ACUTE TOXICITY OF MERCURY ($HgCl_2$) TO NILE TILAPIA, *Oreochromis niloticus*

Nilton Massuo ISHIKAWA [1]; Maria José Tavares RANZANI-PAIVA [2, 3]
Julio Vicente LOMBARDI [2]

ABSTRACT

This paper reports the median lethal concentration ($LC_{50\text{-}96h}$) of mercury to Nile tilapia, *Oreochromis niloticus*, estimated through semi-static acute toxicity test developed with mercury chloride ($HgCl_2$). The experiment was carried out in the Aquatic Toxicology Laboratory – Instituto de Pesca, SP, under controlled conditions of temperature (24.4±2.25 ^{0}C) and photoperiod (10L:14D). Fingerlings (2.46±0.21 cm and 0.41±0.12 g) were kept during 96 hours in 5-liter glass aquaria, according to the following mercury concentrations, set up in three replicates: 0.00 (control), 0.037, 0.185, 0.370, 0.740, 0.925 mg Hg L^{-1}. The value of $LC_{50\text{-}96h}$ was estimated in 0.220 mg Hg L^{-1}.

Key words: mercury; Nile tilapia; *Oreochromis niloticus*; toxicity

TOXICIDADE AGUDA DO MERCÚRIO PARA TILÁPIA-DO-NILO, *Oreochromis niloticus*

RESUMO

Este trabalho relata a concentração letal média ($CL_{50\text{-}96h}$) de mercúrio para tilápia-do-Nilo, *Oreochromis niloticus*, estimada através do teste semi-estático de toxicidade aguda realizado com cloreto de mercúrio ($HgCl_2$). O teste foi conduzido no laboratório de Toxicologia Aquática do Instituto de Pesca – SP, em condições controladas de temperatura (24,4±2,25 ^{0}C) e fotoperíodo (10L:14D). Alevinos (2,46±0,21 cm e 0,41±0,12 g) foram mantidos durante 96 horas em aquários de 5 litros, de acordo com as seguintes concentrações de mercúrio, conduzidas em triplicata: 0,00 (controle); 0,037; 0,185; 0,370; 0,740; 0,925 mg Hg L^{-1}. O valor de $CL_{50\text{-}96h}$ foi estimado em 0,220 mg Hg L^{-1}.

Palavras-chave: mercúrio; tilápia-do-Nilo; *Oreochromis niloticus;* toxicidade

Nota Científica: Recebida em 06/10/2005 - Aprovada em 16/10/2006

[1] *Pós-Graduando - Centro de Aqüicultura da UNESP - e-mail: niltonmi@caunesp.unesp.br*
[2] *Pesquisador Científico – Instituto de Pesca/APTA/SAA, SP*
[3] *Address/Endereço: Instituto de Pesca - Av. Francisco Matarazzo, 455; CEP: 05001-900 - São Paulo-SP-Brazil*

INTRODUCTION

The aquatic environment is constantly exposed to various pollutants, and the group of heavy metals has been the focus of many studies with deep concern. Some particular heavy metals, such as mercury (Hg), are especially included in investigations due to their high toxicity. This element is classified as one of the most toxic metals, which are introduced into the natural environment by human interferences (BUHL, 1997). Inorganic mercury is the most common form of the metal released in the environment by industries, presenting a stronger acute effect on fish tissues than that of the organic form of mercury (SUNDERLAND and CHMURA, 2000). Some papers have reported situations where high mercury levels were detected in water, mainly nearby gold extraction locations (MAURICE-BOURGOIN *et al*., 2000; DOLBEC *et al*., 2001) and industrial zones (KIME, 1998; SUNDERLAND and CHMURA, 2000). Consequently, aquaculture is vulnerable to this pollutant, since supplied ponds water generally comes from rivers, dams or other sources that can possibly be contaminated by mercury. Fish contaminated by Hg suffers pathological alterations, with consequent inhibition of metabolic processes, hematological changes, and decline in fertility and survival (MICRYAKOV and LAPIROVA, 1997).

The aim of this study was to determine the acute toxicity of mercury chloride to one particular species of fish, Nile tilapia (*Oreochromis niloticus*), which is very representative in the global productions from aquaculture and fisheries.

MATERIAL AND METHODS

The bioassay was conducted in May 2002, in the Aquatic Toxicology Laboratory, Instituto de Pesca – SP, Brazil, with controlled conditions of water temperature (24.40±2.25 °C) and photoperiod (10L:14D cycle). The used fish species was Nile tilapia, *Oreochromis niloticus*. Fingerlings with a mean weight of 0.41±0.12 g and mean total length of 2.46±0.21 cm were purchased from a commercial aquaculture facility. The acclimatization period was of 48 h, in a 50-L glass aquarium. During this period, fish were fed a dry commercial food (pellets with 25% of crude protein). Afterwards, fingerlings were transferred to 5-L glass aquaria, which were internally covered with a plastic film to prevent contamination by residues from previous experiments. Plastic film was also placed on the top of the aquarium to prevent evaporation. Air pumps and individual air stone diffusers provided aeration. The experiment was carried out at a stocking density of 10 fish/aquarium.

The chemical product used in this study was inorganic mercury chloride ($HgCl_2$) Synth Laboratory™ with 99% purity. The stock solution (370 mg Hg L^{-1}) was prepared by dissolving a calculated quantity of active ingredient (0.5 g $HgCl_2$ in 1,000 mL of dechlorinated tap water). A series of five concentrations of Hg was prepared by adding a calculated volume from the stocky solution into test containers, considering the equivalent on mercury (Hg). Therefore, nominal concentrations were: 0.037, 0.185, 0.370, 0.740, and 0.925 mg Hg L^{-1} (range determined by preliminary tests). One container was kept as unexposed control group. Test was carried out with three simultaneous replicates. No food was supplied during the experiment. Test solutions were replaced by fresh ones of the same respective concentrations every 24 h until 96 h of testing, according to the renewal method recommended in APHA *et al*. (1998).

Temperature (°C), pH and electric conductivity (μS cm^{-1}) were recorded individually in each test aquarium at exposure times of 24, 48, 72 and 96 hours. Hardness and alkalinity (mg $CaCO_3$ L^{-1}) and total ammonia (mg L^{-1}) were determined by standard methods (APHA *et al*., 1998) only at the end of the experiment.

Mortalities were recorded at 24, 48, 72 and 96 h of exposure, and dead organisms were removed regularly from the test solutions. The data obtained were statistically analyzed using the Trimmed Spearman Karber method (HAMILTON *et al*., 1977) for estimating the median lethal concentration (LC_{50}), and 1/100 of the $LC_{50\text{-}96h}$ was taken as the safe Hg concentration (SPRAGUE, 1971).

RESULTS AND DISCUSSION

Physical and chemical variables analyzed in the test solutions showed no statistical differences among the range of five concentrations, neither between concentration range and control group. The average values for these variables were: temperature, 24.40±2.25 °C; pH, 7.31±0.53; electric conductivity, 73.88±2.02 μS cm^{-1}; hardness, 24.66±1.10 mg $CaCO_3$ L^{-1}; alkalinity, 20.54±1.24 mg $CaCO_3$ L^{-1}; and total ammonia, 1.63±0.32 mg L^{-1}. All of these variables results were in conformity to the standards that are recommended in APHA *et al.* (1998) for toxicity tests.

Clinical signs of tilapia affected by mercury exposure were observed in the first experimental hours, mainly at the higher concentrations (0.925; 0.740 and 0.370 mg Hg L^{-1}). The following aspects were registered with the same intensity for fish associated to those three higher mercury concentrations: hyperactivity, darkening of the body, and aggressiveness, followed by dyspnea and death. Similar behaviors have also been reported by HIRT and DOMITROVIC (1999) in *Aequidens portalegrensis* exposed to $HgCl_2$ (0.64, 1.12 and 2.0 mg Hg L^{-1}). BANO and HASAN (1990) observed signs of mercury (0.2 mg Hg L^{-1}) poisoning in cat fish (*Heteropneustes fossilis*), such as respiratory distress, tremor, ataxia and incoordination of moviments.

PANDEY *et al.* (1994) described alteration in liver and intestine of *Liza parsia* exposed to $HgCl_2$ (0.2 mg Hg L^{-1}) for 15 days. Similarly, OLIVEIRA RIBEIRO *et al.* (2002) reported serious injuries in gill and olfactory epithelium of *Salvelinus alpinus* exposed to 0.15 mg Hg L^{-1}. According to ALLEN (1994), the exposure of *Orechromis aureus* to 0.5 mg Hg L^{-1} caused a raise in the number of leucocyte and erythrocyte within 24 hours. GILL and PANT (1985) also related hematological anomalies in *Barbus conchonius* exposed to 0.18 mg Hg L^{-1} in acute test.

Mortalities recorded along 96 hours exposure are registered in table 1. Despite of mortality had been high at replicate "A" from control group, the average (96 h) was 20%, and it is considered as acceptable in such short term test (KLEMM *et al.*, 1994) for estimating the LC_{50}.

Table 1. Cumulative mortality (%) of Nile tilapia, *Oreochromis niloticus,* expressed according to different exposure times to mercury concentration s

Concentration (mg Hg L^{-1})	***Time* (hour)**												**Average 96h**
	24			**48**			**72**			**96**			
	A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	
0.00 (control)	10	0	0	30	0	0	30	0	0	50	0	10	20
0.037	0	0	0	0	0	0	0	10	0	0	10	0	4
0.185	0	0	10	0	0	20	0	10	20	0	20	50	24
0.370	0	20	60	30	30	60	90	60	80	90	70	90	84
0.740	100	100	100	100	100	100	100	100	100	100	100	100	100
0.925	100	100	100	100	100	100	100	100	100	100	100	100	100

A, B, C = replicates

The values of LC_{50} determined for Nile tilapia in the present study, according to the different exposure times, are shown in table 2. The $LC_{50\text{-}96h}$ was compared with the results from other studies developed on mercury toxicity to fish (Table 3). RAMAMURTHI *et al.* (1982) and CHARUWAN-SOMSIRI (1982) estimated higher $LC_{50\text{-}96h}$ for *Tilapia mossambica* and *Oreochromis niloticus*, respectively (Table 3). The higher values obtained by RAMAMURTHI *et al.* (1982) and CHARUWAN-SOMSIRI (1982) may be attributed to some differences in standard techniques that were adopted in their experiments, such as the larger size of the test-organisms (3.5 cm) used by CHARUWAN-SOMSIRI (1982). According to BUHL (1997) and BOENING (2000), older and larger aquatic organisms are more resistant to toxicants.

Table 2. Median Lethal Concentration (LC_{50}) of mercury in Nile tilapia, *Oreochromis niloticus*

Exposure Time **(hour)**	***LC_{50}*** **(mg Hg L^{-1})**	***95% Confident Limit*** **(mg Hg L^{-1})**
24	0.42	0.37 – 0.48
48	0.37	0.31 – 0.43
72	0.28	0.23 – 0.33
96	0.22	0.18 – 0.28

Table 3. Acute toxicity of mercury in various fish species

Reference	*Species*	$LC_{50\text{-}96h}$ (mg Hg L^{-1})
Present study	***Oreochromis niloticus***	**0.220**
CHARUWAN-SOMSIRI (1982)	*Oreochromis niloticus*	3.710
RAMAMURTHI *et al.* (1982)	*Tilapia mossambicus*	0.739
BUHL (1997)	*Ptychocheilus lucius*	0.168
BUHL (1997)	*Gila elegans*	0.108
BUHL (1997)	*Xyrauchen taxanus*	0.090
DAS *et al.* (1980)	*Heteropneustes fossilis*	0.350
HIRT and DOMITROVIC (1999)	*Aequidens portalegrensis*	0.660
KHANGAROT (1981)	*Channa marulius*	0.314
KIM (1979)	*Cyprinus carpio*	0.100
KIM (1979)	*Misgurnus anguillicaudatus*	0.150
NAMMALWAR (1989)	*Liza macrolepis*	0.360
REHWOLDT *et al.* (1972)	*Cyprinus carpio*	0.180
REHWOLDT *et al.* (1972)	*Roccus americanus*	0.220
REHWOLDT *et al.* (1972)	*Fundulus diaphanous*	0.110
SHYONG and CHEN (2000)	*Variocorhinus barbatulus (V. barbatus)*	0.168
SHYONG and CHEN (2000)	*Zacco barbata*	0.161
SLABBERT and VENTER (1999)	*Poecilia reticulata*	0.200

The higher $LC_{50\text{-}96h}$ determined by RAMAMURTHI *et al.* (1982) may be related to a high water hardness of 35 mg $CaCO_3$ L^{-1}, compared to an average of 24.5 mg $CaCO_3$ L^{-1} in the present study. These data confirm those of AMEND (1970), who reported a reduction in Hg toxicity related to an increase in water hardness. On the other hand, the LC_{50} determined in the present study was very similar to those reported to other fish groups, such as *Cyprinus carpio* and *Roccus americanus* (REHWOLDT *et al.* 1972), *Varichorhinus barbatulus (V. barbatus), Variocorhinus barbatulus (V. barbatus)* and *Zacco barbata* (SHYONG and CHEN, 2000), *Ptychocheilus lucius* (BUHL, 1997), *Misgurnus anguillicaudatus* (KIM, 1979), and *Poecilia reticulata* (SLABBERT and VENTER, 1999).

A safe concentration was estimated in the present study and compared with water quality standards reported by some Environmental Agencies (Table 4). CONAMA (2005) recommends a maximum concentration of 0.0002 mg Hg L^{-1} in water supplies used for rearing fish species destined for human consumption in Brazil. This value is very similar to those recommended by Malaysia National Water Quality Standards (DOE-UM, 1986), however both recommendations are far lower than the safe mercury concentration values determined in this study.

Table 4. Some estimatives of Hg levels considered as safe water quality requirement for fish

	Hg (mg L^{-1})
Present study ($LC_{50\text{-}96h}$ x 0.01)	**0.0022**
Conselho Nacional do Meio Ambiente (CONAMA, 2005)	0.0002
Malaysia National Water Quality Standards (DOE-UM, 1986)	0.0001
Taiwan Water Quality Standards (TAIWAN EPA, 1985)	0.0020

CONCLUSIONS

The data obtained in the present work demonstrate that mercury is highly toxic for tilapia fingerlings and express the risk of this metal to the environment. The environmental contamination with this metal can represent a great threat for the fish populations and also a serious problem for the aquaculture.

ACKNOWLEDGEMENTS

This work was financially supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Brazil (Proc. n. 01/03182-5).

REFERENCES

ALLEN, P. 1994 Changes in the haematological profile of the cichlid *Oreochromis aureaus* (Steindachner) during acute inorganic mercury intoxication. *Comp. Biochem. Physiol., 108C*(1): 117-121.

AMEND, D.F. 1970 Retention of mercury by salmon. *Prog. Fish. Cult., 32:* 192-194.

APHA; AWWA; WPCF 1998 *Standard methods for the examination of water and wastewater*. 20.ed. In: APHA – American Public Health Association; AWWA – American Water Works Association; WPCF – Water Pollution Control Federation (Ed.). Washington, DC. 140p. (Capítulo 8)

BANO, Y. and HASAN, M. 1990 Histopathological lesions in the body organs of catfish, *Heteropneustes fossilis* following mercury intoxication. *J. Environ. Science and Health, 25*(1): 67–85.

BOENING, D.W. 2000 Ecological effects, transport, and fate of mercury: A General Review. *Chemosphere, 40:* 1335-1351.

BUHL, K.J. 1997 Relative sensitivity of three endangered fishes, Colorado Squawfish, Bonytail, and Razorback Sucker, to selected metal pollutants. *Ecotoxicol. Environ. Safety, 37*: 186-192.

CHARUWAN-SOMSIRI, L. 1982 Acute toxicity of mercury, copper and zinc to the Nile tilapia (T*ilapia nilotica*, Linnaeus, 1757). *Thai. Fish. Gazette, 35*(3): 313-318.

CONAMA 2005 *Resolução nº 357*, de 17 de março de 2005. Brasília: Ministério do Meio Ambiente. Conselho Nacional do Meio Ambiente. Publicação D.O.U. 18/03/2005. Disponível em: http://www.mma.gov.br/port/conama/res/res05/res35705.pdf Acesso em: 18/abril/2006.

DAS, K.K.; DASTIDAR, S.G.; CHAKRABARTY, S.; BANERJEE, S.K. 1980 Toxicity of mercury: a comparative study in air-breathing fish and non air-breathing fish. *Hydrobiologia., 68*: 225-229.

DOE-UM 1986 *Water quality criteria and standards for Malaysia*. Executive Summary. Final Report. Department of Environment, Ministry of Science, Technology and Environment, Malaysia/ Consultant group on water quality, Institute of Advanced Studies, Univ. of Malaya, Kuala Lumpur, Malaysia, 1, V-Xll.

DOLBEC, J.; MERGLER, D.; LARRIBE, F.; ROULET, M.; LEBEL, J.; LUCOTTE, M. 2001 Sequential analysis of hair mercury levels in relation to fish diet of an Amazonian population, Brazil. *The Science of the Total Environment, 271:* 87-97.

GILL, T.S. and PANT, J.C. 1985 Mercury-induced blood anomalies in the freshwater teleost. *Water, Air and Soil Pollution., 24*: 165-171.

HAMILTON, M.A.; RUSSO, R.C.; THURSTON, R.V. 1977 Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. *Environ Sci. Technol., 11*: 714-719.

HIRT, L.M. and DOMITROVIC, H.A. 1999 Toxicidad y respuesta histopatológica en *Aequidens portalegrensis* (Pisces, Cichlidae) expuesto a bicloruro de mercurio en ensayos de toxicidad aguda y subletales. REUNIÓN DE COMUNICACIONES CIENTÍFICAS Y TECNOLÓGICAS, UNIVERSIDAD NACIONAL DEL NORDESTE, Corrientes, 1999. *Anais...* Tomo IV (Ciências Veterinárias). p.19-22.

KHANGAROT, B.S. 1981 Effect of zinc, copper and mercury on *Channa marulius* (Hamilton). *Acta Hydrochim. Hydrobiol., 9*(6): 639-649.

KIM, J.M. 1979 The toxicity of mercury and cadmium on two freshwater fishes, carp and loach. *Bulletin Kordi, 1*(1): 15-21.

KIME, D.E. 1998 *Endocrine disruption in fish*. Sheffield: University of Sheffield. Kluwer Academic Publishers. 416p.

KLEMM, D.J.; MORRISON, G.E.; NORBER-KING, T.J.; PELTIER, W.H.; HEBER, M.A. 1994 *Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms*. 2.ed. Cincinnaty: EPA-600/4-91-003, Environmental Monitoring and Supporting Lab, US Environmental Protection Agency. 341p.

MAURICE-BOURGOIN, L.; QUIROGA, I.; CHINCHEROS, J.; COURAU, P. 2000 Mercury distribution in waters and fishes of the upper Madeira Rivers and mercury exposure in Riparian Amazonian populations. *The Science of the Total Environment, 260*: 73-86.

MICRYAKOV, V.R. and LAPIROVA, T.B. 1997 Influence of salts of some heavy metals on the differential blood count in juvenile *Acipenser baeri*.. *J. Ichthyol., 37*(6): 458-462.

NAMMALWAR, P. 1989 Biochemical changes resulting from bioassay of heavy metals mercury and cadmium to the grey mullet *Liza macrolepis* (Smith). In: INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE, 1., Tóquio, 1989. *Anais*… p.47.

OLIVEIRA-RIBEIRO, C.A.; BELGER, L.; PELLETIER, E.; ROULEAU, C. 2002 Histopathological evidence of inorganic mercury and methylmercury toxicity in the Arctic charr (*Salvelinus alpinus*). *Environmental Research, 90:* 217-225.

PANDEY, A.K.; MOHAMED M.P.; GEORGE, K.C. 1994 Histopathological alterations in liver and intestine of *Liza parsia* (Hamilton-Buchanan) in response to mercury toxicity. *J. Adv. Zool., 15*(1): 18-24.

RAMAMURTHI, R.; NAIDU, K.A.; SUBBIAH, M.B.; BALAJI, N.; RAO, M.V.R. 1982 Toxicity of mercury to some freshwater organisms. *Geobios Jodhpur, 9:* 89-90.

REHWOLDT, R.; MENAPACE, L.W.; NERRIE, B.; ALESSANDRELLO, D. 1972 The effect of increased temperature upon the acute toxicity of some heavy metal ions. *Bull. Environ. Contam. Toxicol., 8*: 91-96.

SHYONG, W.J. and CHEN, H.C. 2000 Acute toxicity of copper, cadmium, and mercury to the freshwater fish *Varicorhinus barbatulus* and *Zacco barbata*. *Acta Zool. Taiwanica, 11*(1): 33-45.

SLABBERT, J.L. and VENTER, E.A. 1999 Biological assays for aquatic toxicity testing. *Wat. Sci. Tech., 39*(10-11): 367-373.

SPRAGUE, J.B. 1971 Measurement of pollutant toxicity to fish. III. Sublethal effects and safe concentrations. *Water Res., 3:* 793-821.

SUNDERLAND, E.M. and CHMURA, G.L. 2000 An inventory of historical mercury emissions in Maritime Canada: Implications for present and future contamination. *The Science of the Total Environment, 256*: 39-57.

TAIWAN EPA 1985 *Water quality standards*. Taipei: Environmental Protection Agency. 547327.