Acute Intraperitoneal Mercury Chloride Contamination and Distribution in Liver, Muscle and Gill of a Neotropical Fish *Hoplias malabaricus* (BLOCK, 1794)

Taise Bomfim de Jesus¹*, Priscila Gontijo Aguiar de Almeida², Cristiane dos Santos Vergilio², André Luiz dos Santos Machado² and Carlos Eduardo Veiga de Carvalho²

¹Universidade Estadual de Feira de Santana, Av. Transnordestina, s/n; 44036-900; Feira de Santana - BA - Brasil.  
²Laboratório de Ciências Ambientais; Universidade Estadual do Norte Fluminense Darcy Ribeiro; Av. Alberto Lamego, 2000; 28015-602; Campos dos Goytacazes - RJ - Brasil

ABSTRACT

The present study investigated the distribution of mercury chloride in muscle, liver and gills of *Hoplias malabaricus* contaminated through intraperitoneal injection (6 µg in 0.1mL of PBS) for a period of 24, 48, 72 and 96h. The liver, gill and muscle were analyzed for mercury content in an ICP/AES (Varian Liberty II) with vapor generating accessory (VGA 77). The muscle and liver tissues presented the same contamination pattern increasing concentrations in 24 h of exposure with a decrease in Hg concentration with 72 h and a new increase in Hg concentrations with 96 h of exposure. The Hg concentrations in contaminated organisms were always higher than the control although only for liver samples the difference was statistically significant. Liver samples always presented higher Hg contents when compared with gill and muscle samples.

Key words: Inorganic mercury, liver, acute effects, intraperitoneal contamination

INTRODUCTION

Though mercury (Hg) occurs naturally in the environment, anthropogenic activities have affected its global cycle by mobilizing the increasing amounts of this metal in the environment.  

Currently, such mobilization of Hg is larger than from natural processes (Fitzgerald and Lamborg, 2005). The different chemical forms of Hg have varying toxicities and organic species of Hg. Especially methylmercury (MeHg), are more toxic because of their ability to pass the blood-brain barrier (Honda et al., 2006). Considering the environmental compartments through which Hg is transferred, aquatic ecosystems are very susceptible to MeHg contamination, as they host active populations of Hg methylating bacteria (Fitzgerald et al., 2007). Uptake and accumulation studies of inorganic mercury in fishes have described the relatively slow absorption and low bioavailability of these metals from water or food and the importance of both administrative routes in determining the accumulation of this metal (Olson et al., 1973; Williams and Giesy, 1978; Huckabee et al., 1979; Stary et al., 1981; Norrgren et al., 1985; Borg et al., 1988; Harrison and Klaiverkamp, 1989; Glynn, 1991). The disposition of inorganic...
mercury in fishes has been characterized after water, oral, and intraperitoneal administration, with the pattern of tissue distribution varying depending on the administrative route. Regardless of the exposure route, the liver and kidney tended to accumulate the highest quantities of these metals (Weisbart, 1973; Sorenson, 1990). Thus, the estimates of the biological half-life after water or oral dosing may be based downward because a significant portion of the eliminated metal may not have been absorbed internally. Consequently, an improved understanding of the distribution and elimination of mercury after intravascular injection would be useful in better characterizing the persistence of these compounds in fishes.

The lack of information on the mercury kinetic within fish constrains the understanding of its distribution in these organisms and also the evaluation of potential risks for the human and animal health.

This work studied the acute exposures to HgCl₂, in fish to evaluate and compare the average Hg concentration measured in liver, muscle and gill of Hoplias malabaricus exposed to inorganic mercury. The necessary increase the biological knowledge about this tropical species and the pharmacokinetic of the metal contamination for further use in field assessment.

MATERIALS E METHODS

Hoplias malabaricus specimens were collected, between July and October 2006 from the lakes located at Itaocara municipality, northwest of Rio de Janeiro State (21º40'44''S e 42º 04' 53 ''W). The lakes are located in a forested area with little influence of anthropogenic and point sources of pollutants. No agricultural activities were placed in the surroundings or in the lake catchment.

After the sampling, the specimens were transported to the lab in plastic bags. In the lab, the wounded and sick specimens (about 3% of the sampling specimens) were discarded in order to use only healthy specimens in the experiment. The organisms were then placed in aquarium with continuous water flux for seven days for acclimitization. Table 1 presents the number, average weight and standard length (with the standard deviation) of the specimens used in the experiment for each of the exposure times.

| Time(h) | M | L | G | Weight (g) | Tested Concentration (µg.g) |
|---------|---|---|---|-----------|-----------------------------|
| 24      | 8 | 8 | 8 | 99 ± 34   | 0.0606                      |
| 48      | 8 | 6 | 8 | 93 ± 17   | 0.0645                      |
| 72      | 8 | 6 | 7 | 87 ± 23   | 0.0689                      |
| 96      | 8 | 4 | 4 | 116 ± 11  | 0.0517                      |

The mercury chloride solution was prepared diluting 0.015g of HgCl₂ in 0.5mL of 4 N HCl and made to 250mL with PBS (Phosphate Buffer Solution). This solution was used in the acute intraperitoneal contamination experiment. The volume of mercury chloride solution that was injected into the peritoneal cavity of the studied fish was 0.1mL with 6 µg of Hg/fish. The concentration tested in relation to the weight of each individual is described in Table 1. The mean concentration tested in each fish was 0.0614 µg.g. After the injection of the Hg solution, the fish was placed in the aquarium and in the intervals of 24, 48, 72 and 96 h the specimens were measured, weighed and opened in order to remove the liver, muscular and gill tissue samples. The control samples were also collected from the fishes that were injected with 0.1mL PBS only. Aliquots of approximately 1g (w/w) of liver, gills and muscle samples were submitted to strong acid digestion in triplicate following a modified methodology described by Bastos et al. (1998). All Hg determinations were done in a Varian ICP-AES (model Liberty II) with cold vapor generating accessory (VGA 77). In order to verify the possible contamination of the extracts, chemical blanks were prepared and analyzed for each group of 10 samples.

The precision of the methodology was tested through the analysis of certified reference material.
Acute Intraperitoneal Mercury Chloride Contamination and Distribution

RESULTS AND DISCUSSION

Laboratory studies on the analysis of stress responses in the tissues of organisms exposed to metal can help to understand the mechanism through which metals exert their toxicity on the organisms. These results could be used to explain the impact of heavy metal toxicity on the organisms in the field. Mercuric chloride has been studied extensively as a model of nephrotoxicity for several animal species. However, little information is available on the toxic effect of HgCl$_2$ (Boujbiha et al., 2009).

The average Hg concentration measured in the liver was always higher (744 µg.kg$^{-1}$) than the concentrations measured in the gills (339 µg.kg$^{-1}$) and muscle (68 µg.kg$^{-1}$), and a significant difference ($p = 2.2 \times 10^{-5}$) was observed among the studied tissues (Table 2).

The Hg concentration in muscle of the organisms, initially increased in the first 48 h of contamination (58 µg.kg$^{-1}$ and 90 µg.kg$^{-1}$). After 72 h, a decrease in Hg concentrations was observed (53.3 µg.kg$^{-1}$) and at 96 h another increase in Hg concentration was observed (68.8 µg.kg$^{-1}$) (Fig. 1).

Table 2 - Average mercury concentrations (µg.kg$^{-1}$) in muscle, gill and liver of Hoplias malabaricus in all studied exposure times.

| E.T.* | Liver   | Gill    | Muscle  |
|-------|---------|---------|---------|
| Control | 127±24  | 54±13   | 57±5    |
| 24     | 370±156 | 276 ± 108 | 58±6.4 |
| 48     | 714±248 | 267 ± 27 | 90±13.9 |
| 72     | 633±235 | 284 ± 78 | 57±13.4 |
| 96     | 1260±753| 529 ± 273| 68±12.0 |

*Exposure time in hours.

Figure 1 - Average mercury concentrations (µg.kg$^{-1}$) in fish muscle (Hoplias malabaricus) at different times of exposure (hours).

The total mercury values measured in the muscles of the control specimens collected from the lagoons of Itaocara-RJ were considered low when compared to the total mercury values found for the fish of the same species collected from the Paraíba do Sul River (averages from 137 to 235 µg.kg$^{-1}$), which was the source of fish for this region (Yallouz et al., 2000). Yet, comparing the mercury...
values measured in the muscles of “traíra” injected with HgCl\textsubscript{2} to the WHO values, the findings were below the established limit (500ng.g).

No significant difference was observed (p=0.56) when Hg concentrations from the contaminated and control muscle samples were compared. This could be explained by the short time of exposure of individuals to mercury, since the Hg is transported by the blood to the liver and other tissues to then be stored in the muscle.

The Hg concentration results in the gills (Fig. 2) showed a more homogeneous pattern of Hg in the control samples (average 54.4 ± 13.7µg.kg\textsuperscript{-1}) as well as in the first three periods of exposure (24, 48 and 72 h) (average 276 ± 8.4 µg.kg\textsuperscript{-1}). After 96 h, the gills showed Hg concentrations three times higher than the previous time of exposure and 10 times higher when compared to average concentrations of the control. When comparing the gill Hg concentrations of the contaminated specimens among the distinct exposure times, a significant difference (p=0.004293) was observed. A significant difference was also observed when Hg concentrations in the control and contaminated samples were compared (p = 5.79 x 10\textsuperscript{-5}).

![Figure 2 - Average mercury concentrations (µg.kg\textsuperscript{-1}) in fish gill (Hoplias malabaricus) at different times of exposure (hours).](image)

The high mercury concentrations observed in the gill of the fishes under a regime of 96 h exposure were probably a consequence of its after metabolism release to the water and reabsorption by the gills.

Mercury distribution in the liver of contaminated specimens followed the same pattern described for the gill, although the observed concentrations were higher. Therefore, an increase of Hg concentrations within 48 h after the contamination was observed (370µg.kg\textsuperscript{-1} in 24 h; 714µg.kg\textsuperscript{-1} in 48 h) with a decrease in concentrations after 72 hours of exposure (633µg.kg\textsuperscript{-1}) and a second increase in Hg concentrations after 96 h of exposure time (1.260µg.kg\textsuperscript{-1}, Fig. 3). A significant difference was observed between the liver contaminated samples and liver control samples (p= 2.7 x 10\textsuperscript{-5}).

![Figure 3 - Average mercury concentrations (µg.kg\textsuperscript{-1}) in fish liver (Hoplias malabaricus) at different exposure times (hours).](image)
The average observed Hg concentration in the liver and muscle of the fishes differed significantly (p = 2.2 x 10^-6). The average values were 744µg.kg^-1 and 68µg.kg^-1, respectively (Table 2).

The average Hg concentrations obtained from the contaminated organs followed the sequence: Liver > Gill > Muscle. According to Frodello et al. (2000) and Gonzalez et al. (2005), the demethylation from the organic to the inorganic Hg occurs in the hepatocytes. Besides that, the bile released by the liver during the digestion might cause the dispersion of the Hg to other tissues (Allen et al., 1988). Similar results were mentioned by Simon and Boudou (2001) in Ctenopharyngodon idella contaminated with HgCl₂ and MeHg and by Kenedy (2003) in Carassius auratus exposed to inorganic Hg originated from the dental amalgam. Oliveira-Ribeiro et al. (1996) and Schultz et al. (1996) also observed the same tendency in Trichomycterus zonatus exposed to HgCl₂ in water and Ictalurus punctatus injected with HgCl₂ respectively. Similar results were obtained by Liao et al. (2005) with higher Hg concentrations observed in liver followed by kidney and muscle. In both the studies (present and Liao et al., 2005), the Hg concentrations observed in all the tissues were a consequence of the exposure time and the injected concentration. In natural environment, high concentrations of Hg in the liver and kidneys of aquatic animals were observed by Frodello et al. (2000) in the species of Cetacea, by Régine et al. (2006) in Acnodon oligacanthus, Pseudodancistrus barbatus, Semaprochilodus vari, Doras micropoeus, Hoplias aimara and Cynodon gibus, by Afonso et al. (2007) in Aphanopus carbo and by Raldua et al. (2007) in Barbus graelsii and Alburnus alburnus.

Schultz and Newman (1997) observed the same trend as the present study after the first administration of inorganic Hg to a channel catfish. Most of the Hg eventually became concentrated in the liver with only trace quantities accumulating in skeletal muscle. However, some species presented the inverse, as Cyprinus carpio (Goldstein et al., 1996), Leporinus friderici and Leporinus fasciatus (Régine et al., 2006), which showed higher Hg accumulation in the skeletal muscle instead of the previously mentioned organs. Gonzalez et al. (2005) studied the bioaccumulation of Hg in Danio rerio fed with a diet containing MeHg (5.0 and 13.5mgHg.g^-1).

According to Régine et al. (2006), who compared the Hg distribution in different fishes, there was a great difference based on the feeding habit of each species, besides that, the structural and functional characteristics of each species could modify the organotropism of Hg. Cano (2001) highlighted that the chemical form of Hg (organic or inorganic), concentration, the contamination path (exposure or ingestion), and depuration/excretion mechanism could interfere the Hg kinetics within organisms.

The muscle tissue has been indicated for the assessment of mercury contamination levels in the fishes from impacted environments, because of the preference of the organic form for that tissue (Akagi, 1995; Richardson et al., 1995). However, as it could be observed on the concentrations obtained from the different tissues here analyzed, the muscle tissue was not the best tissue to assess the level of contamination by inorganic mercury. Carvalho et al., (2009) analyzing the mercury levels in the muscles of “Tilapia” observed that the concentrations of mercury in this tissue could be insignificant in comparison to other organs. This observation was relevant, since the fish tissue consumed by humans was the muscle tissue and “Tilapia” showed resistance to the absorption of mercury in the muscle, even when exposed to high concentrations of that element, a conclusion similar to the one reached in the present study.

The distribution of mercury in the fish tissues results in the actions and interactions between the factors such as the exposure condition (importance of the entrance path via water/diet or intraperitoneal injection) and the chemical forms of the metal (inorganic mercury Hg(II)/MMHg) (Boudou and Ribeyre, 1997; Jackson, 1998; Wiener et al., 2003; Régine et al., 2006). Thus, the intraperitoneal administration path of the HgCl₂ contributed to the high total concentrations of Hg observed in the liver and gill of the fish. After the intraperitoneal injection, the chemical quickly enters the bloodstream through the portal vein, which drains the blood directly to the liver, where the mercury and nutrients are absorbed by the digestive system, metabolized and distributed through the systemic bloodstream, which carries the non-oxygenated blood that passes through the heart and is pumped to the gills, where it is oxygenated. Only then the blood is distributed to the body.

On the other hand the muscular tissue did not present affinity with inorganic mercury forms,
Although this tissue was the main storage site of the organic mercury forms (Oliveira-Ribeiro et al., 1996; Olson et al., 1978; WHO, 1990).

Apparently, mercury distribution took approximately 24 h in most organs, except the head, where the peaks of radioactivity in an experimental situation were only achieved in two or three days (HSDB, 2000). This feature was important to determine the first time of exposure of fish to the metal in the experiment in question. Variations in the concentrations of Hg in the liver and muscle are common when animals are subjected to a single dose of the contaminant. Schultz et al., (1996) had observed a decrease in Hg concentrations in the muscle after 24 h of exposure in a study developed with inorganic Hg intravascular administration in Ictalurus punctatus (Rafinesque, 1818) for 12, 24, 48 and 72 h. This behavior would normally be interpreted as the result of excretion of the metals from the animal. Although Oliveira et al., (1996), studying the effect of the exposure of Trichomycterus zonatus of inorganic mercury in the water in successive daily doses, observed a continuous growth of the concentration of metal with the time of exposure, with no decrease in any of the exposure times.

Part of Hg eliminated by the bile in the intestine suffers enterohepatic cycle and is probably reabsorbed into the blood. This process is surely responsible for the decrease in the mercury elimination rate and in the increase in its accumulation, and it also could explain the second rise in concentrations observed in the 96 h exposure (WHO, 1990). The mercury accumulation pattern in different tissues of H. malabaricus indicates a degree of organ specificity, which may be related to the differences in physiological functions (Krishnakumar et al. 1990; James et al. 1993).

This result is similar to other work that report the toxicity of mercury in tropical or subtropical carnivores fishes, submitted to acute tests in laboratory. The highest mercury concentrations found in the liver, when compared to muscle and gills, could affect the histology and consequently the functionality of the body. Therefore, this would cause inefficiency in the metabolism and excretion of these toxic substances.

REFERENCES

Afonso, C., Lourenço, H.M., Dias, A., Nunes, M.L., Castro, M. (2007), Contaminant metals in black scabbard fish (Aphanopus carbo) caught off Madeira and the Azores. Food Chem., 101, 120-125.

Akagi, H., Malm, O., Braches, F.J.P. (1995), Human exposure to mercury due to mining in the Amazon, Brazil-a review. Environmental science, 3, 199-211.

Allen, P., Yoke, S., Keong, W.M. (1988), Acute effects of mercury chloride on intracellular CSH level and mercury distribution in the fish Oreochromis aureus. Bull. Environ. Contam. Toxicol. 40, 178-184.

Bastos, W.R., Malm, O., Pfeiffer, W.C., Cleary, D. (1998), Establishment and analytical quality control of laboratories for Hg determination in biological and geological samples in the Amazon, Brazil. Cienc. Cult., 50, 255-260.

Borg, K., Gottofrey, J., and Tjalve, T. (1988). Effects of some chelating agents on the uptake and tissue distribution of 203Hg$^{2+}$ in the brown trout (Salmo trutta): Studies on ethyl- and isopropylxanthate, diethyl-diisopropylidithiophosphate, dimethyl- and diethylidithiocarbamate and pyrinethione. Arch. Toxicol. 62, 387–391.

Boudou, A. and Ribeyre, F. (1997), Aquatic ecotoxicology: From the ecosystem to the cellular and molecular level. Environmental Health Perspectives, 105, 21-35.

Boujbiha, M.A, Hamden, K., Guermazi, F., Bouslana, A., Omezzine, A., Kammoun, A. and El Feki, A. (2009), Testicular toxicity in mercury chloride treated rats: Association with oxidative stress. Reproductive Toxicology, 28, 81-89.

Cano, E.S. (2001), Toxicología del mercurio. Actuaciones preventivas en Sanidad laboral y ambiental. In: Jornada Internacional sobre el impacto ambiental del mercurio utilizado por la minería aurífera artesanal en Iberoamérica, Lima. Anais eletrônicos... Lima, Disponível em: http://www.gama-peru.org/jornadahg/espanol.pdf. Acesso em: 14 nov. 2006.

Carvalho, G.G.A., França, J.G., Dias, D.C., Lombardi, J.V., Paiva, M.J.R., Carvalho, S., Sarriés, G.A., Ferreira, J.R. (2009), Bulletin of Environmental Contamination and Toxicology, 82, 300-304.

Fitzgerald, W.F., Lamborg, C.H., (2005), Geochemistry of mercury in the environment. In: H.D. Holland and K.K. Turekian, Editors, Treatise on Geochemistry, Environmental Geochemistry, 9, Elsevier, San Diego, USA, pp. 108–148.
Krishnakumar, P.K., Damodaran, R. and Nambisan, H., Harrison, S. E., and Klaverkamp, J. F. (1989). Uptake, distribution and depuration of mercury in the green mussel Perna viridis (Linneaus). Proc. Indian Acad. Sci. 99, 345-352.

Jackson T.A. (1998). Mercury in aquatic ecosystems. In: Langston WJ, Bebianno MJ, editors. Metal metabolism in aquatic environments. London, Chapman and Hall, p. 97–159.

James, R., Sampath, K., Devakiamma, G. 1993. Accumulation and Depuration of mercury in a Catfish Heterooneustes fossilis (Pisces: Heterooneustidae) exposed to sublethal doses of the element. Asian Fisheries Science. 6, 183-191.

Liao, C.Y., Zhou, Q.F., Shi, J.J.B. and Fu, G.B., Jiang (2005), Mercury Accumulation and Distribution in Medaka after the Exposure to Sublethal Levels of Methylmercury. Bull. Environ. Contam. Toxicol., 75, 584–591.

Mela, M. (2004), Uso de biomarcadores na avaliação dos efeitos do metilmercúrio em Hoplias malabaricus (BLOCK, 1794) (Traíra). 2004. 124f. Dissertação (Mestrado em Biologia cellular e molecular) Departamento de Biologia Celular, Setor de Ciências Biológica, Universidade Federal do Paraná, Curitiba.

Norrgren-Karlsson, L., and Runn, P. (1985). Cadmium dynamics in fish: Pulse studies with 109 Cd in female zebrafish, Brachydanio rerio. J. Fish. Biol. 27, 571–581.

Oliveira Ribeiro, C.A., Guimaraes, J.R., Pfeiffer, W.C. (1996), Accumulation and distribution of inorganic mercury in a tropical fish (Trichomycterus zonatus). Ecotoxicol. Environ. Saf., 34, 190-195

Olson, K.R., Bergman, H.L., Fromm, P.O. (1973), Uptake of methyl mercuric chloride and mercuric chloride by trout: A study of uptake pathways into the whole animal and uptake by erythrocytes in vitro. J. Fish. Res. Board. Can., 30,1293–1299.

Olson, K.K., Squibb, K.S., Cousins, R.J. (1978), Tissue uptake, subcellular distribution, and metabolism of CH3HgCl by rainbow trout, Salmo gairdneri. J. Fish. Research Board Can., 35, 381-390

Raldúa, D., Díez, S., Bayona, J.M., Barceló, D. (2007), Mercury levels and liver pathology in feral fish living in the vicinity of a mercury cell chlor-alkali factory. Chemosphere, 66, 1217-1225.

Régine, M.B., Gilles, D., Yannick, D., Boudou, A. (2006), Mercury distribution in fish organs and food regimes: Significant relationships from twelve species collected in French Guiana (Amazonian basin). Sci. Total. Environ., 368, 262–270

Richardson, G.M., Egyed, M., Currie, D.J. (1995), Does acid rain increase human exposure to mercury? A Review and analysis of recent literature. Environmental toxicology and chemistry, 14, 809-813.
Schultz, R.I., Newman, C.M. (1997), Methylmercury Toxicokinetics in channel catfish (Ictalurus punctatus) and largemouth bass (Micropterus Salmoides) after intravascular administration. Environ. Toxicol. and Chem. 16 (5), 990–996.

Schultz, R.I., Peters, L.E., Newman, C.M. (1996), Toxicokinetics and disposition of inorganic mercury and cadmium in channel catfish after intravascular administration. Toxicol. and Applied Pharm., 140, 39-50.

Simon, O., Boudou, A. (2001), Direct and trophic contamination of the herbivorous carp ctenopharyngodon idella by inorganic mercury and methylmercury. Ecotoxicology and Environmental Safety, v. 50, 48-59.

Sorensen, E. (1991), Mercury. CRC Press, Boca Raton, FL.

Stary, J., Havlik, B., Kratzer, K., Prasilova, J., and Hanusova, J. (1981). Mercury circulation in aquatic environment. Part 4: The accumulation inorganic mercury and phenylmercury by Fish (Poecilia reticulata (Peters)). Acta Hydrochim. Hydrobiol. 9, 545–553.

Wiener JG, Krabbenhoft DP, Heinz GH, Scheuhammer AM. (2003).Ecotoxicology of mercury. In: Hoffman DJ, Rattner BA, Burton GA, Cairns J, editors. Handbook of ecotoxicology. Boca Raton, Lewis Publ; p. 409– 63.

WHO. (1990), World Health Organization. Environmental Health Criteria 101. Methylmercury. Geneve, Switzerland: World Health Organization.

Williams, D.R., Giesy, JR (1978), Relative importance of food and water sources to cadmium uptake by Gambusia affinis (Poeelliidae). Environ. Res. 16, 326-332.

Yallouz, A.V., De Campos, R.C., Paciornik, S. (2000), A low-cost non-instrumental method for semiquantitative determination of mercury in fish. Fres. J. Anal. Chem. 366, 461-465.

Received: December 01, 2008;
Revised: August 12, 2009;
Accepted: November 11, 2010.