Bull. Environ. Contam. Toxicol. (2003) 70:118–123

DOI: 10.1007/s00128-002-0164-9

Bulletin of Environmental Contamination and Toxicology

Chromium Induced Alterations in Some Biochemical Profiles of the Indian Major Carp, *Labeo rohita* (Hamilton)

S. S. Vutukuru

Environmental Toxicology Laboratory, Centre for Environment, Jawaharlal Nehru Technological University, Mahaveer Marg, Hyderabad 500 028, Andhra Pradesh, India

Received: 19 December 2001/Accepted: 18 August 2002

Natural waters are often contaminated by untreated wastes of industrial, technological and agricultural origin containing various metallic compounds. Heavy metals due to their bio-accumulative and non-biodegradable properties constitute a core group of aquatic pollutants. Chromium particulates enter the aquatic medium through effluents discharged from tanneries, textiles, electroplating, metal finishing, mining, dyeing and printing industries, ceramic, photographic and pharmaceutical industries etc. They concentrate in the tissues of aquatic biota and are known to produce cumulative deleterious effects. In nature chromium occurs in divalent, trivalent and hexavalent forms. Hexavalent chromium predominates over the trivalent form in natural waters. Knowledge of acute toxicity of a xenobiotic often can be very helpful in predicting and preventing acute damage to aquatic life in receiving waters as well as in regulating toxic waste discharges. The 96h LC_{50} tests can be used to obtain toxicity data as rapidly and inexpensively as possible. A perusal of the available literature reveals that studies on the acute effects of hexavalent chromium on the biochemical constituents of fishes are scanty (Buhler *et al.* 1977, Arillo *et al.* 1982, Sastry and Sunitha, 1984 and Ambrose *et al.* 1994.)

Chromium was proved to be highly toxic to *Labeo rohita* and the harmful effects caused by chromium to the fish include depression in metabolic rate, histopathological and hematological alterations (Sesha Srinivas and Balaparameswara Rao, 1996, 1998, 1999). Hexavalent chromium potentially possesses toxicological, carcinogenic and mutagenic properties (Langard and Norseth, 1979). Heavy metals concentrated in the tissues of fish enter human beings through food chain and due to their cumulative action causes potential health hazards sometimes even lethal (Ui, 1972). The toxic effects may result from the bioconcentration of metals and their consequent binding with biologically active constituents of the body such as lipids, amino acids, enzymes and proteins (Passow *et al.* 1961). In this context, an attempt was made to investigate the acute effects of hexavalent chromium on some biochemical profiles of the Indian major carp, *Labeo rohita* since it is a widely cultured edible fish and also forms an important link in the aquatic food chain. Biochemical profiles in fish and other aquatic organisms under heavy metal stress serve as important bioindicators in the monitoring of aquatic environment.

MATERIALS AND METHODS

Labeo rohita were obtained from the Government Fish farm at Nidubrolu town (16^{o}. 5'N; 80^{o}.5'E) and healthy fish were acclimated in the laboratory for 5-7 days in plastic pools of 100 L capacity containing well-aerated unchlorinated ground water before they were used for experiments. The feeding and maintenance of the fish and the physico-chemical characteristics of water used for acclimation, controls and experimentation were given earlier (Sesha Srinivas and Balaparameswara Rao, 1996). Biochemical analysis was carried out on fingerlings of *Labeo rohita* (total length 5.9-6.5cm and total weight 2.5-3.1 g). Twenty fish in two batches of ten each were exposed separately for 24h and 96h to the 96hr LC_{50} concentration of chromium (39.40 mg/l) which was determined earlier (Sesha Srinivas and Balaparameswara Rao, 1996). Five fish were sacrificed both at the end of 24h and also at 96h, blotted dry and weighed. They were later dissected to isolate the whole liver (30-40 mg), muscle (250 mg) and whole gill (80-100 mg) tissues. The tissues were dried for 24hr in a hot air oven at 50^{o}C. The dry tissues were weighed to the nearest mg. The tissues of the control fishes (n=5) were also processed similarly for biochemical analysis. The biochemical parameters viz., glycogen, total lipid and total protein were analyzed by adopting standard protocols (Kemp *et al.* 1954; Pondey *et al.* 1963 and Lowry *et al.* 1951), respectively. The biochemical constituents were expressed as mg per gram dry weight of the tissue and only the arithmetic mean values (n = 5) are presented to express the results. The mean values of the control and the exposed fishes are compared following '*t*' test (Bailey, 1959).

RESULTS AND DISCUSSION

The glycogen, total lipid and total protein levels in liver, muscle and gill of control fish and of *Labeo rohita* exposed to the 96hr LC_{50} concentration of chromium for 24 and 96hrs were presented in Tables 1 and 2, respectively. It is clear from the results that there is a decline in different biochemical constituents. The glycogen concentration of the liver, muscle and gill of the exposed fishes was 19.16 mg, 5.72 mg and 3.48 mg per gram dry weight, respectively, showing a decrease of 8.80%, 23.32% and 15.74% from control at the end of 24h exposure (Table 1) and 7.70 mg, 2.26 mg and 1.39 mg per gram dry weight showing a decrease of 54.91%, 56.37% and 39.04% from control at the end of 96h, respectively (Table 2).

The results of the '*t*' test show that the decrease in the glycogen level from control is significant in liver and muscle except in gill at the end of 24h. This decrease was, however, highly significant in all three tissues at the end of 96h exposure.

The total lipid concentration of liver, muscle and gill of the exposed fishes was 52.71, 123.78 and 50.23 mg per gram dry weight of the tissue showing a significant decrease of 21.50%, 8.84% and 15.22% from control at the end of 24h exposure and 32.94 mg, 92.50 mg and 40.48 mg per gram dry weight showing a

significant decrease of 43.65%, 29.87% and 26.53% from control at the end of 96h, respectively. The total protein concentration of liver, muscle and gill was 120.71 mg, 272.50 mg and 195.55 mg per gram dry weight, respectively and it decreased by 6.10%, 0.81% and 3.81% from control, respectively, in fishes exposed for 24h.

Table 1. Effect of 96hr LC_{50} concentration of hexavalent chromium (39.40 mg/l) on total glycogen, total lipid and total protein concentration of liver, muscle and gill of *Labeo rohita* (mg per gram dry weight) at the end of 24hr exposure (n=5)

Bio-chemical Constituent	Tissue	Control $\overline{X} \pm$ S.D	Experiment $\overline{X} \pm$ S.D	Percent Change	*'t'* Value	Result
Glycogen	Liver	21.01±1.02	19.16±0.46	-8.80	5.347	P<0.05**
	Muscle	7.46±0.54	5.72±0.39	-23.32	5.058	P<0.05**
	Gill	4.13±0.16	3.48±0.48	-15.74	1.873	P>0.05#
Total Lipids	Liver	67.15±7.85	52.71±2.11	-21.50	4.122	P<0.05**
	Muscle	135.78±5.01	123.78±2.65	-8.84	4.463	P<0.05**
	Gill	59.25±6.50	50.23±3.64	-15.22	2.612	P<0.05*
Total Protein	Liver	128.55±12.90	120.71±5.81	-6.10	1.220	P>0.05#
	Muscle	274.70± 7.65	272.50± 7.43	-0.81	0.449	P>0.05#
	Gill	203.29±3.91	195.55±2.10	-3.91	1.750	P>0.05#

* Significant, ** Highly significant, # Not significant

Table 2. Effect of 96hr LC_{50} concentration of hexavalent chromium (39.40) mg/l) on glycogen, total lipid and total protein content of liver, muscle and gill of *Labeo rohita* (mg per gram dry weight) at the end of 96hr exposure (n=5)

Bio-chemical Constituent	Tissue	Control $\overline{X} \pm$ S.D	Experiment $\overline{X} \pm$ S.D	Percent Change	*'t'* Value	Result
Glycogen	Liver	17.08±1.25	7.70±0.95	-54.91	13.243	P<0.001**
	Muscle	5.18±0.17	2.26±0.27	-56.37	16.685	P<0.001**
	Gill	2.28±0.44	1.39±0.14	-39.04	4.363	P<0.001**
Total Lipids	Liver	58.46±3.16	32.94±1.98	-43.65	14.394	P<0.001**
	Muscle	131.89±1.31	92.50±4.64	-29.87	18.950	P<0.001**
	Gill	55.10±2.60	40.48±1.24	-26.53	11.200	P<0.001**
Total Protein	Liver	122.39±4.41	102.83±5.56	-15.98	5.138	P<0.001**
	Muscle	267.46±8.64	252.55±5.74	-5.58	2.993	P<0.05 *
	Gill	192.79±2.06	171.78±3.64	-10.90	13.598	P<0.001**

** Highly Significant, * Significant

The results of the present study indicated that the glycogen, total lipid and total protein concentration of liver, muscle and gill were depleted in *Labeo rohita* exposed to 96h LC_{50} concentration of chromium. A decrease in the glycogen content of fishes exposed to metallic stress was observed by many investigators (Quyyum and Shaffi, 1977, Gill and Pant, 1981, Radhakrishnaiah *et al.* 1992, Mary Chandravathy and Reddy, 1995 and James *et al.* 1995). The decrease in the glycogen concentration of the tissues of *Labeo rohita* may be due to its enhanced utilization since glycogen forms the immediate source of energy to meet energy demands under metallic stress. DeZwaan and Zandee (1973) stated that decrease in glycogen concentration might be due to the prevalence of hypoxic or anoxic conditions, which normally enhances glycogen utilization. Enhanced utilization of glycogen and its consequent depletion in tissues may be attributed to hypoxia since it increases carbohydrate consumption. Under hypoxic conditions, the animal derives its energy from anaerobic breakdown of glucose, which is available to the cells by the increased glycogenolysis (Mary Chandravathy and Reddy, 1995). Our studies on the respiration of *Labeo rohita* exposed to chromium have also shown decreased oxygen consumption indicating hypoxia (Sesha Srinivas and Balaparameswara Rao, 1999). Behavioral manifestations of acute toxicity like copious mucous secretion, surfacing and darting movements, darkening of skin on dorsal side, loss of scales and loss of equilibrium were observed in *Labeo rohita* under chromium stress (Sesha Srinivas and Balaparameswara Rao, 1996). Chromium induced hypoxia probably might have resulted in a shift to anaerobic glycolytic pathway by increased glycogenolysis. Depleted glycogen levels following chromium stress reported in *Cyprinus carpio communis* (Ambrose *et al.* 1994) under hypoxic conditions also supports this view. A consistent decrease in tissue glycogen reserves observed in this study suggests impaired glycogenesis. Further, the decline in glycogen might be partly due to its utilization in the formation of glycoproteins and glycolipids, which are essential constituents of various cells and other membranes.

Decrease in tissue lipid and proteins were also observed in *Labeo rohita* exposed to chromium. Earlier studies have also shown that lipid and protein concentration of vital organs like gills, liver, muscle and kidney depleted in fishes exposed to chromium (Arillo *et al.* 1982, Sastry and Sunitha, 1984 and Ambrose *et al.* 1994). The decrease in tissue lipid and proteins might be partly due to their utilization in cell repair and tissue organization with the formation of lipoproteins which are important cellular constituents of cell membranes and cell organelles present in cytoplasm (Harper, 1963). Damage to the gill tissue was also observed in *Labeo rohita* exposed to chromium (*Sesha Srinivas and Balaparameswara Rao, 1998*) and the decrease in the lipid concentration observed in the present study can also be attributed to its utilization in cell repair and tissue organization.

The depletion in tissue proteins of *Labeo rohita* may be due to impaired or low rate of protein synthesis under metallic stress as has been reported earlier (Syversen, 1981 and Prasanta Nanda and Milan Kumar Behera, 1996) or due to their utilization in the formation of mucoproteins which are eliminated in the form

of mucous. Further, direct and / or indirect utilization of proteins and lipids for energy needs was also reported (Nagai and Ikeda, 1971). Also, the utilization of proteins in cell repair and organization as causes of their depletion in the tissues cannot be ruled out. The present study showed that chromium is toxic to *Labeo rohita* and induced alterations at the biochemical level, more pronounced changes occurring at the end of 96h and thus it is time-dependent. Also, the metal induced alterations may probably effect the enzyme mediated biodefence mechanisms of the fish. Further studies are required to elucidate the impact of chromium on detoxifying enzymes for assessing the fish health.

Acknowledgments I thank Prof.M.Balaparameswara Rao, Director, Centre for Aquaculture Research and Education, Nagarjuna University for his valuable guidance and Dr.P.S.R.Anjaneyulu, Head, Department of Aquaculture, Nagarjuna University, Nagarjuna Nagar for his help and providing necessary facilities to carry out this work and also Dr.J.Venkateswara Rao, Scientist E1, Toxicology Unit, Biology Division, Indian Institute of Chemical Technology, Hyderabad, for his valuable suggestions. I also thank Prof.Y.Anjaneyulu, Head, Centre for Environment, Jawaharlal Nehru Technological University, Hyderabad for his guidance and help.

REFERENCES

Ambrose T, Cyril Arun Kumar L, Vincent S, Roselyn Lambert (1994) Biochemical responses of *Cyprinus carpio communis* to the toxicity of tannery effluent. J Ecobiol 3: 213-216

Arillo A, Margiocco C, Melodia F, Mensi P (1982) Biochemical effects of long-term exposure to chromium, cadmium and nickel on Rainbow trout, *Salmo gairdnerii.* Chemosphere 11: 47-58

Bailey NTJ (1959) Statistical Methods in Biology. The English Language Book Society, Great Briton

Buhler DR, Stokes RM, Caldwell RS (1977) Tissue accumulation and enzymatic effects of hexavalent chromium in *Salmo gairdnerii.* J Fish Res Bd Canada 34: 9- 18

DeZwaan A, Zandee DI (1973) Body distribution and seasonal changes in glycogen content of the common sea mussel, *Mytilus edulis.* Comp Biochem Physiol 43: 53-55

Gill TS, Pant JC (1981) Effect of Sub-lethal concentrations of mercury in a teleost, *Puntius conchonius*: Biochemical and hematological responses. Indian J Expt Biol 19: 571-573

Harper AH (1963) Review of Physiological Chemistry. Language Medical Publications Co Ltd Japan

James R, Sampath K Siva Kumar V, Babu S, Shanmuganandam P (1995) Toxic effects of copper and mercury on food intake, growth and proximate chemical composition in *Heteropneustes fossilis.* J Environ Biol 16: 1-6

Kemp A, Andienne JM, Kits Van Hejningen (1954) A colorimetric micromethod for the determination of glycogen. Biochem J 56: 640-648

Langard S, Norseth T (1979) Chromium In: Friberg L, Gunnar, FN , Vehinir. BV (ed) Hand Book on Toxicology of Metals. Elsevier, North-Holland, Biochemical press, Netherlands, p383-394

Lowry OH, Rosebrough NJ, Lewis Forr A, Randall RJ (1951) Protein measurement with the Folin-phenol reagent. Biochem J 193: 265-275

Mary Chandravathy V, Reddy SLN (1996) Lead nitrate exposure changes in carbohydrate of freshwater fish, *Anabas Scandens.* J Environ Biol 17: 75-79

Nagai M, Ikeda S (1971) Carbohydrate metabolism in Fish. I. Effects of starvation and dietary composition on the blood glucose level and hepatopancreatic glycogen and lipid contents in *Cyprinus carpio.* Bull Japan Soc Sci Fish 37: 404-409

Passow H, Rothestein A, Clarkson TW (1961) The General pharmacology of heavy metals. Pharmocol Rev 13:183-224

Pondey SV, Khan AP, Subramaniam TAV (1963) Microdetermination of lipids and serum fatty acids. Analyst Biochem 6: 120-125

Prasanta Nanda and Milan Kumar Behera (1996) Nickel induced changes in some hemato-biochemical parameters of a catfish, *Heteropneustes fossilis* (Bloch.). Environ Ecol 14: 82-85

Quyyam MA, Shaffi SA (1977) Changes in tissue glycogen in a freshwater cat fish, *Heteropneustes fossilis* due to mercury intoxication. Curr Sci 46:652-653

Radhakrishnaiah K, Venkataramana P, Suresh A, Sivaramakrishna B (1992) Effects of lethal and sub-lethal concentrations of copper on glycolysis in liver and muscle of freshwater teleost fish, *Labeo rohita* (Ham). Indian J Comp Animal Physiol 14: 30-33

Sesha Srinivas V, Balaparameswara Rao M (1996) Acute toxicity of chromium to the freshwater fish. *Labeo rohita* (Ham). Indian J Comp Animal Physiol 14: 30-32

Sesha Srinivas V, Balaparameswara Rao M (1998) Chromium induced alterations in the gill of the freshwater fish. *Labeo rohita* (Ham). Indian J Comp Animal Physiol (In press)

Sesha Srinivas V, Balaparameswara Rao M (1999) Chromium induced alterations in the oxygen consumption of the freshwater teleost fish, *Labeo rohita.* Pollut Res 18: 377-380

Standard Methods for the Examination of Water and Waste water (1995) American Public Health Association, American Water Works Association, Water Environment Federation, 19th Edition, Washington DC

Syversen TLM (1990) Effects of methyl mercury on protein synthesis *invitro.* Actal Pharmacol Toxicol 49: 422-426.

Ui J (1972) The changing chemistry of the oceans. Almquist and Wickesells, Stockholm.