Bull. Environ. Contam. Toxicol. (1994) 53:733–739

Environmental Contamination and Toxicology

Hepatotoxic Effects of Hexachlorocyclohexane on Carbohydrate Metabolism of a Freshwater Fish *Channa punctatus* (Bloch)

D. S. Reddy, V. V. Ghanathay, S. L. N. Reddy, K. Shankariah

Department of Zoology, Osmania University, Hyderabad-500 007, A. P., India

Received: 30 April 1993/Accepted: 12 March 1994

In the developing countries recently a vast number of pesticides have been liberally used in agriculture to combat the pest menace to produce higher yield of crops. Among these pesticides HCH (1,2,3,4,5,6 hexachlorocyclohexane) is the most indiscriminately used insecticide affecting the nontarget organisms such as fish, crabs, etc. (Srinivas Reddy et al 1983). Due to the persistent nature of HCH in the environment, it undergoes biomagnification through food-chain thereby causing serious concern to the human health. Studies on the toxicological impact of HCH on the freshwater crabs indicated remarkable changes in the enzyme profiles of different metabolic segments (Geethanjali 1985). Reports reveal that organochlorides induce alterations in the carbohydrate metabolism (Bakthavasthalam and Srinivas Reddy 1983). Toxicological influence of HCH on the biochemical mechanisms concerned with carbohydrate metabolism is not yet completely understood. Hence an attempt is made to determine the sublethal toxic impact of HCH on the enzyme and metabolic profiles of carbohydrate metabolism in the liver of freshwater edible fish Channa punctatus.

MATERIALS AND METHODS

Channa punctatus (35 ± 5 gm) were collected from local fisheries and acclimatized to the laboratory conditions for a period of 15 d and fed with commercial fish food. Toxicity tests were conducted in aquaria containing 20 L of dechlorinated tap water with the following characteristics: temperature 26 ± 2°C; pH 7.2 - 7.4; alkalinity 102 mg/L; hardness of water 112 mg/L (as $CaCO_3$); dissolved oxygen 7.8-8.0 mg/L; carbon dioxide 2.08 mg/L. Technical grade HCH (96%) stock solution was prepared in acetone and mixed in water in required dilutions. To determine the LC_{50} value, the fish were exposed to six serial concentrations of HCH. Each concentration was repeated six times with parallel controls. The recorded mortality data were subjected to probit analysis. The LC_{50} value 5 mg/L for 15 d was determined (Finney 1964).

Correspondence to: S. L. N. Reddy

The fish were divided in two groups of 24 eacn. Group I served as control and group II was subsequently exposed to a sublethal concentration of HCH (1/3 of LC_{50}, i.e., 1.66 mg/L) for a period of 15 d. The toxicant and control water were renewed every 24 hr after feeding. The fish were starved a day prior to experimentation to reduce metabolic differences, if any, due to differential feeding. Six fish each from experimental and control group were sacrificed on day 1, 5, 10 and 15 of exposure. The liver tissue was isolated and transferred to deep-freezer and stored for 3 hr at 0^0C for biochemical analysis. All biochemicals were procured from Sigma Chemical Co., St. Louis,USA.

The glycogen and glucose were estimated by the method of Nicholas (1956). The glycogen was precipitated from 1 mL supernatant of 10% trichloroacetic acid extracts by the addition of 5 mL of absolute alcohol. The tubes were incubated at 37^0C for 3 hr. The glycogen precipitated was pelleted by centrifugation at 3000 rpm for 15 min. The residue was dissolved in a known volume of double distilled water and used as a source for glycogen while the supernatant (1 mL) was taken into another tube for the estimation of glucose. Both the supernatant and the residue were treated with 5 mL of freshly prepared anthrone reagent and boiled in a waterbath for 15 min and cooled to room temperature. The absorbance was read at 620 nm in a spectrophotometer against a reagent blank. The values were expressed as milligrams of glucose per gram wet weight of the tissue. Phosphorylase 'a' & 'ab' activity was assayed by the method of Cori et al (1955) by the determination of inorganic phosphate liberated from glucose-1-phosphate. The tissues were homogenized in aqueous medium containing sodium fluoride (0.1 M) and EDTA (0.037 M) of pH 6.5 and centrifuged at 2500 rpm for 15 min; the clear supernatant was used for enzyme assay. Lactate was estimated by the method of Barker and Summerson (1941). One mL supernatant of 5% trichloroacetic acid (TCA) tissue extract was processed with $CuSO_4$ and $Ca(OH)_2$. The resultant extract was treated with 0.1 mL of p-hydroxybiphenyl reagent and color development was read at 540 nm in spekol against reagent blank. Pyruvate was estimated by the method of Friedman and Hanger (1942). 10% homogenate of liver was prepared in 10% TCA and then centrifuged at 2500 rpm for 15 min. To 1 mL of TCA filtrate, 1.0 mL of 2,4-dinitrophenyl hydrazine (0.001 N HCl) was added and tubes were kept for incubation at room temperature for 37^0C. Color was extracted with 5.0 mL of 4 N NaOH. After 10 min the color was read at 545 nm in spekol against reagent blank. Assays of respiratory enzymes, lactate dehydrogenase, succinate dehydrogenase and malate dehydrogenase were carried out according to Nachlas et al (1960). The reaction mixture contained in 2 mL volume of 100 µmoles of phosphate buffer (7.4 pH), 2 µmoles of INT and 100 µmoles of specific substrate (sodium sucinate or sodium lactate or sodium malate) of pH 7.4. For SDH, LDH and MDH 0.1 µ mole of NAD was also added as coenzyme. NAD-NADP isocitrate dehydrogenase (ICDH) was assayed by the method of Korenberg and Pricer (1951). The incubation mixture in addition to above components contains 10 µ moles of $MgCl_2$ and 0.2

μmoles of NADP or NAD and DL-isocitrate as specific substrate. Glucose-6-phosphate dehydrogenase (G-6-PDH) was assayed by the method of Lohr (1974). The reaction in a volume of 2 mL contained 100 μmoles of triethanolamine buffer (pH 7.4), 4 μmoles of INT, 0.1 μmole of glucose -6-phosphate, 0.3 μmoles of NADP. The reaction of all dehydrogenases was initiated at 37°C by adding 0.5 mL of 5% sucrose homogenate and was stopped by the addition of 5 mL of glacial acetic acid and color was extracted in 5 mL of toluene. The extracted formazan was measured at 495 nm in spectrophotometer. The protein content in the enzyme source was estimated by the method of Lowry et al (1951) using bovine serum albumin as standard. The results were subjected to statistical analysis i.e. standard deviation and standard error. Student 't' test was used to compare the differences between control and experimental groups.

RESULTS AND DISCUSSION

The impact of HCH intoxication on glycogen, glucose, phosphorylase 'a' and 'ab', pyruvate, lactate, lactate dehydrogenase, glucose-6-phosphate dehydrogenase, succinate dehydrogenase and malate dehydrogenase levels in liver were investigated. The results presented in Tables 1 and 2 clearly indicate that the carbohydrate metabolism was significantly altered throughout the exposure period. The alterations were found to be time- dependent.

Glycogen decreased progressively throughout the exposure period (Table1). Decrease in the glycogen levels in liver suggests the possibility of glycogenolysis. Studies indicating such depletion in fish models (Mishra and Srivastava 1984) during organochlorine toxicity offers an excellent support to the decreasing levels of glycogen in the present study. As evidence to the glycogenolysis the activities of phosphorylase 'a' and 'ab' in the present study were found elevated throughout the exposure time. Endosulfan an organochlorine pesticide exhibited a similar response in freshwater crabs (Nagender Reddy et al 1991) suggesting the nonspecific action of organochlorine pesticides in inducing the glycogenolysis. Involvement of adrenal hormones for the breakdown of glycogen cannot be ruled out in this study. Furthermore, stressful situations in fish elicit a neuroendocrine response, which in turn induces disturbances in the carbohydrate metabolism (Nakano et al 1967).

Though glycogenolysis is well indicated during HCH intoxication in this study, the decrease in the glucose clearly suggests an imbalance between glycogenolysis and glycolysis. Increase in the activity of phosphorylase throughout the exposure period suggests the increased oxidation of glucose through glycolytic pathway. As evidence of this, glucose was found reduced throughout the exposure period. Depletion in the glucose could also be attributed to its sequestration into blood (Gopal et al 1980). A number of reports on mammalian models and fish models indicate glycogenolysis and hyperglycemia

Table 1. Activity of glycogen, glucose, phosphorylase 'a' and 'ab' and pyruvate, lactate levels in the liver of Channa punctatus exposed to sublethal concentration 1.66 mg/L of HCH (number of fish =6).

Parameters	Exposure time in days							
	1		5		10		15	
	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental
Glycogen	3.128 ±0.018	2.476 ±0.015 (-20.84)	3.161 ±0.011	2.393* ±0.015 (-24.29)	3.147 ±0.017	2.191* ±0.091 (-30.37)	3.059 ±0.019	1.831** ±0.014 (-40.14)
Glucose	5.455 ±0.013	4.288* ±0.014 (-21.39)	5.389 ±0.014	4.417* ±0.015 (-18.03)	5.422 ±0.018	4.892 ±0.017 (-10.93)	5.482 ±0.015	3.980** ±0.014 (-27.39)
Phosphorylase'a'	5.177 ±0.013	5.924 ±0.014 (+14.42)	5.179 ±0.015	6.436* ±0.016 (+24.27)	5.199 ±0.018	6.601*** ±0.016 (+26.96)	5.210 ±0.059	13.624** ±0.102 (+161.49)
Phosphorylase'ab'	10.281 ±0.069	11.217 ±0.087 (+9.10)	10.743 ±0.017	12.014* ±0.093 (+11.83)	10.012 ±0.032	11.852* ±0.048 (+18.38)	10.379 ±0.137	12.631** ±0.129 (+21.70)
Pyruvate	0.127 ±0.015	0.131 ±0.013 (+ 3.15)	0.131 ±0.015	0.135 ±0.020 (+3.05)	0.123 ±0.015	0.078*** ±0.016 (-36 58)	0.126 ±0.014	0.059** ±0.014 (-53.17)
Lactate	0.049 ±0.015	0.047 ±0.019 (-4.06)	0.051 ±0.015	0.085* ±0.010 (+66.66)	0.051 ±0.013	0.097*** ±0.013 (+90.19)	0.051 ±0.013	0.111** ±0.016 (+117.65)

Value indicated in parenthesis is percent change over controls. Mean values are expressed as μ moles of formazan/mg. protein/hour. Experimental value significantly different from control with statistical significance:* p <0.05; **<0.01 :*** < 0.001. ± SD is Standard deviation.

Table 2. Activity of lactate dehydrogenase, glucose-6-phosphate dehydrogenase, NAD and NADP-isocitrate dehydrogenase (ICDH), succinate dehydrogenase and malate dehydrogenase in the liver of Channa punctatus exposed to sublethal concentration 1.66 mg/L of HCH (number of fish = 6).

Parameters	Exposure time in days							
	1		5		10		15	
	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental
Lactate dehydrogenase	0.513 ±0.010	0.488* ±0.018 (- 8.38)	0.530 ±0.017	0.423* ±0.016 (-20.18)	0.523 ±0.016	0.316** ±0.013 (-39.57)	0.504 ±0.004	0.253 ±0.016 (- 49.80)
Glucose-6-P -dehydrogenase	5.286 ±0.014	5.296 ±0.014 (+12.11)	5.229 ±0.020	6.287** ±0.018 (+20.23)	5.259 ±0.017	6.831*** ±0.016 (+29.89)	5.281 ±0.019	7.004*** ±0.014 (+32.62)
NAD-Isocitrate dehydrogenase	4.079 ±0.037	4.923 ±0.093 (+20.69)	4.122 ±0.019	4.023 ±0.022 (-2.40)	4.143 ±0.073	3.020 ±0.019 (-27.10)	4.064 ± 0.017	2.332** ±0.019 (-42.61)
NADP-Isocitrate dehydrogenase	5.488 ±0.026	5.933 ±0.025 (+8.11)	5.557 ±0.014	6.207*** ±0.013 (+11.69)	5.292 ±0.022	6.025*** ±0.021 (+13.85)	5.362 ±0.012	6.256** ±0.022 (+16.67)
Succinate dehydrogenase	4.657 ±0.016	5.525 ±0.015 (+18.64)	4.674 ±0.023	3.850* ±0.132 (-17.63)	4.618 ±0.017	2.957*** ±0.015 (-35.97)	4.683 ±0.177	2.949 ±0.0.040 (-37.03)
Malate dehydrogenase	0.585 ±0.014	0.723 ±0.014 (+23.59)	0.607 ±0.021	0.568** ±0.017 (-6.43)	0.590 ±0.018	0.284** ±0.014 (-51.86)	0.614 ±0.019	0.269** ±0.018 (-56.18)

Value indicated in parenthesis is percent change over controls. Mean values are expressed as μ moles of formazan/mg. protein/hour. Experimental value significantly different from control with statistical significance:* p <0.05; **<0.01 :*** < 0.001. ± SD is Standard deviation.

by activation of the phosphorylase enzyme system during pesticide poisoning (Shaffi 1979). The drop in the glucose levels in the present study clearly deviates from the general observation.

Glycogen and glucose are converted to pyruvate through glycolytic mechanism. Results of lactate, pyruvate and lactate dehydrogenase (Table 2) clearly suggests the enhanced rates of glycolytic reactions during HCH intoxication. Signifcant reduction of pyruvate levels during advanced stages of toxicosis suggests its conversion to lactate, which is clearly evidenced in the accumulation of lactate throught the exposure period. Lactate accumulation is clearly reflected in the activity patterns of lactate dehydrogenase in the present study. Lactate and pyruvate levels form meaningful biochemical indices of oxygen debt (Huckabee 1958) and mitochondrial redox state and also indicate the possibility of gluconeogenesis. Progressive enhancement of glucose-6-phosphate dehydrogenase in the present study (Table 2) confirms the operation of gluconeogenesis to provide additional amount of glucose to augment energy demands due to the increased anoxic conditions in the fish.

All the oxidative enzymes, viz., NAD and NADP dependent isocitrate dehydrogenase and succinate and malate dehydrogenases in the present study exhibited an enhancement over the control during 1st day of exposure, indicating higher metabolic rates during the initial stages of HCH toxicosis. Such an enhancement in the oxidative enzymes could be attributed to the stress imposed by the toxicant on the fish. Observations recorded on the initial elevation in oxygen consumption in Colisa lalia after lindane intoxication (Ramalingam and Srinivas Reddy 1982) extends the supporting evidence to enhanced rates in the activities of oxidative enzymes during the early stages of toxicosis. Inhibition of oxidative enzymes such as SDH, MDH, ICDH during subsequent exposure span suggests the decline in the operation of TCA cycle. The decrease in the enzyme activity might be due to the close association of HCH with mitochondrial disorganization combined with an inhibition of oxidative enzymes. Reduction in the oxidative enzymes could also be attributed to the reduced supply of oxygen particularly due to the histopathological manifestations resulting in structural damage to the respiratory tissue gill during HCH poisoning in the same animal model (Ganathay 1990). NADP-isocitrate dehydrogenase activity, which was found progressively enhanced throughout the exposure in the present investigation, may be to provide the reduced NADP molecules to be utilized during HCH induced stress.

The results conclude that there a clear shift from aerobic metabolism to anaerobic metabolism as a major compensatory mechanism to combat the HCH toxicosis. The parameters studied can form meaningful biochemical indices of HCH poisoning.

Acknowledgments: Authors DSR, GANATHAY are grateful to CSIR, New Delhi, for providing a Fellowship.

REFERENCES

Bakthavasthalam R, Srinivas Reddy Y (1983) Intoxication effects of lindane (γ- BHC) on the carbohydrate metabolism in the climbing perch Anabas testudineus (Bloch). Pestic Biochem Physiol 20:340-346

Barker SB, Summerson WH (1941) The colorimetric determination of lactic acid in biological material. J Biol Chem 138:535-554.

Cori GT, Illingworth B, Keller PG (1955) Muscle phosphorylase. In: Colowick SP, Kaplan NO (ed) Methods in Enzymology, Academic Press, NewYork, p 200-209

Finney DJ (1964) Probit Analysis, 2nd ed. Cambridge University, London

Friedemann TE, Haugen GE (1942) Pyruvic acid I. Collection of blood for the determination of pyruvic acid and lactic acids. J Biol Chem 144 : 67-77.

Geethanjali K (1985) Effects of organochloride pesticide BHC on the physiology of edible fresh water crab Oziotelphusa senex senex. M.Phil Thesis, S.V. Univeristy, Tirupati, India

Gopal K, Anand M, Khanna N and Mishra D (1980) Endosulfan induced changes in blood glucose of catfish Clarias batrachus. J Adv Zool 2:68-74

Huckabee WF (1958) Relationship of pyruvate and lactate during anaerobic metabolism II exercise and formation of oxygen debt.J Clin Invest 37 : 255-259

Korenberg A, Pricer WF (1951) Di and Triphospho pyridine nucleotide isocitrate dehydrogenase in yeast. J Biol Chem 189:123-136

Lohr GW, Waller HD (1974) Glucose-6-phosphate dehydrogenase. In: Methods of Enzymatic analysis, by Bergmeyer 2nd Edn. Academic press, NewYork, p 636-646

Lowry OH, Rosembrough NJ, Farr AL, Randall RJ (1951) Protein estimation with the folin-phenol reagent. J Biol Chem 193:265-275

Mishra J, Srivastava AK (1984) Effects of chlorodane on the blood and tissue chemistry of a teleost fish Heteropneustes fossilis. Cell Mol Biol 30 : 519-523

Nachlas MM, Margulius SI, Seligman AM (1960) Sites of electron transfer to tetrazolium salts in the succinoxidase system. J Biol Chem 235 : 2739-2743

Nagender Reddy A, Venugopal NBRK, Reddy SLN (1991) In vivo effects of endosulfan on glycogen metabolism of freshwater field crab Barytelfusa guerini. Pestic Biochem Physiol 40:176-180

Nakano T, Tomlinson N (1967) Catecholamines and carbohydrate concentration in rainbow trout Salmo gairdneri in relation to physical disturbance. J Fish Res Bd Can 241:1701-1705

Nicholas V, Roe JH, Longley RW, Joseph HR (1956) The determination of glycogen in liver and muscle by use of anthrone reagent. J Biol Chem 220: 583-593

Ramalingam R, Srinivas Reddy Y (1982) Kinetics of dose response relationship in the bimodel respiration of Colisa lalia exposed to lindane. Water Res 16(1):1-6

Shaffi SA (1979) The acute toxicity of heptachlor for fresh water fishes. Toxicol Lett 4:31-38

Srinivas Reddy P, Bhagyalaxmi A, Rammurthi R (1983) In vivo acute physiological stress induced by HCH on the levels of glycogen and total carbohydrates in various tissues of Oziotelphusa senex senex the Indian rice field crab. Toxicol Lett 22:335-338

Srivastava AK, Mishra J (1982) Effects of lindane on carbohydrate metabolism and on blood chloride in Indian catfish Heteropneustes fossilis. Hydro Biol 24:175-180

Vinodghanathy V (1990) In vivo effects of BHC on some aspects of physiology and histopathology of fish Channa punctatus Ph.d thesis, Osmania University, Hyderabad, India