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* and *in vivo* effects of PCBs on the ATPase enzyme system. We have found some effects which are similar, yet distinctive, to chemically-related compounds of the DDT type. In earlier research we have shown inhibition of Mg^{2+}ATPase and Na^{+}-K^{+}ATPase.

Tissues from blue gill fish, *Lepomis machrochirus*, were dissected, homogenized, and fractionated by centrifuging at 13,000 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° C until the ATPase assay. ATPase activity was determined using the enzymatic or continuous procedure in which catalysts pyruvate kinase and lactic dehydrogenase were used and NADH is oxidized to NAD for spectrophotometric determination at 340 nm. 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. 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.

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Four PCB preparations (Aroclor 1221, Aroclor 1242, Aroclor 1254 and Aroclor 1268) and a polychlorinated terphenyl, Aroclor 5460 were used in these investigations. 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. Reaction temperature was 37°C, except for muscle where the temperature was maintained at 27°C. The amount of solvent added with the PCBs had no effect on the ATPase activities.

Four tissues of fish (brain, muscle, liver and kidney) were used to test the effects of the PCBs. Oligomycin - insensitive Mg^{2+}ATPase from muscle homogenate was most sensitive in in vitro tests. Aroclors 1242 and 1254, 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 and 1.2 for Aroclor 1254. Mg^{2+}ATPase from mitochondria (oligomycin-sensitive) was not as sensitive to the PCBs as to DDT-type compounds. The I_{50} for Aroclor 1242 was 2.0 ppm on mitochondrial 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 (>6x) that for Mg^{2+}ATPase.

The in vivo studies were conducted with fat head minnows, Pimephales promelas which were chronically exposed to Aroclor 1242 and 1254 for 4 months. Comparisons of ATPase activity showed some 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 showed the greatest inhibition of Mg^{2+}ATPases

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both in vitro and in vivo and its greatest effect was in kidney tissue. A maximum inhibition of Aroclor 1242 in treated fish (in vivo) was 75% on mitochondrial Mg^{2+}ATPase and 36% on Na^{+}-K^{+}ATPase. In contrast, Aroclor 1254 did not inhibit mitochondrial Mg^{2+}ATPase in kidney, but in one group treated with 8.3 ppm inhibited 30% Na^{+}-K^{+}ATPase in kidney. Somewhat more consistent results showing inhibition were obtained from brain tissues.

Overall, PCB treated fish showed more variation of effects on the ATPases, therefore the precision of the results obtained in vitro could not be duplicated in in vivo studies. However, the greatest effects were on Mg^{2+}ATPase, with lesser effects on other components of the ATPase system. Aroclors in the intermediate range of chlorination appeared to have the greatest inhibitory effect, especially Aroclor 1242.

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