Histopathological and Pathomorphological Effects of Mercuric Chloride on the Gills of Persian Sturgeon, *Acipenser persicus*, Fry

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Abstract Histopathological and pathomorphological effects of 15 ppb mercuric chloride on Persian sturgeon, *Acipenser persicus*, were investigated using histological and electron microscopy observations. Light microscopy showed that the gill epithelial hypertrophy, wrinkling and hyperplasia in lamellar epithelia and lamellae fusion occurred after 48 h of exposure. Gill epithelia also showed occasional necrosis, which had almost been completed and blood emerged from the capillaries. However, occasional necrosis in some regions of the filament, both with blood emerging and with no bleeding, was observed by using electron microscopy. These injuries were well observed in inter-lamellar regions of the filament and also wrinkling of the lamellar epithelium. Ultrastructural observations showed some cellular disorders in gill epithelium of the Persian sturgeon, *A. persicus*, fry. In addition, increase in apical vesicles of the chloride cells and necrosis in apical surfaces of some chloride cells, hypertrophy and necrosis of the chloride cells’ mitochondrion and endoplasmic reticulum also were some of the other cellular disorders observed through transmission electron microscopy. In conclusion, the gills of *A. persicus* fry were sensitive to low concentrations of inorganic mercury (HgCl₂).

Key words: *Acipenser persicus*, Fish, Gill, Histopathology, Mercuric chloride, Pathomorphology

1 INTRODUCTION

Heavy metals are introduced into the marine environment in a number of ways. They may be deposited in the sea ‘naturally’ as a consequence of erosion from ore-bearing rock, windblown dust, volcanic activity and forest fires. Increasingly, however, they are introduced via contaminated rivers, marine outfalls and through the deliberate dumping of wastes in coastal waters (Schindler, 1991; Agusa et al., 1994).

Mercury pollution in aquatic ecosystems has received a great deal of attention since the discovery of mercury as the cause of Minimata disease in Japan in the 1950s (Allen et al., 1988). The fate of mercury in the environment depends on the chemical form of mercury released and the environmental conditions. Elemental mercury, inorganic mercury and methyl-mercury are the three most important forms of mercury in natural aquatic environments. Most mercury is released into the environment as inorganic mercury, which is primarily bound to particulates and organic substances and might not be available for direct uptake by aquatic organisms (Beckvar et al., 1996).

Toxicity is influenced by the form of mercury, environmental media, environmental conditions, sensitivity or tolerance of the organism, and its life history stage. Inorganic mercury is less acutely toxic to aquatic
organisms than methyl-mercury, but the range in sensitivity among individual species for either compound is large. Toxicity was found to be greater at elevated temperatures, lower oxygen content, reduced salinities in marine environments, and in the presence of metals such as zinc and lead (Oliveira and Torres, 1995; Beckvar et al., 1996).

Once in the aquatic ecosystem, part of the inorganic mercury can be microbiologically converted into methyl-mercury and taken up by aquatic organisms. Fish accumulate mercury directly from food and the surrounding water and can bio-concentrate large amounts of this metal (Rainbow, 1985).

The respiratory system provides the most extensive interface of a fish with water and is frequently the first system to be affected by dissolved pollutants (Heath, 1995). Mercury causes strong toxicological effects on the cell membrane and many aspects of its toxic action have been attributed to its ability to cross the cell membrane and to disrupt cellular ion transport processes (Stinson and Mallatt, 1989). Many changes in water quality are rapidly reflected in fish gill structure, since this organ is continuously and directly exposed to the external environment (Tkatcheva et al., 2004). Numerous types of gill damage have been documented in fish experimentally exposed to toxicants or in populations sampled from polluted environments (Alazemi et al., 1996; Pawert et al., 1998; Thophon et al., 2003). Most of the gill histo-pathological changes are largely non-specific as confirmed by the occurrence of similar alterations under a wide range of toxicant-exposure conditions (Mallatt, 1985). Hyperplasia with lamellar fusion, telangiectasia, edema with epithelial lifting and desquamation, are typical lesions of gills in response to organochlorines, petroleum compounds, organophosphates, carbamates, herbicides and heavy metals (Alazemi et al., 1996; GlobalTox, 1997; Jiraungkoorskul et al., 2003; Thophon et al., 2003; Dezfuli et al., 2006; Giari et al., 2007) and suggest an impairment to the respiratory and osmoregulatory functioning of the gills.

Because of important functions of fish gills, such as respiration, acid-base balance, excretion, and osmoregulation, and because they are the most important organs for the uptake of inorganic mercury in fish (Allen et al., 1988), they are considered very efficient indicators of water quality (Roncero et al., 1990; Kirk and Lewis, 1993).

Histopathology is an important component of several measures of an organism’s health and histo-pathological markers have been recommended for field application, more often as a generalized, non-specific response to several stressful stimuli (Teh et al., 1999). Persian sturgeon, *Acipenser persicus*, is one of the vulnerable species of Caspian Sea sturgeons. The adults live in the Caspian Sea (10–13 ppt) but spawning occurs in freshwater rivers (such as ‘Sefid-rood’, Iran); fry live in rivers for a couple of months and then they return back to the Caspian Sea. The Caspian Sea is an enclosed water body that is fed from several freshwater rivers, many of them carrying different land source pollutants, such as heavy metals. Although some investigation has been provided on the histopathological effects of inorganic mercury on teleost fish liver, kidney, gill, olfactory epithelium and spleen (Handy and Penrice, 1993; Skak and Baatrup, 1993; Oliveira Ribeiro et al., 1996, 2002; Samson and Shenker, 2000), there is a paucity of information concerning the effects of water-borne pollutants on Caspian Sea sturgeon fry. The purpose of this work is to describe the acute histopathological effects of inorganic mercury from water on *Acipenser persicus* fry, using histopathological observations and electron microscopy (TEM and SEM).

## 2 MATERIALS AND METHODS

### 2.1 Fish and experiments

*Acipenser persicus* fry (3g ± 0.3 and 3.30-8.12 cm ± 0.5), were obtained from Shahid Rajaei Hatchery (Sari, Iran) during June, 2006 and
adapted to experimental conditions (running dechlorinated tap water at 25°C (with a 12 h-D, 12 h-L photoperiod) for a minimum period of 15 days. The experiment began with the transfer of 60 fish to a 100-l aquarium containing 15 ppb HgCl₂ (Merck). Control fish were placed in another aquarium containing uncontaminated water and both groups were not fed during the experiment.

From each group, six samples were collected after 48 h, and their gills were dissociated and fixed for histological and ultrastructural examinations. Gill samples were fixed in Bouin’s solution for 24 h, dehydrated in a graded series of ethanol, and embedded in Paraplast (Merck). Three-micrometer-thick sections were obtained and stained in hematoxylin/fushin for examination in light microscopy (Martoja and Martoja-Pierson, 1967; Khodabandeh et al., 2005a; Khodabandeh et al., 2008; Khodabandeh et al., 2009).

2.2 Electron microscopy
For scanning electron microscopy (SEM), gill samples were placed in cold 4% glutaraldehyde in 0.1 M phosphate buffer, pH = 7.4, containing 5% sucrose. After an initial 12-h fixation, followed by rinsing in 0.1 M phosphate buffer containing 5% sucrose, the samples were post-fixed for 1 h in 2% osmium tetroxide in 0.1 M phosphate buffer, pH = 7.4, containing 5% sucrose (Glauert, 1974; Khodabandeh et al., 2005b).

For transmission electron microscopy (TEM), gills were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer for 24 h at room temperature, pH = 7.4. They were then rinsed in sodium cacodylate buffer and post-fixed for 1 h in a mixture (v/v) of 2% osmium tetroxide and 0.45 M sodium cacodylate buffer at room temperature. Samples were washed in distilled water and dehydrated in a graded series of ethanol and propylene oxide, then embedded in Spurr’s resin. Semi-thin and ultra-thin sections were cut on a Reichert OMU3 ultramicrotome. The first sections were stained with toluidine blue. Ultra-thin sections were contrasted with 2% uranyl acetate in 70% alcohol and lead citrate, and they were observed on a JEOL 1200 EX2 transmission electron microscope at 70 kV (Glauert, 1974; Khodabandeh et al., 2005b; Ghanizadeh Kazerouni and Khodabandeh, 2010).

3 RESULTS
During the course of the experiment, no mortality was observed in all experimental groups. Gill histological micrographs of the control group showed that the gill was made up of filaments arranged in double rows and the lamellae arise from these filaments (Fig. 1A). The lamellae are lined by squamous epithelium called pavement cells (Fig. 1B). Below the squamous epithelium lamellar blood sinuses separated by pillar cells are shown (Fig. 1B). Between the lamellae, the filament was lined by a thick stratified epithelium. This region contains chloride cells (Fig. 1B).
Fig. 1 Histology of *A. persicus* gill in control (A,B) and mercuric chloride-contaminated groups (C–H). Filament and lamellae (A); cellular structure of the filament and lamellae (B). Hypertrophy of the epithelial cell in lamellae (C: black arrow); wrinkling of the epithelium (D,E: black arrows); epithelial hyperplasia and epithelial necrosis without blood emerging (F: black arrows); lamellae fusion (G); necrosis in epithelium with blood emerging (H). BS: blood sinuses; CC: chloride cell; F: filament; L: lamellae; PC: pavement cell; PiC: pillar cell; RBC: red blood cell.

In the HgCl$_2$-contaminated fish gills, the epithelium of lamellae showed some histopathological changes. The most important of these was hypotrophy of some epithelial cells in lamellae (Fig. 1C) and wrinkling of the lamellar epithelium (Fig. 1D,E). Also, histopathological lesions such as lamellae fusion and lamellae surface hyperplasia were observed (Fig. 1F-H).
Fig. 2 Gill scanning electron micrographs of *A. persicus* in the control group (A) and in the mercuric chloride-contaminated group (B–F). Filament and lamellae (A); epithelial necrosis with and without blood emerging (B,D: arrows); wrinkling of the lamellar epithelium (E: arrows); epithelium necrosis in interlamellar region (F: arrows). F: filament; L: lamellae; IL: interlamellar region.

Scanning electron micrographs also showed occasional necrosis in some regions of the filament, both with blood emerging and with no bleeding (Fig. 2C–F). These injuries were well observed in interlamellar regions of the filament (Fig. 2F). With regard to histological observations, the wrinkling of the lamellar epithelium was clearly observed through scanning electron micrographs (Fig. 2C–F).
Numerous apical vesicles in chloride cell, partial necrosis of the apical part of the cell (A,B). Swelling of the mitochondrion and necrosis in endoplasmic reticulum of the chloride cells (C,D). AP: apical; N: necrosis; TM: turgid mitochondria; V: vesicle; ER: endoplasmic reticulum.

Transmission electron micrographs also showed some intercellular disorders in gill epithelial cells of the Persian sturgeon fry (Fig. 3A, D). Increase in apical vesicles of the chloride cells (high activity of transportation between cells and the surrounding environment) and necrosis in apical surfaces of some chloride cells were some observed alterations (Fig. 3A, B). Swelling and necrosis of the chloride cell mitochondrion and endoplasmic reticulum also were some of the other cellular disorders observed through TEM, in HgCl$_2$-exposed fish gills (Fig. 3C, D).
4 DISCUSSION
In the present work we studied some histological damage in *A. persicus* gills such as tissue necrosis, chloride cells hypertrophy and emergence of blood.

Severe gill damage of fish exposed to high levels of water-borne organic and inorganic Hg was previously described by Handy and Penrice (1993) and Oliveira Ribeiro *et al.* (1996). In addition, Gregory *et al.* (2002) reported the effects of dissolved mercury on the gills of mollusca (*Perna perna*), reinforcing the physiological importance of this respiratory structure to aquatic organisms and its vulnerability to dissolved toxicants once this organ is in direct and constant contact with the surrounding water. The damage observed in the present work certainly could interfere with the efficiency of gas exchange, as described by Jagoe *et al.* (1996a). According to Jagoe *et al.* (1996b), the effects of sub-lethal and more realistic doses of mercury on fish have not yet been explored in detail, but the authors described the effects of low and moderate doses (75, 150 and 300 nM) of inorganic Hg on the gill physiology of *Micropterus salmoides*. Circulation anomalies and the proliferation of epithelial cells with lamellar fusion or excessive mucus secretion were the most evident consequences to the mosquito fish, *Gambusia hobrooki*, when it was exposed to water-borne inorganic Hg (Jagoe *et al.*, 1996a); the physiological consequence was a decrease of gas exchange. The fusion between the secondary lamellae described for *Trichomycterus zonatus* (Oliveira Ribeiro *et al.*, 2000) under the same experimental conditions as those described in this work, reinforces the need for understanding the interference of chemical and physical conditions with the bioavailability of dissolved inorganic Hg to biological membranes.

Although alterations to these vital organs have been used as a morphological biomarker to assess environmental pollution, many toxicants can be associated with pathological gill events, and it becomes difficult to relate gill damages to a particular pollutant in a contaminated environment (Takashima and Hibiya, 1995). Alterations described in this work to gills exposed to mercuric chloride are not specific to Hg and may also be observed with some other contaminants or as a chronic response to parasitic or bacterial infections. Gill damage at tissue and cellular levels provides a sensitive but not specific indication of the general quality of water in a contaminated environment. These types of damage seem reversible even at acute dosages and cannot be seen as indicators of long-term chronic exposure.

In present study, lamellar fusion, gill epithelial hyperplasia and epithelial necrosis with blood emergence were some of the effects of exposure to mercuric chloride that may be causes of respiratory and osmoregulatory disorders. These alterations also may play a defensive role against contamination rather than have an irreversible toxic effect. However, these modifications can produce adverse effects on fish health, and may increase their susceptibility to secondary infectious diseases and even death (Hawkins *et al.*, 1984).

Gill chloride cells are the most active cells of the fish gill, and absorb and secrete many ions and electrolytes (Evans *et al.*, 2005). Because of these functions, increase in the apical vesicles of chloride cells of *A. persicus* gills could be a result of an increase of their ion exchange activity. In the present study many of the chloride cells showed high amounts of apical vesicles, which may be responsible for excreting mercuric chloride. Also, in apical surfaces of these cells, the cell membrane was partially necrotic.

Evans (1987) noted that chloride cells were the cells most affected by different environmental pollutants (heavy metals, herbicides, oil pollution, acid rain, etc.), and after exposure to pollutants severe disorders were observed in these cells. These phenomena may be the result of high transport activity of these cells. Results of the present study showed that, like many other species (Baker, 1969;
In conclusion, this study confirms that both histopathological and pathomorphological aspects of *A. persicus* gills were affected by exposure to mercury. This phenomenon confirmed those reported in other fish species under a wide range of exposure conditions. Therefore, fish gills are the most significant organs for detection of environmental pollution by mercury components.

5 REFERENCES


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