+} ATPase and 0·6 ppm for oligomycin-insensitive Mg^{2+} ATPase, both from muscle homogenates.

Detailed studies of the inhibition of Na^+–K^+ ATPase by Aroclor 1242 showed it to be a less effective inhibitor of Na^+–K^+ ATPase than Mg^{2+} ATPase. This feature is shared by DDT and DDE, but differs from some cyclodiene compounds, such as chlordane and heptachlor, which are effective inhibitors of Na^+–K^+ ATPase.§

The stimulation of Mg^{2+} ATPase by Aroclor 1268 and to a lesser extent by Aroclor 1221 is also unusual for compounds closely related to DDT. Thus the responses of ATPases to PCBs, although similar to DDT-type insecticides in that the Mg^{2+} ATPases were most affected, were quite different in their actions on the two types of Mg^{2+} ATPase (mitochondrial and oligomycin-insensitive). In fact, certain PCBs (1221 and 1268, the least toxic) have opposite effects on mitochondrial Mg^{2+} ATPase as compared to DDT.

Mg^{2+} ATPases are involved in oxidative-phosphorylation resulting in the production of ATP. ATP is sometimes called the universal energy carrier and the site is within mitochondria.[37] This enzymatic portion has discrete biochemical differences, as evidenced by its high sensitivity to oligomycin and our more recent results *in vitro* with DDT. The oligomycin-insensitive portion may also contain small amounts of Mg^{2+} ATPase from mitochondria, but the remainder may be somewhat more variable. Its differential role is under study. The present results with PCBs add a discriminating feature which should be useful in interpreting toxicity differences between DDT types of compounds and the PCBs.

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