

The Sensitivity of Fish ATPases to Polychlorinated Biphenyls*

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In spite of widespread concern about the possible hazards of polychlorinated biphenyls (PCBs), little is known about the action of these materials. We have therefore examined the in vitro (1, 2) and in vivo (3) effects of PCBs on the adenosine triphosphatase (ATPase) enzyme system. We have found some effects which are similar, yet distinctive, to chemically related compounds of the DDT type. A background of earlier research with DDT and closely related chemicals has shown the greatest inhibition on Mg^{2+} ATPase and less on Na^+ - K^+ ATPase (4-7). Subsequent research showed that mitochondrial Mg^{2+} ATPase of fish and insects was most sensitive to DDT (8). The results from testing several PCBs on the ATPase enzyme system are presented in this manuscript.

Materials and Methods

Tissues from bluegill fish, *Lepomis macrochirus*, were dissected, homogenized, and fractionated by centrifuging at $13,000\times g$ for 20 minutes, and the sediments were resuspended in 0.32 M sucrose, 1 mM EDTA, and 10 mM imidazole. The fraction obtained (B) contained mitochondrial and nerve ending particles. Each preparation was appropriately diluted and the samples were quick frozen in liquid nitrogen and stored at $-20^\circ C$. until the ATPase assay. ATPase

activity was determined using the enzymatic or continuous procedure in which catalysts pyruvate kinase and lactic dehydrogenase were present, and NADH (reduced nicotinamide adenine dinucleotide phosphate) is oxidized to NAD (nicotinamide adenine dinucleotide phosphate) for spectrophotometric determination at 340 nm. The procedure is detailed in Pullman et al (9), and Fritz and Hamrick (10), and in Yap and Cutkomp (11). Mg^{2+} ATPase activity was measured when one mM ouabain was in the reaction mixture. Ouabain is a cardiac glycoside which specifically inhibits the Na^+ - K^+ ATPase. 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%) per ml reaction mixture. Oligomycin insensitive Mg^{2+} ATPase is generally more prominent in microsomes (endoplasmic reticulum and plasma membrane) and is present to a small extent in mitochondria.

Four PCB preparations (Aroclor 1221, Aroclor 1242, Aroclor 1254 and Aroclor 1268) and a polychlorinated terphenyl, Aroclor 5460 were investigated for effects on the ATPase systems. Aroclors 1221, 1242 and 1254 were dissolved in ethanol, and Aroclors 1268 and 5460, in acetone, and added to the reaction mixture by slowly releasing the solution from a Hamilton micro-syringe under the liquid surface of the vortex of a rapidly-stirred reaction mixture. This prevented sedimentation and promoted the formation of colloidal mixtures. Reaction temperature was $37^\circ C$, except for muscle homogenates, where the temperature was maintained at $27^\circ C$. The amount of solvent added (never exceeding 5 μl) with the PCBs had no effect on the ATPase

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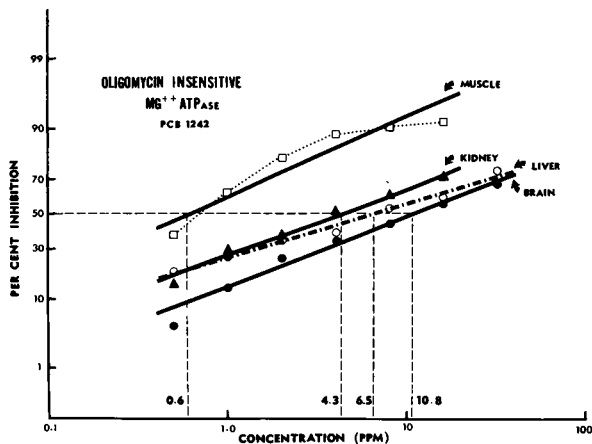


FIGURE 1. Aroclor 1242 inhibition (Probit Units) of oligomycin-insensitive Mg^{2+} ATPase from fish homogenates. ATPase activity of untreated in $\mu\text{moles } P_i \text{ mg}^{-1} \text{ protein hr}^{-1}$ for brain 14.5 ± 0.03 ; kidney 16.1 ± 0.02 ; liver 10.4 ± 0.5 ; muscle 46.2 ± 9.6 . Unbroken lines computed; dotted line connects actual points.

activities. Four tissues of fish (brain, muscle, liver and kidney) were selected as enzyme sources.

Results and Discussion

Those PCBs tested in vitro were generally most effective as inhibitors of oligomycin-insensitive Mg^{2+} ATPase, particularly from muscle homogenates. Aroclors 1242 and 1254, which

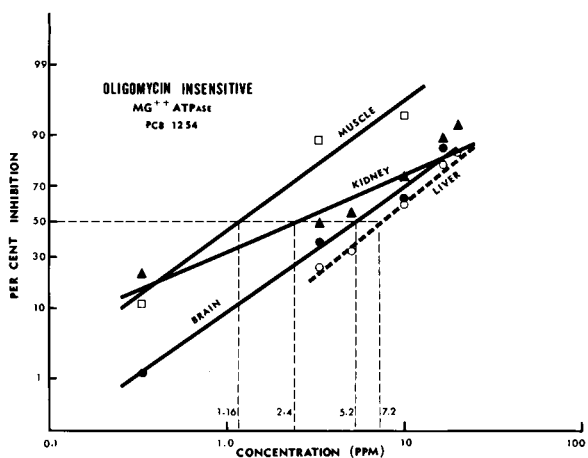


FIGURE 2. Aroclor 1254 inhibition (Probit Units) of oligomycin-insensitive Mg^{2+} ATPase from fish homogenates. ATPase activity of untreated in $\mu\text{moles } P_i \text{ mg}^{-1} \text{ protein hr}^{-1}$ for brain 14.2 ± 0.4 ; kidney 17.3 ± 3.0 ; liver 10.0 ± 0.05 ; muscle 63.7 ± 5.5 .

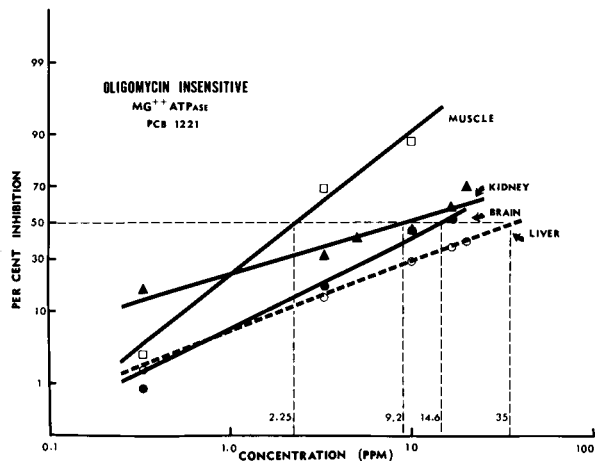


FIGURE 3. Aroclor 1221 inhibition (Probit Units) of oligomycin-insensitive Mg^{2+} ATPase from fish homogenates. ATPase activity of untreated in $\mu\text{moles } P_i \text{ mg}^{-1} \text{ protein hr}^{-1}$ for brain 14.2 ± 0.4 ; kidney 17.3 ± 3.0 ; liver 10.7 ± 0.07 ; muscle 63.7 ± 5.5 .

are in the intermediate range of chlorination, were more effective than 1221 and 1268. Compared at 50% inhibition, the values were 0.6 ppm for Aroclor 1242 (Fig. 1), 1.2 ppm for Aroclor 1254 (Fig. 2) and 2.3 ppm for Aroclor 1221 (Fig. 3), the smallest value indicating the most effective. Mg^{2+} ATPase from mitochondria (oligomycin-sensitive) was not affected as much by the PCBs as by DDT-type compounds. The I_{50} for Aroclor 1242 was 2.0 ppm on mitochondrial Mg^{2+} ATPase from muscle, 4.0 ppm from kidney (Table 1 and ref. 2) and 3.5 ppm from brain

Table 1. Percent inhibition of mitochondrial Mg^{2+} ATPase activity by AROCLOR 1242 in fish kidney and muscle homogenates.

Concentration (ppm)	Per cent inhibition*	
	Kidney	Muscle
0.5	+13.7	+26.5
1.0	+10.4	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	27.2	5.6
Sp. Act \pm S.E.	± 2.5	± 1.1

* (+) Values represent the per cent enzyme activation.

Table 2. Fifty percent inhibition values (in ppm) of ATPases determined from homogenates of blue gill fish brain. Summarized from references 2 and 8. (Lower values denote greater effectiveness.)

ATPases	In vitro tests	
	DDT	Aroclor 1242
Total Mg ²⁺	15.0	8.3
Mitochondrial Mg ²⁺	0.5	3.5-5.6 (max. 64%)
Oligomycin-insensitive Mg ²⁺	>7.5 (30%)	10.8
Na ⁺ -K ⁺	>15.0 (30%)	18.5

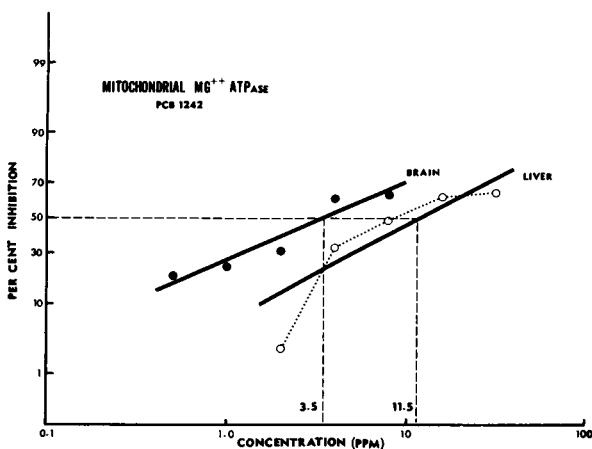


FIGURE 4. Aroclor 1242 inhibition (Probit Units) of mitochondrial Mg²⁺ ATPase from fish homogenates. ATPase activity of untreated in $\mu\text{moles P}_i \text{ mg}^{-1} \text{ protein hr}^{-1}$ for brain 13.2 ± 1.1 ; liver 29.1 ± 0.004 . Unbroken lines computed; dotted line connects actual points.

Table 3. Percent inhibition of ATPases in homogenates of mitochondrial fraction of kidney of 4-month Aroclor 1242 treated fat-head minnows.

ppb of 1242	ATPases		
	Mg ²⁺ ATPase		
	Mitochondrial	Oligomycin-insensitive	Na ⁺ -K ⁺ ATPase
0.9	56	23	21
2.8	75	21	36
8.3	45	35	18

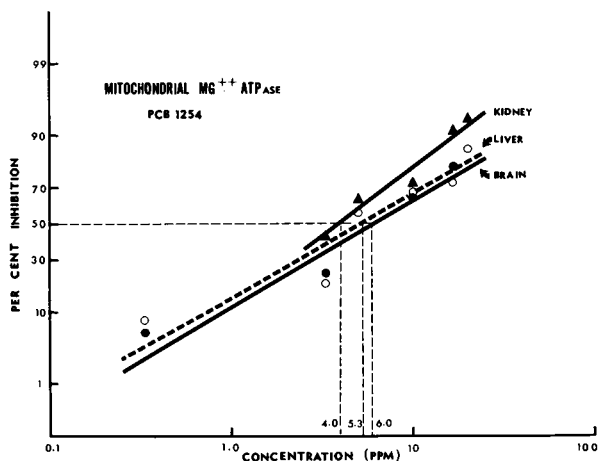


FIGURE 5. Aroclor 1254 inhibition (Probit Units) of mitochondrial Mg²⁺ ATPase from fish homogenates. ATPase activity of untreated in $\mu\text{moles P}_i \text{ mg}^{-1} \text{ protein hr}^{-1}$ for brain 13.5 ± 0.35 ; kidney 28.6 ± 0.85 ; liver 39.0 ± 1.25 .

(Fig. 3). The I₅₀ for DDT was 0.5 ppm in brain (Table 2). In contrast to the effective inhibitors, two of the poorest inhibitors, Aroclor 1221 and Aroclor 1268 (see ref. 2), showed some stimulation of Mg²⁺ATPase.

Na⁺-K⁺ATPase from fish brain homogenates was inhibited by Aroclor 1242, but the concentration required was about 5X that for mitochondrial Mg²⁺ATPase. Aroclor 1242 is compared with DDT in Table 2, showing this PCB to be a better inhibitor of Na⁺-K⁺ATPase, but poorer on mitochondrial Mg²⁺ATPase.

The in vivo studies were conducted with fat-head minnows, *Pimephales promelas*, which were chronically exposed to Aroclor 1242 and 1254 for four months following hatching. Comparisons of ATPase activity showed considerable inhibition and some stimulation over a concentration range in which fish were exposed to 0.31, 0.93, 2.8 and 8.3 ppb. Aroclor 1242 compared with Aroclor 1254 showed the greatest inhibition of Mg²⁺ATPases, as it had in in vitro studies. The inhibition in the chronic in vivo study was greatest on mitochondrial Mg²⁺ATPase from kidney (Table 3). Inhibition of mitochondrial Mg²⁺-ATPase was also greatest in brain and liver homogenates. Comparisons with muscle homogenates were not feasible in the small, chronically exposed minnows. The result contrasted with the inhibitory effect on oligomycin-insensitive

Mg²⁺ATPase which occurred in vitro. A maximum inhibition by Aroclor 1242 in treated fish (in vivo) was 75% on mitochondrial Mg²⁺ATPase and 35% on Na⁺-K⁺ATPase. In contrast, Aroclor 1254 did not inhibit mitochondrial Mg²⁺ATPase in kidney but gave 30% Na⁺-K⁺ATPase inhibition in the group of fish treated with 8.3 ppb (3). Results from brain tissues showed more consistent inhibitory effects than did other tissues.

Overall, PCB-treated fish showed more variation of enzymic effects than in vitro determinations. Thus, although the precision of the results was greater in vitro than in vivo, both approaches seem desirable for assessing the mode of action. In both cases the greatest effects were on Mg²⁺-ATPase, with lesser effects on Na⁺-K⁺ATPase. Of the Aroclors studied, those in the intermediate range of chlorination appeared to have the greatest inhibitory effect on the ATPase system. Aroclor 1242 was a particularly good inhibitor.

Conclusions

The ATPase enzyme system in fish is adversely affected by all PCBs studied. Mg²⁺ATPase is inhibited to the greatest extent in muscle, but effects in kidney, brain, and liver also occurred. Fifty per cent inhibition occurred with less than 1 ppm up to a few ppm. The greatest effects, in vitro, were on oligomycin-insensitive Mg²⁺-ATPase, but on oligomycin-sensitive (mitochondrial) Mg²⁺ATPase in vivo (chronically-exposed fish). The most effective PCB studied, Aroclor 1242, required a somewhat higher concentration than DDT in comparative in vitro studies.

Acknowledgements

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