Bull. Environm. Contam. Toxicol. 20,769-777 (1978)

In vivo Effect on ATPase in Certain Tissues of *Labeo rohita* and *Saccobranchus fossilis*, Following Chronic Chlordane Intoxication

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Voluminous information is available on the residual toxicity and the other environmental impacts of the pesticides, but there is little information available on the mechanism of toxic action of the organochlorine pesticides especially on studies concerning active transport across cellular membranes. One of the initial efforts to explain the action of DDT in active transport was made by MATSUMURA et al. (1969). By using differential centrifugation techniques, they isolated various nerve components of rat brain and localized the source of ATPase sensitive to DDT. Their results indicated that (Na^+, K^+, Mg^{++}) dependent ATPase in the rat brain is specifically sensitive to DDT. They suggested that DDT is casually related to disruption of ion transport mechanisms in the nervous system in vivo. Using similar methods, JANICKI AND KINTER (1971) conclusively showed that DDT inhibits (Na^+, K^+, Mg^{++}) dependent ATPases engaged in active sodium transport functioning to maintain tissue osmolarity.

In long term exposure of organochlorine compounds in vivo, (Na^+, K^+, Mg^{++}) dependent ATPases were inhibited in several fish species (KOCH et al. 1972, DESAIAH et al. 1975). They also noted that in some cases, notably at the lower concentrations of DDT, erratic stimulation occurred. However, stimulation was most pronounced in kidney and liver tissues.

Therefore, the purpose of this study was to determine the effect of various concentrations of chlordane upon the ATPase system in the brain, gill, liver and kidney of two fresh water teleosts, Labeo rohita and Saccobranchus fossilis following chronic chlordane intoxication after 30 and 60 days' treatment.

Materials and Methods

The fishes, Labeo rohita and Saccobranchus fossilis known as 'Rohu' and 'Singli', respectively in vernacular Hindi language belong to the order Cypriniformes and family Cyprinidae and Saccobranchidae respectively. These are easily available in the rivers and ponds of this

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Communication No. 48.

0007-4861/78/0020-0769 $01.80

region. The size of Saccobranchus fossilis selected varies from 150-220 mm (average 190 mm) and weight from 45-72 gm (average 60 gm) while in Labeo rohita, the size varies from 130-165 mm (average 150 mm) and weight varies from 100-110 gm (average 105 gm). The pesticide chlordane (1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene) made by M/s Rallis India Ltd., Bombay, and available in 20% emulsifiable concentration was used for the present study.

After the normal process of acclimatization and washing with 1% light $KMnO_4$ solution so as to avoid the possibility of any infection, the fishes were transferred into the experimental tanks. The concentrations of experimental pesticide were made by adopting the dilution technique (STANDARD METHODS, 13th edition, 1971). The sublethal concentrations of 1/2, 1/3 and 1/6 of the TL_{50} values for Labeo rohita and Saccobranchus fossilis as obtained by VERMA et al. (1977, 1978) were taken for long term exposure of both the fishes. The fishes were fed with an artificial diet and the water was renewed at every five days' interval.

Activity of ATPase was determined in fish tissues by measuring the amount of inorganic phosphate produced when adenosine triphosphate was converted to adenosine diphosphate.

The fish tissues were dissected out and weighed. Each tissue sample was transferred to a cold solution containing 0.25 M sucrose, 0.005 M disodium ethylene diamine tetracetic acid, and 0.003 M histidine buffer (pH 7.4 to yield a 5% W/V concentration). Tissue were homogenized in a ground glass homogenizer in ice. Activity of (Na^+, K^+, Mg^{++}) dependent ATPase was determined at 20-24°C. Glassware was washed immediately after each determination with distilled deionized water and with Fiske and SubbaRow reagents. After each fifth determination, the glassware was washed with hot 1ON HCl. A 0.2 ml aliquot of the tissue homogenate was added to 4.25 ml of the incubation media containing 20 mM histidine buffer (pH 7.4), 100 mM NaCl, and 20 mM KCl. Samples were assayed in triplicate. The reaction was initiated by addition of 50 µL of 100 mM Na_2 ATP and 100 mM MgCl and continued agitation for 30 min at 24°C. ATPase activity was terminated by the addition of 1.0 ml of ice cold 30% trichloroacetic acid. Samples were then transferred for 30 min to a refrigerator at 5°C to allow complete precipitation of the homogenate proteins. The precipitate was sedimented in Remi clinical centrifuge at 3,000 rpm for 3 min. Total ATPase activity was measured with Na^+, K^+, Mg^{++} in the reaction mixture. Mg^{++} ATPase activity was measured

using 1 mM ouabain, a cardiac glycocide, in the reaction mixture, the later being a specific inhibitor of Na^+, K^+ ATPase (McILWAIN 1963). Na^+, K^+ ATPase activity is total activity minus the Mg^{++} ATPase activity.

Inorganic phosphate produced as a result of the cleavage of ATP to ADP was measured by the method of FISKE AND SUBBAROW (1925) as modified by BARTLETT (1958). Color development proceeded at room temperature for 10 min. Protein was determined by the procedure developed by LOWRY et al. (1951), using a spectronic-20 colorimeter.

Results and Discussion

According to the several theories of active transport, ATPase is specifically required for the transport of ions against concentration gradient and across membranes. The mechanism of the action of chlordane on ATPase system may be due to the uncoupling of oxidative phosphorylation which causes a depletion in the phosphorylation product-ATP. This reduction in available free phosphate would be directly proportional to the reduction of total ATP produced.

The sensitivity of ATPases from brain, gill, liver and kidney of Labeo rohita and Saccobranchus fossilis after chronic chlordane intoxication was determined in vivo and the results are tabulated in tables I to IV. The inhibition of total, Mg^{++} and Na^+, K^+ ATPases by chlordane were quite similar to each other and there was an increased inhibition with increased concentration of the toxicant (Table I-IV). DESAIAH AND KOCH (1975) observed that the inhibition of Na^+, K^+ ATPase by Kepone^R and DCPD was quite similar and there was an increased inhibition with increased concentration of the toxicant. CUTKOMP et al. (1971a) also observed similar response with dicofol (Kelthane^R) on blue gill brain Na^+, K^+ ATPase. They also showed that a number of other organochlorines which inhibited Na^+, K^+ ATPase did not show such a progressive response.

In the present investigation, authors observed that at the lowest sublethal concentration (1/6th TL_{50}) in both the fishes, insignificant stimulation was observed in all the three ATPases. However, in 60 days treated Saccobranchus fossilis liver Mg^{++} ATPase showed an insignificant inhibition. KOCH et al. (1972) further noted stimulation in all the ATPases at the lower concentrations of polychlorinated biphenyls in the brain, liver and kidney of Pimephales promelas following a four month exposure period. DESAIAH et al. (1975) observed an activation of Na^+, K^+ ATPase and oligomycin insensitive Mg^{++} ATPase in brain tissue homogenate of Pimephales promelas following chronic DDT administration.

TABLE I

In vivo effect on ATPase in brain and gill of Labeo rohita after chronic chlordane treatment.

Tissue	Concentration mg/l	Specific activity = μ moles of inorganic phosphate liberated/mg of protein/hr.					
		30 days			60 days		
		(Na^+, K^+, Mg^{++}ATPase)	Mg^{++}ATPase	Na^+, K^+ATPase	(Na^+, K^+, Mg^{++}ATPase)	Mg^{++}ATPase	Na^+, K^+ATPase
Brain	None	34.23 ± 1.95[a] (-)[b]	14.78±1.72 (-)	19.45±1.51 (-)	32.56 ± 2.12 (-)	14.24±2.04 (-)	18.32±2.25 (-)
	0.09	14.39 ± 1.48 (57.96)*	5.91±1.68 (59.98)*	8.48±1.95 (56.40)*	13.02 ± 2.02 (60.01)*	5.34±0.31 (62.45)*	7.68±0.51 (58.05)*
	0.06	30.50 ± 1.42 (10.90)	10.32±1.42 (30.16)	20.18±0.92 (+3.78)	20.85 ± 1.90 (35.96)*	9.16±0.95 (35.54)*	11.69±1.82 (36.20)
	0.03	37.84 ± 0.95 (+10.55)	15.98±1.20 (+8.12)	21.86±0.75 (+12.42)	36.62 ± 1.88 (+12.46)	16.31±1.54 (+14.54)	20.31±1.95 (+10.88)
Gill	None	23.56 ± 2.20 (-)	12.42±1.51 (-)	11.14±1.31 (-)	24.04±1.95 (-)	13.54±1.52 (-)	10.50±1.90 (-)
	0.09	11.43 ± 1.86 (51.49)*	6.15±0.32 (50.42)*	5.28±0.71 (52.59)*	10.20±1.52 (57.57)*	5.39±0.72 (60.12)*	4.81±0.96 (54.18)*
	0.06	22.11 ± 1.95 (6.15)	13.04±1.12 (+4.98)	9.07±0.98 (18.54)	18.59±1.95 (22.67)	10.35±1.14 (23.52)	8.24±1.12 (21.48)
	0.03	25.50 ± 1.71 (+8.23)	13.30±1.20 (+7.12)	12.20±1.32 (+9.54)	26.63±1.38 (+10.77)	14.78±1.62 (+9.20)	11.85±1.71 (+12.88)

* Values are significant when determined by Fisher's 't' test at 95% level of confidence.
a Values expressed as mean ± mean standard error.
b Values in parenthesis indicate percent inhibition.
\+ Indicate % stimulation.

TABLE II

In vivo effect on ATPase in liver and kidney of *Labeo rohita* after chronic chlordane treatment.

Tissue	Concentration mg/l	Specific activity = μ moles of inorganic phosphate liberated/mg of protein/hr.					
		30 days			60 days		
		(Na^+, K^+, Mg^{++}ATPase)	Mg^{++}ATPase	Na^+, K^+ATPase	(Na^+, K^+, Mg^{++}ATPase)	Mg^{++}ATPase	Na^+, K^+ATPase
Liver	None	18.00 ± 2.06[a] (-)[b]	12.78±1.84 (-)	5.22±0.31 (-)	19.05 ± 1.82 (-)	12.98±0.92 (-)	6.07 ±0.48 (-)
	0.09	10.72 ± 1.71 (40.44)*	7.31±0.52 (42.82)*	3.41±0.22 (34.54)*	10.58 ± 0.95 (44.46)*	6.72±0.72 (48.20)*	3.86±0.21 (36.42)*
	0.06	17.61 ± 0.98 (2.16)	13.45±0.32 (+5.24)	4.16±0.52 (20.14)	13.10 ± 1.20 (31.23)*	8.82±0.75 (32.08)*	4.28±0.32 (29.54)*
	0.03	19.96 ± 0.95 (+10.88)	14.31±1.01 (+12.04)	5.65±0.71 (+8.24)	20.74 ± 1.52 (+8.87)	14.28±1.71 (+10.09)	6.46±0.78 (+6.40)
Kidney	None	35.72 ± 2.88 (-)	16.20±1.86 (-)	19.52±1.48 (-)	34.98 ± 2.42 (-)	15.82±1.48 (-)	19.16±1.70 (-)
	0.09	18.54 ± 1.72 (48.10)*	7.81±0.92 (51.78)*	10.73±1.42 (45.02)*	17.38 ± 1.90 (50.03)*	7.70±0.48 (51.28)*	9.68±0.42 (49.45)*
	0.06	30.42 ± 1.44 (14.84)	13.84±1.32 (14.54)	16.58±1.52 (15.05)	27.10 ± 1.98 (22.53)*	13.40±1.32 (15.28)	13.70±0.96 (28.48)*
	0.03	40.75 ± 2.02 (+14.08)	19.13±1.71 (+18.12)	21.62±1.98 (+10.78)	38.74± 2.12 (+10.74)	17.78±1.42 (+12.42)	20.96±1.89 (+9.42)

* Values are significant when determined by Fisher's 't' test at 95% level of confidence.
a Values expressed as mean ± mean standard error.
b Values in parenthesis indicate percent inhibition.
\+ Indicate % stimulation.

TABLE III

In vivo effect on ATPase in brain and gill of *Saccobranchus fossilis* after chronic chlordane treatment.

Tissue	Concentration mg/l	Specific activity = μ moles of inorganic phosphate liberated/mg of protein/hr.					
		30 days			60 days		
		(Na^+, K^+, Mg^{++} ATPase)	Mg^{++} ATPase	Na^+, K^+ ATPase	(Na^+, K^+, Mg^{++} ATPase)	Mg^{++} ATPase	Na^+, K^+ ATPase
Brain	None	32.78 ± 2.70[a] (-)[b]	11.46±1.72 (-)	21.32±1.78 (-)	30.64 ± 2.02 (-)	13.98±1.42 (-)	16.66±1.42 (-)
	0.21	15.72 ± 1.92 (52.04)*	5.67±0.78 (50.48)*	10.05±1.72 (52.86)*	12.91 ± 0.98 (57.86)*	6.09±0.21 (56.42)*	6.82±0.31 (59.05)*
	0.14	27.13 ± 1.78 (17.24)	9.38±0.72 (18.16)	17.75±1.31 (14.72)	22.33 ± 1.95 (27.12)*	10.01±0.31 (28.42)*	12.32±0.98 (26.04)*
	0.07	36.52 ± 1.95 (+11.91)	12.67±0.48 (10.56)	23.85±1.02 (+11.87)	33.46 ± 2.04 (+9.20)	14.66±0.95 (+4.88)	18.80±1.12 (+12.84)
Gill	None	22.08 ± 1.42 (-)	13.57±0.95 (-)	8.51±0.58 (-)	23.56 ± 1.48 (-)	12.42±1.10 (-)	11.14±0.62 (-)
	0.21	12.98 ± 1.02 (41.21)*	8.09±0.72 (40.35)*	4.89±0.42 (42.48)*	11.41 ± 0.98 (51.57)*	6.13±0.95 (50.62)*	5.28±0.42 (52.62)*
	0.14	17.71 ± 1.12 (19.79)	10.72±0.58 (20.98)*	6.99±0.32 (17.84)	18.25 ± 1.41 (22.54)*	9.39±0.78 (24.42)	8.86±0.52 (20.44)*
	0.07	23.05 ± 1.12 (+4.39)	14.25±0.72 (+4.98)	8.80±0.28 (+3.42)	25.27 ± 1.72 (+7.26)	13.19±0.68 (+6.20)	12.08±0.65 (+8.40)

* Values are significant when determined by Fisher's 't' test at 95% level of confidence.
a Values expressed as mean ± mean standard error.
b Values in parenthesis indicate percent inhibition.
+ Indicate % stimulation.

TABLE IV

In vivo effect on ATPase in liver and kidney of Saccobranchus fossilis after chronic chlordane treatment.

Tissue	Concentration mg/l	Specific activity = μ moles of inorganic phosphate liberated/mg of protein/hr.					
		30 days			60 days		
		$(Na^+, K^+, Mg^{++}$ATPase)	Mg^{++}ATPase	Na^+, K^+ATPase	$(Na^+, K^+, Mg^{++}$ATPase)	Mg^{++}ATPase	Na^+, K^+ATPase
Liver	None	17.48 ± 1.12[a] (−)[b]	10.08±0.95 (−)	7.40±0.78 (−)	18.04 ± 1.32 (−)	11.54±1.22 (−)	6.50±0.72 (−)
	0.21	11.40 ± 1.08 (34.78)*	6.23±0.72 (38.12)*	5.17±0.46 (30.16)*	10.41 ± 1.00 (42.29)*	5.87±0.98 (49.10)*	4.54±0.31 (30.12)*
	0.14	13.76 ± 1.04 (21.28)*	8.00±0.56 (20.54)	5.76±0.52 (22.16)	13.14 ± 1.02 (27.16)*	8.47±0.52 (26.56)	4.67±0.21 (28.22)
	0.07	19.00 ± 1.12 (+8.69)	11.14±0.78 (+10.54)	7.86±0.60 (+6.20)	18.03 ± 1.24 (0.06)	10.84±0.86 (06.08)	7.19±0.24 (+10.64)
Kidney	None	30.28 ± 2.12 (−)	14.20±1.05 (−)	16.08±1.01 (−)	28.14 ± 1.72 (−)	12.42±1.05 (−)	15.72±1.42 (−)
	0.21	17.48 ± 1.18 (42.27)*	7.92±0.90 (44.24)*	9.56±0.95 (40.54)*	16.74 ± 1.30 (40.51)*	7.65±0.98 (38.40)*	9.09±1.08 (42.18)*
	0.14	26.69 ± 1.78 (11.86)	12.68±1.42 (10.72)	14.01±0.78 (12.86)	24.49 ± 1.52 (12.97)	12.84±1.05 (+3.42)	11.65±0.98 (25.87)
	0.07	32.04 ± 1.84 (+5.81)	14.90±0.56 (+4.98)	17.14±0.99 (+6.58)	31.67 ± 1.78 (+12.54)	14.36±1.06 (+15.62)	17.31±1.16 (+10.12)

* Values are significant when determined by Fisher's 't' test at 95% level of confidence.
a Values expressed as mean ± mean standard error.
b Values in parenthesis indicate percent inhibition.
\+ Indicate % stimulation.

Authors in the present investigation observed inhibition of all the three ATPases in both fish Labeo rohita and Saccobranchus fossilis in all tissues, following chronic chlordane treatment. However, inhibition of ATPases in all the tissues of Labeo rohita was found higher as compared to Saccobranchus fossilis, indicating more susceptibility of the former to chlordane. Authors also observed an insignificant activation of gill and liver Mg^{++}ATPase and brain Na^{+}, K^{+} ATPase of 30 days treated Labeo rohita with 0.06 mg/l of chlordane. Authors, also observed that chlordane was equally effective in its inhibitory action on both Na^{+}, K^{+} and Mg^{++}ATPase activities. KOCH (1969/70) also observed the similar behaviour of ATPases for chlordane. It was also observed that maximum inhibition of total ATPase was found in brain and gill, less in kidney and least in the liver of both the fishes.

Sensitivity of ATPase system to organochlorine pesticide both in vivo (DESAIAH et al. 1975) and in vitro (VERMA et al. 1978) has also been shown by KOCH (1969/70) and CUTKOMP et al. (1971). They also observed that different types of organochlorines exert different effects on three different ATPase activities. For example, DDT was more effective on the mitochondrial Mg^{++}ATPase activity (CUTKOMP et al. 1971, DESAIAH et al. 1974).Hence differential sensitivity of ATPases to pesticides can provide a good understanding on the action and effect of these pesticides on active transport mechanism involving ATPase system.

The observed action of chlordane in all tissues of the fish assayed might be related to the ability of the compound to alter the cellular membrane configuration by binding with the fat portion of the membrane. Since ATPase is a structural part of the membrane, the active site of the enzyme would be altered. Movement of substances by active transport would be blocked. If the uptake of chlordane by fish in natural environment reaches tissue concentration equal to those found in this study, the resulting ATPase inhibition may be sufficient to impair organ function.

Acknowledgements

Authors wish to express their thanks to the authorities of D.A.V. (P.G.) College, Muzaffarnagar, for providing necessary research facilities and to CSIR and UGC (New Delhi) for sanctioning the research project/ scheme of which the present work is a part.

References

APHA, AWWA, and WPCF, Standard methods for the examination of water and waste water 13th edition Washington U.S.A. (1971).

BARTLETT, G.R.: J. Biol. Chem. 324, 446 (1958).

CUTKOMP, L.K., H.H. YAP, E.V. VEA, and R.B. KOCH: Life Science, 10, 1201 (1971).

CUTKOMP, L.K., H.H. YAP, E.V. CHENG, and R.B. KOCH: Chem. Biol. Inter. 3, 439 (1971a).

DESAIAH, D., R.B. KOCH, L.K. CUTKOMP, and A. JARVINEN: Arch. Environ. Contam. & Toxicol. 3(1), (1974).

DESAIAH, D., and R.B. KOCH: Bull. Environ. Contam. & Toxicol. 13(2), (1975).

DESAIAH, D., L.K. CUTKOMP, and R.B. KOCH: Arch. Environ. Contam. & Toxicol. 3(2), (1975).

FISHER, R.A.: Statistical Methods for Research Workers ed. Oliver & Boyd, 11th ed. London (1950).

FISKE, C., and Y. SUBBAROW: J. Biol. Chem. 66, 375 (1925).

JANICKI, R.M., and W.B. KINTER: Nature(London) New Biol. 233, 148 (1971).

KOCH, R.B.: Chem. Biol. Inter. 1, 199 (1969/70).

KOCH, R.B., D. DESAIAH, H.H. YAP, and L.K. CUTKOMP: Bull. Environ. Contam. & Toxicol. 7, 87 (1972).

LOWRY, O.H., N.J. ROSEBROUGH, A.L. FARR, and R.J. RANDALL: J. Biol. Chem. 193, 265 (1951).

MATSUMURA, F., T.A. BRATKOWSKI, and K.C. PATIL: Bull. Environ. Contam. & Toxicol. 4(5), 262 (1969).

McILWAIN, H.: Chemical exploration of the brain, Amsterdam: Elsevier (1963).

VERMA, S.R., S.K. BANSAL, and R.C. DALELA: Indian J. Environ. Hlth. 19, 107 (1977).

VERMA, S.R., S.K. BANSAL, and R.C. DALELA: Arch. Environ. Contam. & Toxicol. 7(3), (1978).

VERMA, S.R., S.K. BANSAL, A.K. GUPTA, and R.C. DALELA: Environ. Res. (Communicated) (1978).