Journal Home page : www.jeb.co.in ★ E-mail : editor@jeb.co.in

JEB
Journal of Environmental Biology

ISSN: 0254-8704
CODEN: JEBIDP

Acute effects of mercuric chloride on glycogen and protein content of Zebra fish, *Danio rerio*

S.S.Vutukuru* and Kalpana Basani

Environmental Biotechnology Division, Department of Biotechnology, Sreenidhi Institute of Science and Technology, Hyderabad-501 301, India

**Corresponding Author email : vssrinivas@sreenidhi.edu.in*

Publication Info

Paper received:
11 June 2011

Revised received:
17 December 2011

Re-revised received:
03 March 2012

Accepted:
17 March 2012

Abstract

Presence of mercury and other heavy metals above permissible levels in water bodies across the globe is posing a serious threat to aquatic biota and public health. Occurrence of mercury above the permissible limits in the aquatic ecosystem of Hyderabad city is well established. In this context, we carried out static- renewal bioassays on the zebra fish, *Danio rerio* exposed to different concentrations of mercuric chloride, and the 96-h median lethal concentration (LC_{50}) was found to be 0.077 mgl-1. Behavioral manifestations like loss of scales, hyper secretion of mucus, surfacing and darting movements, loss of balance, irregular swimming patterns were noticed in the fish exposed to 0.077 mgl-1. The present study also examined the toxic effects of mercuric chloride on vital biochemical constituent's total glycogen and total protein. Significant decrease ($p<0.001$) in glycogen and protein content of fish exposed to 0.077 mgl^{-1}

Key words

Danio rerio, Mercury toxicity, Glycogen, Protein, Zebra fish

Introduction

Water pollution by industrial effluents containing organics and heavy metals pose a serious hazard to the aquatic biota and public health (Velma *et al.,* 2009). Mercury is usually discharged into environment through the effluents from thermal power stations, paper and pulp industries, electronic wastes, batteries, electroplating, metal industries, fossil fuel combustion etc. It is also used in the manufacture of paints and dental amalgams (El-Moselhy, 2006). During the last decade, energy demand across the globe has led to the massive expansion of thermal based power plants which have been adding increasing loads of carbon dioxide and mercury as coal combustion byproducts to the environment, evoking major environmental and health concerns (Chen Lie *et al.,* 2007; Guffey and Bland, 2004; Milena Horvat *et al.,* 2003; Carballeira and Fernandez, 2002; Kotnik *et al.,* 2000).

In Hyderabad city, a large number of bulk drug industries, battery industries, electroplating industries were established in the pre-catchments of many aquatic bodies, and the occurrence of mercury and other heavy metals in the aquatic ecosystems is well established. For example, an alarmingly high level of mercury (8.02 mg kg^{-1}) was reported in the sediments of Hussainsagar lake (Suneela *et al.,* 2008). Similar trends were observed in the soils of Kattedan industrial estate in Hyderabad (Govil *et al.,* 2008). Mercury is known to induce alterations in the biochemical contents in piscine models (Martin and Arivoli, 2008 ; Shakoori and Iqbal, 1994). After entering fresh water bodies and the oceans, or settled into sediments and soils, mercury undergoes microbial transformation into the highly toxic form of methyl mercury, and it eventually bioaccumulates in the fish tissues. After bioaccumulation, methyl mercury is introduced into the human population through the dietary intake of fish and seafood products (Parvinder Kaur *et al.,* 2011). However, toxicity of mercury in fish depends on the sources of pollution, growth rate of organism, sex, size and

diversity of species (El-Moselhy, 2006). Mercury primarily targets the gills in fish leading to their death due to its high rate of accumulation, hypoxia and asphyxiation (De Oliveira Ribeiro *et al.,* 2002).

Fish are ideal sentinels for assessing the perturbations in behavior, oxygen uptake and biochemical profiles under toxic chemical exposure (Dube and Hosetti, 2010). Heavy metals accumulate in the tissues of the fish and cause cumulative deleterious effects at various functional levels ultimately leading to their death (Vutukuru *et al.,* 2009). Earlier reports suggest that mercury cause toxic effects in a number of tissues and organs of aquatic biota depending on its chemical form as well as the level, duration and the route of exposure (USEPA, 2001). The occurrence of such subtle alterations at the biochemical level has the potential to serve as 'early warning signals'.

Zebra fish (*Danio rerio)* is a unique freshwater fish and commonly inhabits native aquatic bodies of Hyderabad. It is one of the most widely accepted vertebrate models for toxicological testing (Hill *et al.,* 2005). Zebra fish requires a short generation time, exhibits higher fecundity with transperant embryos, making it an ideal model for investigating chemical toxicity. Moreover, it has nearly 70% homology with the human genome. The toxic effects of mercury to embryonic zebra fish at various functional levels are well documented (Lixin Yang *et al.,* 2010, Mario *et al.,* 2010; Navarro *et al*., 2009; Smith *et al.,* 2010; Weber, 2006). Recent research (Choong Yong Ung *et al.,* 2010) have reported that mercuric chloride is toxic to adult zebra fish in the range of 0.2 to 0.6 mgl^{-1} and induces hepatotoxicity, gene deregulation and histological damage. Little data are available on the toxic effects of mercury on the survival and biochemical profiles of *Danio rerio* in India. In light of the above, the present study aimed to investigate the acute toxicity of mercury and its impact on the glycogen and protein content of *D. rerio*.

Materials and Methods

Specimens of zebra fish (4 - 5 cm long; weight 1.25 ± 0.25 g) were used for toxicity tests. These were procured from a local aquarium shop, Hyderabad, transported in polythene bags to the laboratory and immediately transferred into plastic tubs of 20l capacity containing well-aerated ground water. The fish were allowed to acclimatize for 15 days before the experiments. They were fed with rice bran and ground nut oil cake *ad libitum* during the acclimation period. Only healthy and active fishes were used for experiments. The temperature, pH, dissolved oxygen, hardness of the water used for acclimatization were 29^0 C, 7.2 ± 0.2, 5.0-6.5 mgl^{-1} and 220 mgl^{-1}, respectively.

Determination of median lethal concentration (96 hr LC_{50}): The acute toxicity of mercuric chloride to *D. rerio* was determined using a standard 24 hr static renewal technique (OECD, 1992). Desired concentrations of mercuric chloride were prepared by adding 1% mercuric chloride stock solution to known volume of water. The initial experiment was conducted on a minimum of two random concentrations (0.03 and 0.10 mg l^{-1}**)** to determine the mortality of the fishes within the range of 5-95%. The tests were repeated thrice to check for the reproducibility of the results.

Subsequent tests were conducted using four concentrations of mercuric chloride *i.e.* 0.05, 0.07, 0.08 and 0.10 mgl^{-1} the mortality in these different concentrations ranged from 5 to 95%. Thirty fish (three batches of ten each) were exposed to each concentration in 10 l plastic troughs separately. Controls without toxicant were also run simultaneously. Behavioral manifestations and the condition of the fishes were noted every 24 hr up to 96 hr. The fish that failed to respond even to strong tactile stimuli were considered dead and removed immediately. The number of dead fishes was recorded for each concentration of the toxicant, and the data were used to determine the median lethal concentration (LC_{50}) by means of probit analysis (Finney, 1953). The corresponding results were generated with a computer program.

Estimation of glycogen and protein : A group of 12 fish were exposed to LC_{50} does *i.e.* 0.077 mg l^{-1} of mercuric chloride and 3 fish each were sacrified at 24, 48, 72 and 96 hrs. 3 fish were kept as control. The total glycogen and total protein were analysed in gills, muscles and visera as per the method of Kemp *et al.* (1954) and Lowry *et al.* (1951), respectively.

Statistical analyses: Students't' test was employed for significance level between control and treated groups. Values were expressed as mean ± SD.

Results and Discussion

High concentration of mercury (8.02 mg kg^{-1}) were reported from water bodies in and around Hyderabad (Suneela *et al.,* 2008) against the permissible levels of 0.001 to 0.002 mg l^{-1} in water. Mercury forms salts in two ionic states mercury (I) and mercury (II). Mercuric salts (mercury (II) are common in the environment than mercurous salts (mercury (I). Once in water these salts become soluble in water, are bioavailable and considered toxic (USEPA, 2001). The concentrations of mercuric chloride tested in the present study were 0.03, 0.05, 0.06, 0.07 and 0.10 mg l^{-1}. The mortality in these different concentrations ranged from 6.66, 10, 36.6, 43.3 and 96.6% and was dependent on both time and concentration. At each concentration, 30 fishes were

examined and the number of dead fish recorded as 2 in 0.03 mg l^{-1} in 0.05 mg l^{-1}, 11 in 0.07 mg l^{-1}, 12 in 0.08 mg $l^{-1}$and 29 in 0.1 mg l^{-1}. The 96 hr LC_{50} for mercuric chloride was derived from regression equation Y= 14.168 + 8.185x and it was found to be 0.077 mgl^{-1}. The lower and upper fiducial limits at 95% confidence level were 0.0654 to 0.0854.

Whilst the 96 hr LC_{50} values for freshwater fish ranged between 33 and 400 µg l^{-1}, their lethal responses to mercury in acute nominal concentrations started from approximately as low as 30 µg l^{-1} (Boening, 2000). In the present investigation, 0.077 mgl^{-1} LC_{50} in zebra fish suggested that the metal was highly toxic to the fish.

In the present investigation, significant decline in the total glycogen content of gill, muscle and viscera was observed in the fish tissues exposed to 0.077 mg l^{-1} at the end of 24, 48, 72 and 96 hrs demonstrating a time-concentration response (Table 1). A decrease in the glycogen content of the fishes exposed to metallic stress has been reported by several authors (Mona Saki *et al.,* 2010; Hadi *et al.,* 2009; Martin and Arivoli, 2008; Sobha *et al.,* 2007; Emad *et al.,* 2005). The decrease in the glycogen content in the tissues of *Danio rerio* may be due to its enhanced utilization as it forms the immediate source of energy under metallic stress. However, the glycogen decrease was found to be maximum in gills (22.66 to 53%) at the end of 24-96 hr. This could be due to the fact that the metal potentially damages the respiratory epithelium (De Oliveira Ribeiro *et al.,* 2002, Chun-Yang Liao *et al.,* 2006). Recent report (Vutukuru *et al.,* 2011) indicates that glycogen content decreases drastically in fish under hypoxic or anoxic conditions.

The protein content of gills decreased by 13.01, 28.8, 36.24, 46.76% in 0.077 mg l^{-1} as compared to control at the end of 24, 48, 72 and 96 hrs, respectively. The t-test showed that the protein decrease in gills of the fish exposed to 0.077 $mgl^{-1}$was insignificant at the end of 24 hr while significant changes was observed at the end of 48, 72 and 96 hrs (Table 2).

The total protein content in muscle decreased by 8.16, 18.44, 34.73 and 47.14% in 0.077 mgl^{-1} as compared control at the end of 24 hr, 48 hr, 72 hr and 96 hr, respectively. Insignificant decrease in total protein content in muscle of fish exposed to 0.077 mgl^{-1} was observed at the end of 24 hr whereas this decrease was significant at the end of 48, 72 and 96 hr showed a significant decrease in the muscle protein suggesting that even low concentrations of mercury is able to cause toxic effects in zebra fish. The protein content in viscera of the fish exposed to 0.077 mgl^{-1} showed a significant decrease from the control at the end of 24, 48, 72 and 96 hrs. Depletion of glycogen and protein contents up to 53 and 47% was observed in all the three tissues. The data suggests that under acute metallic stress, diversification of energy occurs to accomplish the impending energy demands. It is also possible that proteins breakdown into free amino acids under the toxic impact of mercuric chloride. Earlier studies also showed that appreciable decline in the protein content of vital organs like gills, liver and muscle of fishes exposed to mercury and other heavy metals (Martin and Arivoli, 2008, Sobha *et al.,* 2007; Snehalatha Das *et al.,* 2001) (Table 2).

The decrease in tissue 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 (Vutukuru, 2003). Gill damage in fish under mercury stress has also been reported (De Oliveira Ribeiro *et al.,* 2002). The decrease in the protein content observed

Table 1 : Total glycogen content (mg g^{-1}) in gills of *Danio rerio* exposed to lethal concentration of mercuric chloride

Concentration (0.07mg l^{-1})	Duration (hr)	Control fish	Treated fish
Gills	24	6.83±0.55	5.28±0.59 (-22.6)
	48		5.04± 0.46 (-26.2)
	72		4.61± 0.38 (-39.8)
	96		3.24± 0.52 (-53.0)
Muscle	24	13.33±0.72	10.49± 0.85 (-21.2)
	48		9.91± 0.76 (-21.6)
	72		8.66± 0.81 (-35.03)
	96		7.24± 0.80 (-44.33)
Viscera	24	16.20±0.46	14.71± 0.61(-9.19)
	48		14.19± 0.60 (-12.4)
	72		13.83± 0.62 (-14.62)
	96		11.04± 0.56 (-31.85)

Values are mean of three replicates ± S.D.

Table 2 : Total protein content (mg l^{-1}) in muscle of *Danio rerio* exposed to lethal concentration of mercuric chloride

Concentration (0.07mg l^{-1})	Duration (hr)	Control fish	Treated fish
Gills	24	30.13±2.15	26.21±2.76 (-13.01)
	48		21.43± 2.7 (-28.80)
	72		19.21± 2.78 (-36.24)
	96		16.04± 2.76 (-46.76)
Muscle	24	63.31±2.16	58.14± 2.84 (-8.16)
	48		51.63± 2.80 (-18.44)
	72		41.32± 2.86 (-34.73)
	96		33.46± 2.84 (-47.14)
Viscera	24	49.93±2.03	40.43± 2.65 (-13.85)
	48		37.15± 2.72 (-20.83)
	72		32.10± 2.73 (-31.60)
	96		27.67± 2.67 (-41.03)

Values are mean of three replicates ± S.D.

in the present study may be attributed to its utilization in cell repair and tissue organization.

Depletion in tissue proteins of *D. rerio* may be partly due to impaired or low rate of protein synthesis under metallic stress (Nanda and Behera, 1996). Copious mucous secretion observed in the present study suggests the probable utilization of proteins in the formation of mucoproteins that are eliminated in the form of mucous. Further, direct and /or indirect utilization of proteins for energy needs also cannot be ruled out. The present study showed that even low levels of mercury induced biochemical alterations in *D. rerio*, with more pronounced changes occurring at the end of 96 hr indicating that the decrease in proteins is time-concentration dependent. Eventually, mercury induced alterations may probably affect the enzyme mediated biodefense mechanisms of the fish.

Acknowledgments

The authors thank the Executive Director, Principal, Dean R&D and Management of SNIST for their support, encouragement to carry out this research as a part of in house M.Tech project and laboratory facilities.

References

Boening, D.W.: Ecological effects, transport and fate of mercury: A general review. *Chemosphere*, **40**, 1335-1351, (2000).

Carballeira, A. and J.A. Fernández: Bioconcentration of metals in the moss *Scleropodium purum* in the area surrounding a power plant: A geotopographical predictive model for mercury. *Chemosphere*, **47**, 1041-1048 (2002).

Chen Lei, Duan Yufeng, Zhuo Yuqun , Yang Liguo, Zhang Liang, Yang Xianghua, Yao Qiang, Jiang Yiman and Xu Xuchang: Mercury transformation across particulate control devices in six power plants of China: The co-effect of chlorine and ash composition. *Fuel Process. Technol*., **86**, 603-610 (2007).

Choong Yong Ung**,** Siew Hong Lam**,** Mya Myintzu Hlaing**,** Cecilia Lanny Winata**,** Svetlana Korzh**,** Sinnakaruppan Mathavan and Zhiyuan Gong: Mercury-induced hepatotoxicity in zebrafish: *in vivo* mechanistic insights from transcriptome analysis, phenotype anchoring and targeted gene expression validation. *BMC Genomics*, **11,** 212 (2010).

Chun-Yang Liao, Jian-Jie Fu, Jian-Bo Shi, Qun-Fang Zhou, Chun-Gang Yuan and Gui-Bin Jiang: Methylmercury accumulation, histopathology effects, and cholinesterase activity alterations in medaka (*Oryzias latipes*) following sub-lethal exposure to methyl mercury chloride. *Environ. Toxicol. Pharmacol*., **22**, 225-233 (2006).

De Oliveira Ribeiro, C.A., L. Belger, Pelletier and C. Rouleau: Histopathological evidence of inorganic mercury and methyl mercury toxicity in the arctic charr (*Salvelinus alpinus*). *Environ. Res.,* **90**, 217–225 (2002).

Dube, P.N. and B.B. Hosetti: Behaviour surveillance and oxygen consumption in fresh water fish *Labeo rohita* exposed to sodium cyanide. *Biotechnol. Animal Husbandry*, **26,** 91-103 (2010).

El-Moselhy, Kh.M.: Bioaccumulation of mercury in some marine organisms from lake Timsah and bitter lakes (Suez canal, Egypt). *Egyptian J. Aqu. Res.,* **32**, 124-134 (2006).

Emad, H., Abou El-Naga, Khalid M. El-Moselhy and Mohamed A. Hamed: Toxicity of cadmium and copper and their effect on some biochemical parameters of marine fish *Mugil seheli*. *Egyptian J. Aqu. Res.,* **31**, 60-71 (2005).

Finney, D.J.: Probit analysis. 2nd Edn. Cambridge University Press, Cambridge, England (1953).

Govil, P.K., J.E. Sorlie, N.N. Murty, D. Sujata, G.L.N. Reddy, Kim Rudolph Lund, A.K. Krishna and K. Ramamohan: Soil contamination of heavy metals in the Katedan Industrial Development Area, Hyderabad, India. *Env. Monit. Assess*., **140**, 313-323 (2008).

Guffey, F.D. and A.E. Bland: Thermal pretreatment of low-ranked coal for control of mercury emissions. *Fuel Process. Technol*., **85**, 521-531 (2004).

Hadi, A.A., A.E. Shokr and S.F. Alwan: Effects of aluminium on the biochemical parameters of fresh water fish, *Tilapia zilli*. *J. Sci. Appli.,* **3**, 321-325 (2009).

Hill, A.J., H. Teraoka, W. Heideman and R.E. Peterson: Zebra fish as a model vertebrate for investigating chemical toxicity**.** *Toxicol. Sci*., **86**, 6-19 (2005).

Kemp, A., J.M. Andienne and K.V. Hejningen: A colorimetric micromethod for the determination of glycogen. *Biochem. J*., **56**, 640-648 (1954).

Kotnik, J., M. Horvat, V. Mandic and M. Logar: Influence of the O tanj coal-fired thermal power plant on mercury and methyl mercury concentrations in Lake Velenje, Slovenia. *Sci. Tot. Environ*., **259**, 85-95 (2000).

Lowry, O.H., N.J. Rosebrough, A.L. Forr and R.J. Randall: Protein measurement with the Folin-phenol reagent. *Biochem. J*., **56**, 640-648 (1951).

Mario, Roberto Senger, Denis Broock Rosemberg, Kelly Juliana Seibt, Renato Dutra Dias, Maurício Reis Bogo and Carla Denise Bonan: Influence of mercury chloride on adenosine deaminase activity and gene expression in zebrafish (*Danio rerio*) brain. *Neuro toxicology*, **31**, (2010).

Martin, P. Deva Prasath and S. Arivoli: Biochemical study of freshwater fish *Catla catla* with reference to mercuric chloride. *Iran. J. Environ. Hlth. Sci. Eng.*, **5**, 109-116 (2008).

Milena, Horvat, Nata a Nolde, Vesna Fajon, Vesna Jereb, Martina Logar, Sonja Lojen, Radojko Jacimovic, Ingrid Falnoga, Qu Liya, Jadran Faganeli and Damjana Drobne: Total mercury, methylmercury and selenium in mercury polluted areas in the province Guizhou, China. *Sci. Tot. Environ*., **304**, 231-256 (2003).

Nanda, P. and M.K. Behera: Nickel induced changes in some hemato-biochemical parameters of a catfish, *Heteropn. fossilis* (Bloch). *Eviron. Ecol*., **14**, 82-85 (1996).

Navarro, A., L. Quirós, M. Casado, M. Faria, L. Carrasco, L. Benejam, J. Benito, S. Díez, D. Raldúa, C. Barata, J.M. Bayona and B. Piña: Physiological responses to mercury in feral carp populations inhabiting the low Ebro River (NE Spain), a historically contaminated site. *Aquat. Toxicol*., **93**, 150-157 (2009).

OECD: Guideline for testing of chemicals. Fish, acute toxicity test adopted by the Council on 17th July, 1992.

Parvinder Kaur, Michael Aschner, and Tore Syversen: Biochemical factors modulating cellular neurotoxicity of methylmercury. *J. Toxicol.,* **20**, 1-9 (2011).

Shakoori, A.R. and M.J. Iqbal: Biochemical changes induced by inorganic mercury on the blood, liver and muscle of fresh *Ctenopharyngodon idella*. *J.Ecotoxicol.Environ.Moni*., **4**, 81-92 (1994).

Smith, L.E., M.J. Carvan, J.A. Dellinger, J.K. Ghora, D.B. White, F.E. Williams and D.N. Weber: Developmental seleno methionine and methyl mercury exposures affect zebrafish learning. *Neurotoxicol. Teratol*., **32**, 246-255 (2010).

Snehalata Das, Sunil K. Patro and B.K. Sahu: Biochemical changes induced by mercury in the liver of penaeid prawns *Penaeus indicus* and *P.monodon* from Rushikulya estuary, east coast of India. *Indian J. Marine Sci.*, **30**, 246-252 (2001).

Sobha, K., A. Poornima, P. Harini and K. Veeraiah: Study on biochemical changes in the fresh water fish *Catla catla* exposed to the heavy metal toxicant cadmium chloride. *Kathmandu Unive. J. Sci., Engi. Technol.*, **1**, 84-91 (2007).

Suneela, M., G. Radha Krishna, K. Vamsi Krishna, V. Manga Sai, V. Bhargav, K. Shyam Sundar Reddy, D.S.S. Srinivas and J.S. Srinivas: Water and sediment analysis of Hussainsagar lake, Hyderabad. Proceedings of Raal 2007: The 12th World Lake Conference (Eds.: M. Sengupta and R. Dalwani) p. 304-306 (2008).

USEPA: Water Quality Criterion for the Protection of Human Health: Methyl Mercury**.** U.S. Environmental Protection Agency, Washington, D. C., EPA 0823-R-01-001 (2001).

Velma, V., S.S. Vutukuru and P.B. Tchounwou: Ecotoxicology of hexavalent chromium in freshwater fish: A critical review. *Rev. Environ. Hlth*., **24**, 129–145 (2009).

Vutukuru, S.S.: Chromium induced alterations in some biochemical profiles of the freshwater teleost fish, *Labeo rohita* (Hamilton). *Bull. Environ. Contam. Toxicol*, **70**, 118-123, (2003).

Vutukuru, S.S.: Acute effects of hexavalent chromium on survival, oxygen consumption, haematological parameters and some biochemical profiles of the Indian major carp, *Labeo rohita*. *Int. J. Environ. Res. Public Hlth*., **2**, 456-462 (2005).

Vutukuru, S.S., Juveria, V. Himabindu and Y. Anjaneyulu: Physiological stress responses as biomarkers of sensitivity in *Esomus danricus* exposed to copper. *Asian J. Microbiol. Biotech. Env. Sci*., **11**, 969-975 (2009).

Vutukuru, S.S., V.V. Vinay Prabhakar and Y. Anjaneyulu: Stress responses as biomarkers of sensitivity in *Labeo rohita* exposed to lime treated sludge filtrate from paper and pulp industry. *Asian J. Microbiol. Biotech. Env. Sci*., **13**, 495-504 (2011).

Weber, D.N.: Dose-dependent effects of developmental mercury exposure on C-start escape responses of larval zebra fish, *Danio rerio. J Fish Biol.,* **69**, 75–94 (2006).