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Morphological evidence of neurotoxicity in retina after methylmercury exposure

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ABSTRACT

The visual system is particularly sensitive to methylmercury (MeHg) exposure and, therefore, provides a useful model for investigating the fundamental mechanisms that direct toxic effects. During a period of 70 days, adult of a freshwater fish species *Hoplias malabaricus* were fed with fish prey previously labeled with two different doses of methylmercury (0.075 and 0.75 $\mu g\, g^{-1}$) to determine the mercury distribution and morphological changes in the retina. Mercury deposits were found in the photoreceptor layer, in the inner plexiform layer and in the outer plexiform layer, demonstrating a dose-dependent bioaccumulation. The ultrastructure analysis of retina revealed a cellular deterioration in the photoreceptor layer, morphological changes in the inner and outer segments of rods, structural changes in the plasma membrane of rods and double cones, changes in the process of removal of membranous discs and a structural discontinuity. These results lead to the conclusion that methylmercury is able to cross the blood–retina barrier, accumulate in the cells and layers of retina and induce changes in photoreceptors of *H. malabaricus* even under subchronic exposure.

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1. Introduction

Recently we published the localization of mercury in *Danio rerio* after trophic and acute exposure to methylmercury (Mela et al., 2010) where some physiological effects on retina could be hypothesized, but cellular effects in retina under realistic exposure conditions were not yet published. The toxic compound methylmercury (MeHg) is a form of mercury commonly encountered in the environment, responsible for a specific range of neurological effects. Chronic exposure to MeHg may contaminate the biota with risk to wild vertebrate and human central nervous system, notably in the visual system (Kusmic and Gualtieri, 2000; Saldana et al., 2006; Chang, 2007). This led MeHg pollution to be considered a continuous environmental hazard to human health, especially *via* fish-eating. In 2007 mercury was ranked third on the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) priority list of hazardous substances (Giari et al., 2008).

The visual system is susceptible to the toxicological effects of mercuric ions (Iwata and Abe, 1986; Canto-Pereira et al., 2005; Ventura et al., 2004, 2005, 2008; Bridges et al., 2007; Rand et al., 2009; Costa et al., 2008a,b; Feitosa-Santana et al., 2007; Barboni et al., 2008, 2009). *In vivo* studies in laboratory animals have shown that vision loss can result after exposure to mercury (Fox and Sillman, 1979; Evans and Garman, 1980; Shaw et al., 1980; Tessier-Lavigne et al., 1985; Warfvinge and Bruun, 1996). In adult monkeys exposed to mercury vapor by inhalation, autometallographic techniques show that mercury accumulates in the ocular tissues and remains there for a long period of time (Warfvinge and Bruun, 1996). In fish there are only a few studies describing the damage due to mercury exposure on the visual system. Pioneering these studies, Hawryshyn et al. (1982) showed behaviorally that the visual system of the rainbow trout is affected by exposure to mercury. In the last years, our group investigated the effects of MeHg in the retina of a freshwater fish species (*Hoplias malabaricus*) widely distributed in regions of Brazilian Amazon Rivers impacted by mercury. The experimentally exposed animals after acute and subchronic exposure to MeHg showed electrophysiological anomalous responses on horizontal cells (Tanan et al., 2006). In addition, Bonci et al. (2006) described losses of immunoreactivity of parvalbumin amacrine cells (PV-IR) and α protein kinase C bipolar cells (PKC-IR) after acute high doses of MeHg (2 and 6 $\mu g\, g^{-1}$). More recently, Mela et al. (2010) described abundant mercury deposits in the photoreceptor layer, inner nuclear layer, outer

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nuclear layer, plexiform layer, ganglion cell layer and in the cells from the disc region of *D. rerio* retina.

Fish eyes possess a complicated morphological and neural retinal organization compared to other animals. The retina of most fish, as with the majority of other vertebrates, is duplex, containing both rods and cones. Fish may have a scotopic system, with input from the rods, that is responsible for achromatic, high sensitivity, low acuity vision, while a photopic system using the cones is responsible for color vision, low sensitivity, high acuity vision at higher light intensities (Kusmic and Gualtieri, 2000).

According to Evans and Garman (1980), one of the earliest signs of mercury poisoning is an impairment of scotopic (night) vision. This aspect of vision is mediated primarily by rod photoreceptor cells, which are unable to respond appropriately to light following exposure to mercuric compounds (Evans and Garman, 1980; Tessier-Lavigne et al., 1985). In addition, chronic exposure to mercury may result in a perturbation of peripheral vision, followed by a more severe loss of central vision (Evans and Garman, 1980; Finocchio et al., 1980; Saldana et al., 2006). Surprisingly, the retinal target sites due to mercury exposure of vertebrates and the consequent neuronal damages were not completely elucidated yet.

The presence of metals in selected cell-types can be visualized with the aid of histochemical techniques such as autometallography (AMG). This technique allows the localization of metal ions as black silver deposits (BSD) in biological tissues (Danscher, 1994) and has been used to determine which cell-types are involved in metal accumulation in fish (Amaral et al., 2002; Mela et al., 2010).

Due to biomagnification in the food web, MeHg is of particular interest to those human populations whose diet contains significant quantities of predatory fish. Fish absorb MeHg throughout their life (Oliveira Ribeiro et al., 2008) hence, predatory fish, such as, *H. malabaricus* are generally very contaminated (Mela et al., 2007). Traíra, *H. malabaricus* (Bloch), is a freshwater carnivorous fish widely distributed in South America. This species is an interesting biological model for experimental study of dietary exposure to contaminants due to its voracious behavior, its ability to adapt to experimental conditions, and its high food chain position.

Despite the existence of a large number of studies, the knowledge about the toxic mechanisms of mercury and its pharmacokinetics is still a challenge for toxicologists. Studies focusing the distribution of minute traces of mercury in target tissues together with morphological, analytical and behavioral studies are the basis for understanding mercury neurotoxicity. To the best of our knowledge, the current study is the first comprehensive investigation aiming to determine the morphological changes in the retina of a vertebrate after subchronic dietary exposure to methylmercury, to support the diversity of mercury effects reported by the literature.

2. Materials and methods

2.1. Animals

Forty-five mature freshwater fish thraira (*H. malabaricus*) were obtained from a fish farm station located in Paraná State, Southern Brazil, and transported to Federal University of Paraná. Before the experiment, fish were acclimated to experimental conditions for 30 days (one fish for each 30 L aquarium in dechlorinated tap water, $T = 21 \pm 2$ °C, 12:12 h photoperiod). The food supply provided to each fish was one young live *Astyanax* sp, a natural freshwater prey fish species to thraira, from the same fish farm station without polluting sources. Fish were fed once every five days. All procedures using animals were performed according to the NIH guidelines and Federal University of Paraná commission for studies involving human or animal subjects (http://www.bio.ufpr.br/ceea/html/index.html).

2.2. Experimental design – MeHg exposition

After acclimation to laboratory conditions, the fish *H. malabaricus* were randomly separated into three groups. Two groups of fifteen individuals each (158 ± 10.6 g of wet weight and 23 ± 0.5 cm of total length) were exposed to doses of either 0.075 $\mu g\ g^{-1}$ or 0.75 $\mu g\ g^{-1}$ of MeHg. The fish were fed young live specimens of *Astyanax* sp previously injected ip with an aqueous solution of MeHg (CH_3HgCl, Sigma®, in HCl 0.1 M). Since each thraira was kept in one aquarium, it was possible to determine with precision the number of *Astyanax* specimens ingested by each fish. The final dose was based on the total amount of MeHg ingested by the fish. This total amount was the sum of the mercury content that had been injected into 14 *Astyanax* sp fish eaten by the thraira. Doses of 0.075 or 0.75 of MeHg were administered every 5 days and adjusted so as to have daily nominal doses of 0.015 (0.075 $\mu g\ g^{-1}$) and 0.15 (0.75 $\mu g\ g^{-1}$) $\mu g\ g^{-1}$ fish. Since mercury concentrations in the prey used as vehicle were not measured, total ingested MeHg dose was estimated from individual doses assuming that the injected MeHg in the prey was entirely transferred to the predators, as the time between mercury injection in the prey and predation was some few seconds. The total trophically absorption of MeHg by fish was described by Oliveira Ribeiro et al. (1999). The dose 0.075 $\mu g\ g^{-1}$ used in our work was very close to real conditions found in prey fish from Amazonian rivers impacted by mercury, as reported by Mela et al. (2007). The ten-fold higher dose (0.75 $\mu g\ g^{-1}$) was used for comparison. For the third group (control), live *Astyanax* sp were injected with only distilled water. Two days after the last dose, all animals were dark-adapted for 2 h, anesthetized with 0.02% MS222 (ethil-ester-3-aminobenzoic acid, Sigma®) and retina samples were immediately fixed for morphological analysis. A total of thirty retinas per sample were obtained. From each group a number of eight retinas were used as described: (1) autometallography analysis, (2) electron microscopy procedures and (3) scanning electron microscopy procedures. Additionally six flattened whole mount retinas were used for counts of paired cones and cellular maps of density (unpublished data). For each methodology, ten sections were analyzed per retina.

2.3. Autometallography

The retinas were fixed in Bouin fluid for 24 h at 4 °C and washed with ethanol 70% for 24 h. Dehydration was carried out in a series of ethanol baths (90%; 95%; 100%) and in butanol. Tissues were embedded in paraffin at 56 °C for 4 h. Serial sections (10 μm thick) were mounted in albumin coated slides, dried at 40 °C for 24 h, stained with Hematoxylin and Eosin solutions and mounted in Entellan resin. The sections were observed under a Leitz Orthonplan microscope. The autometallographic procedure was modified from Danscher (1994). The basic mechanism can be summarized as the formation of shells of metallic silver around nuclei of trace metals, after covering the biological section with an emulsion (Ilford Nuclear emulsion L4) and placing it in a bath of developer under a safelight. After drying in complete darkness (15 min) from both light and electron microscopy sections were rinsed in developer (Ultrafin tetenal, AGFA) for 15 min, in stop bath (1% acetic acid) for 1 min and in fixer (B&W Fixer, AGE, AGFA) for 10 min. Before every experiment the emulsion was checked to test uniformity of silver grains, by covering a slide without sections. Metal deposits appear as black silver deposits (BSD) indicating the presence of silver shells around the metals (Danscher, 1994; Soto et al., 1998).

2.4. Electron microscopy procedures

For ultrastructure investigations the retinas were fixed in 2% glutaraldehyde, 2% paraformaldehyde, 5 mM $CaCl_2$, 20 mM NaCl

dissolved in 0.1 M cacodylate buffer (pH 7.2–7.4) at room temperature for 2 h. The samples were postfixed in 1% osmium tetroxide in the same buffer for 1 h, dehydrated in a gradient series of ethanol, propylene oxide, and embedded in PoliEmbeb 812 DER736 resin; 200–400 nm slices were contrasted with uranil acetate (5%) for 20 min and lead citrate (Reynolds) for 5 min and observed in a JEOL TEM 1200 EXII electron microscope.

2.5. Scanning electron microscopy procedures

For scanning electron microscopy the retinas were fixed overnight in 3% glutaraldehyde in (0.1 M phosphate buffer, pH 7.4) at 4 °C. Tissue samples were dehydrated using a graded ethanol series until absolute ethanol was reached and a graded ethanol/acetone series until pure acetone was reached, after which they were dried to the critical point using CO_2 as a transition liquid. Filament pairs were glued with silver paint onto the specimen stub, coated with gold in a vacuum sputter, and examined in a JEOL JSM-6360LV scanning electron microscope.

2.6. Histopathological record

Morphological damages were measured according to the injury index described by Bernet et al. (1999), where photoreceptors alterations were classified in three severity factors (minimal, moderate and marked pathological importance). The injury indexes were obtained after the application of a mathematical equation established for each group of lesions:

$$\mathrm{IL} = \sum_{\mathrm{pr}} \sum_{\mathrm{alt}} (a \times w),$$

where pr is the reaction pattern; alt is the alteration; a is the score value; w is the importance factor.

The damages considered to establish the index lesion in the present work were: cell deterioration; changes in membranes which unite the two elements of a double cones; structural changes in plasma membrane; formation of vacuoles; changes in membranous disc elimination; discontinuity in the lamellar membranous disc and morphological changes in the inner and outer photoreceptors segments.

2.7. Statistical procedures

Data were expressed as mean ± standard error of mean. One-way analyses of variance (ANOVA) were used to determine significant differences in the results of the various groups; p-values < 0.05 were considered significant after Dunnett's *post hoc* test.

3. Results

3.1. Experimental conditions

No mortality occurred during the experiment, however the morphological lesions observed in the retinas of *H. malabaricus* revealed important alterations throughout the course of the experiment.

3.2. Mercury distribution in retina

After 14 doses, the retinas from control animals did not reveal the presence of mercury deposits by autometallography analysis (Fig. 1A). Differently, mercury deposits were easily distinguished as black silver deposits (BSD) under the light microscope in exposed fish at both doses analyzed. In retinas from fish exposed to 0.075 μg MeHg g^{-1} the mercury deposits were found in the photoreceptor layer (PL), in the outer plexiform layer (OPL) and in the inner plexiform layer (IPL) (Fig. 1B). In the group exposed to 0.75 μg MeHg g^{-1} mercury deposits were observed in the same sites, however with more intensity (Fig. 1C). These results showed that the higher doses of mercury lead to more deposits in retina of *H. malabaricus*. In both doses the mercury deposits were found evenly distributed throughout the retina. Unstained paraffin sections showed mercury deposits without interference from

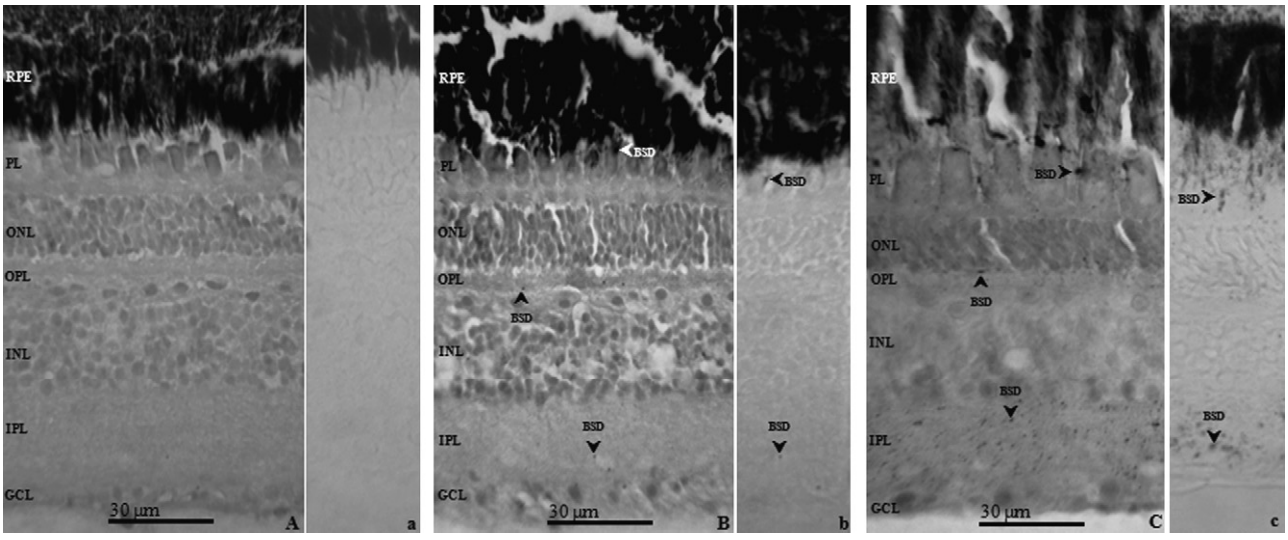

Fig. 1. Autometallographical demonstration of mercury in cross section of retina from *Hoplias malabaricus*. (A) Cross section of retina from *Hoplias malabaricus* control group counterstained with Hematoxylin–Eosin. No mercury is visualized. Retinal pigment epithelium (RPE); photoreceptor layer (PL); outer nuclear layer (ONL); outer plexiform layer (OPL); inner nuclear layer (INL); inner plexiform layer (IPL); ganglion cell layer (GCL). (a) Cross section of unstained retina from control group. No mercury is visualized. (B) Cross section of retina from *Hoplias malabaricus* exposed to 0.075 μg MeHg g^{-1}. Section counterstained with Hematoxylin–Eosin showing black silver deposits (BSD – arrow-heads) in PL, OPL and IPL. (b) Cross section of the unstained retina of *Hoplias malabaricus* exposed to 0.075 μg MeHg g^{-1}, with black silver deposits (BSD – arrow-heads) found in the PL and IPL. (C) Cross section of retina from *Hoplias malabaricus* exposed to 0.75 μg MeHg g^{-1}. Section counterstained with Hematoxylin–Eosin showing highest mercury deposits (BSD – arrow-heads) in PL, OPL and IPL. (c) Cross section of the unstained retina of *Hoplias malabaricus* exposed to 0.75 μg MeHg g^{-1}, with mercury deposits (BSD – arrow-heads) found in the PL and IPL.

the staining (Fig. 1b and c) to compare with slides after staining with Hematoxylin and Eosin. Similarly a slide of unstained paraffin section from control group is presented (Fig. 1a).

3.3. Photoreceptor morphological analysis

The retina consists of various cell types arranged in eight layers and two membranes. From the scleral side to the vitreous side, the second layer is photoreceptor cell layer (Figs. 1A and 3A). Structural and ultrastructural analysis allowed observation of the morphology of photoreceptors in *H. malabaricus*. As most fish do, this species has rods (Fig. 3A and B); long single cones (Fig. 3B), short single cones (Fig. 3B) and double cones (Fig. 3A and B); the double cones are a prominent feature in this species. Rods have slender cylindrical-shaped outer and inner segments. The cones are larger than rods; the outer segment presents a conical shape and the inner segment has a thick spindle shape. Each photoreceptor cell presents an outer segment, with membranous discs containing the visual pigment (Fig. 4A and C). The outer segment is attached to the inner segment by a connecting cilium (Fig. 4A). The inner segment has mitochondria, ribosomes, Golgi bodies, microtubules and cell nucleus. The mitochondrias are arranged perpendicular to the long axis of the photoreceptor cell (Fig. 4A). The double cones are typically composed of two partly jointed elements. Electron microscopy showed that these membranes are juxtaposed until the level of the inner segments and then separated by an extracellular space (Fig. 4B). Long filamentous membrane processes, known as calycal processes, depart from the apical region of the inner segment of both rods and cones to embrace the outer segment, except for the last terminal portion (Fig. 4B). This structure prevents rotational movements of the outer segment with respect to the cilium. The accessory outer segment extends along the cone outer segment (Fig. 4C). In fish cones, this ciliary structure does not contain visual pigment and its function is still debated.

After exposure to MeHg damage in the photoreceptor were found in individuals from both tested group. The lesion index increased significantly and the damage and injuries detected are summarized in Fig. 2. The current results demonstrated that the cell changes were dose-dependent showing that both photoreceptors (rods and cones)

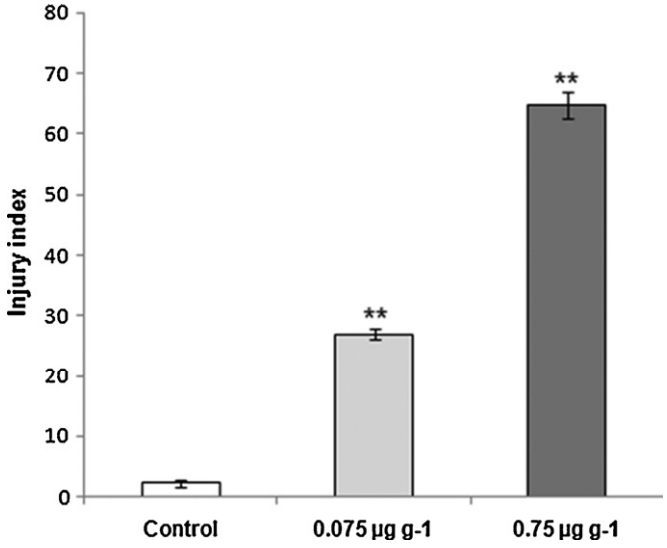

Fig. 2. Summary of photoreceptors damage. Lesion index determined according to Bernet et al. (1999) in *Hoplias malabaricus* after 14 trophic doses of methylmercury (0.075 and 0.75 μg MeHg g^{-1}). Data are expressed as mean ± standard error of mean. **Indicates statistically significant differences ($p < 0.01$) between exposed groups and control group.

of *H. malabaricus* are sensitive to MeHg when experimentally trophically exposed. The morphological analysis in photoreceptors cells revealed that 90% and 60% of retinas presented damage respectively under 0.75 μg MeHg g^{-1} and 0.075 μg MeHg g^{-1}.

The vast majority of the cells exhibited a clear deterioration (Fig. 3F and G) and morphological changes in the outer (Fig. 3D) and in the inner (Fig. 3E) segment of rods. The disc membrane of the rods did not show continuity with the plasma membrane of the outer segment (Fig. 4G). In the cones, the discs are invaginations of the plasma membrane and have direct contact with the extracellular space. In retinas from fish exposed to 0.075 μg MeHg g^{-1} we found clear structural changes in this membrane (Fig. 4H). In addition, plasma membrane changes were observed in membranes which unite the two elements of double cones in fish exposed to 0.75 μg MeHg g^{-1} (Fig. 3C).

The photoreceptor discs regenerate at the base of the outer segment to replace older discs phagocytized at the apex of the outer segment by retinal epithelial pigment cells (Fig. 4C). However, we observed a change in this disc elimination in both groups exposed to methylmercury. In retinas exposed to 0.075 μg MeHg g^{-1}, a removal of membranous discs occurred laterally rather than at the apical region (Fig. 4E). Additionally, in the group exposed to 0.75 μg MeHg g^{-1} the disc elimination often occurred with the formation of vacuoles (Fig. 4D and F). Finally, there was a discontinuity in the structure of lamellar discs in the rods at the lower dose tested (0.075 μg MeHg g^{-1}) (Fig. 4I).

4. Discussion

The localization of mercury in mammal tissues by AMG is a consolidated method described by Danscher and Möller-Madsen (1985), however data about mercury localization on neotropical fish tissues are scarce, even though the largest and the richest hydrographic basin in biodiversity on Earth, the Amazon basin, is actually impacted by mercury from different sources (Rabitto et al., 2011). In the current study, the autometallography was applied to assess the cell-types of the retina selectively involved in metal accumulation after methylmercury exposure, bringing important information toward the understanding of mercury neurotoxicity in the vertebrate retina and its impact on vision.

The accumulation of MeHg in the retina of *D. rerio* after trophic and subchronic exposure was described by Mela et al. (2010), and showed that methylmercury accumulates mainly in the photoreceptor layer, inner nuclear layer, outer nuclear layer and in the optic disc region. In the current study, *H. malabaricus* accumulated deposits of mercury in the photoreceptor layer and in the inner and outer plexiform layers, but not in nuclear layers. Although these two species are teleosts, we observed that mercury accumulation occurred differently in their retinas. The reason for this difference in target cells of retina for mercury could only be speculated, suggesting that one or more retinal trapping mechanisms present in *D. rerio* are not the same found to retina of *H. malabaricus*, since the same mercury accumulation or distribution was not observed. Another hypothesis is cited by Oliveira Ribeiro et al. (2002), suggesting a different sensitivity of tropical fish species to mercury exposure. According to these authors, this could be due to the different metabolic rates of tropical species, resulting in different tissue damage when compared to others species. However, further studies are needed to understand the mechanisms involved in mercury accumulation in the retina of different fish species, which could help understand mercury neurotoxicity in these organisms.

The mechanisms by which mercuric ions gain access to the photoreceptor cells inducing subsequent vision loss are still unclear. According to Bridges et al. (2007) mercury can access photoreceptors cells by the retinal pigment epithelium (RPE), a single layer of epithelial cells positioned strategically between the

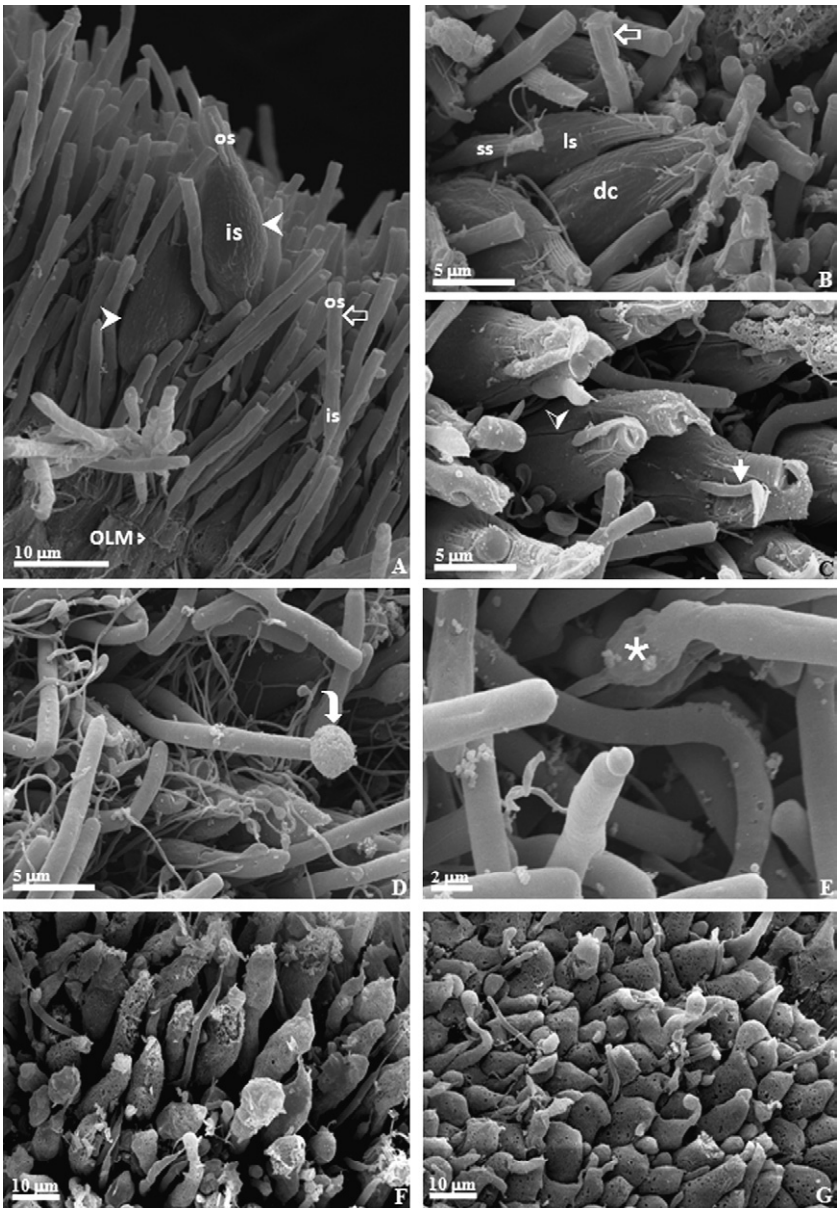

Fig. 3. Scanning electron micrograph showing the photoreceptor in retina of *Hoplias malabaricus*. (A) View of retina of control fish. Double cones (arrow-head), rods (open arrow) and outer limiting membrane (OLM) formed by prolongations of Müller cells (▷). The photoreceptors have an inner segment (is) and an outer segment (os). (B) Retina of control fish. Three different types of cone photoreceptors can be seen: (ss) small single cone (ls) large single cone (dc) double cone and a rod (open arrow). (C) Retina of exposed group (0.75 µg MeHg g^{-1}). Note the changes in the plasma membranes which unite the two elements of a paired cone (⩗). Observe the calycal process of a cone outer segment (⇩). (D) Retina of exposed group (0.075 µg MeHg g^{-1}). Observe the changes in the morphology of the outer segment of a rod (⤵). (E) Retina of exposed group (0.75 µg MeHg g^{-1}). Observe the changes in the morphology of a rod inner segment (↙). (F) Retina of exposed group (0.075 µg MeHg g^{-1}). Clear cell deterioration in the photoreceptors layer. (G) Retina of exposed group (0.75 µg MeHg g^{-1}). Cell deterioration in the photoreceptors layer.

choriocapillaris and the neural retina, containing mechanisms involving transporters of essential nutrients. Bok (1993) demonstrated that these mechanisms are mediated mainly by specific protein transporters present on the basolateral and apical plasma membranes which can be used to transport also methylmercury when conjugated with cysteine (Cys) or homocysteine (Hcy) or other toxic compounds (Bridges et al., 2007). This hypothesis is corroborated by the studies of Warfvinge and Bruun (1996), where the presence of mercury was observed in photoreceptor cells confirming the importance of this route for retina toxicity. Additionally, some previous studies have demonstrated functional changes in cones and rods, assuming a possible accumulation of mercury in these cells (Rice and Gilbert, 1982; Fox and Sillman, 1979; Hawryshyn et al., 1982). The current work corroborates this idea since mercury deposition was found in the photoreceptor layer, showing strong evidence of relation between photoreceptor damage and mercury localization in retina of vertebrates.

Another possible route to explain the distribution of mercury within the retina is through the complex system of blood vessels located at the inner limiting membrane in direct contact with the Müller cells and ganglion cell layer. There is evidence that both routes are important to mercury deposition (Alvarez et al., 2007; Bridges et al., 2007). So, after crossing the inner limiting membrane, mercury can also be distributed to other cell layers – the inner and outer plexiform layers, as found in the present study. The outer plexiform layer is the first synaptic site in the retina. In this layer connection between photoreceptors, bipolar, amacrine and horizontal cells take place. The inner plexiform layer is the second synaptic site in the retina. These connections occur between bipolar, amacrine, horizontal and ganglion cells. Previous

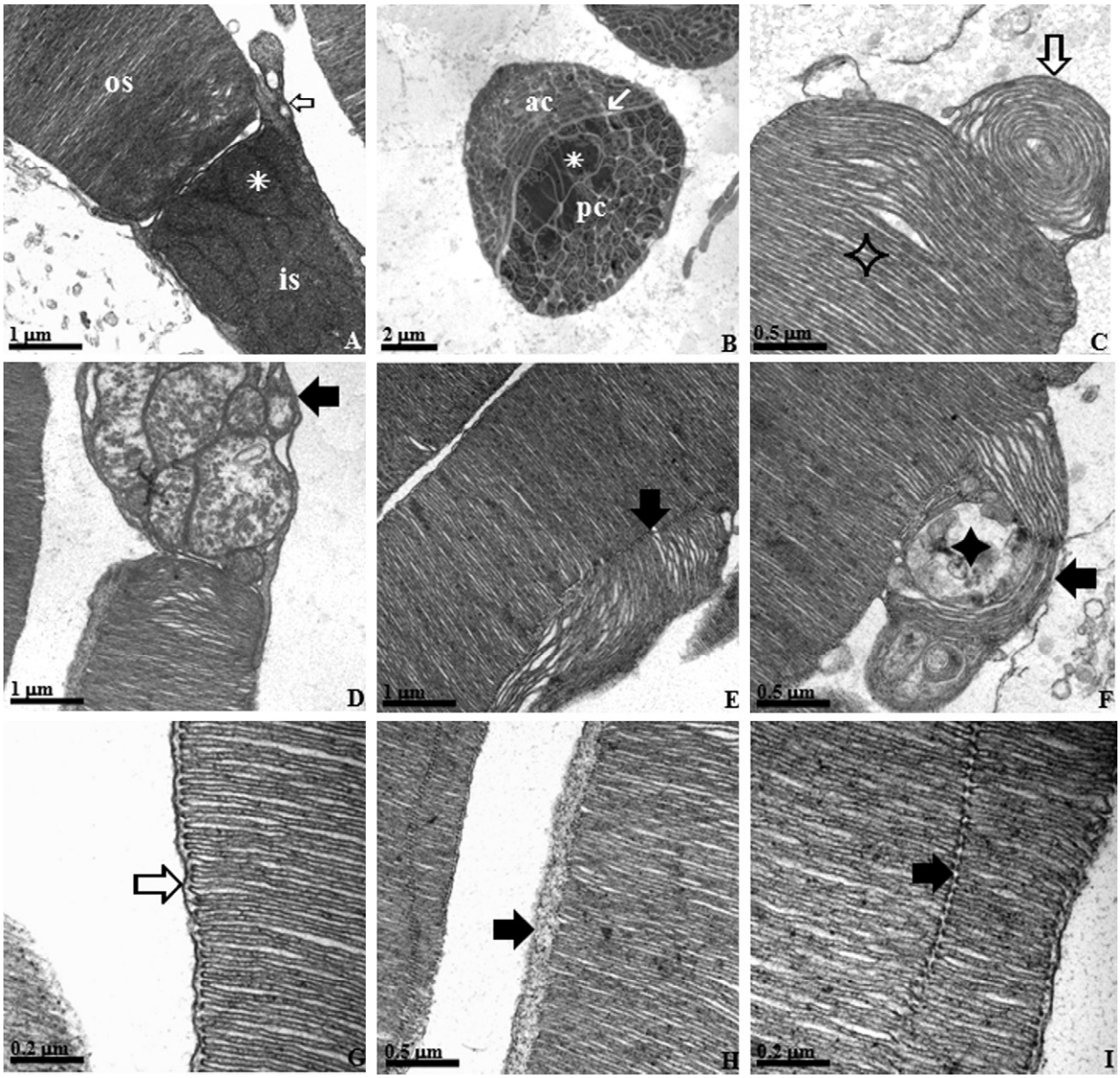

Fig. 4. Electron micrograph of the photoreceptors in retina of *Hoplias malabaricus*. (A) Radial section of photoreceptor from control group showing outer segement (os), inner segment (is), mitochondria (asterisk) and connecting cilium (open arrow). (B) Tangential section of double cone from control group. Detail of principal cone (pc) and accessory cone (ac). The membranes of paired cones are juxtaposed at the level of the inner segments (↙). Observe the mitochondria (asterisk). (C) Photoreceptor with lamellar disks (✧) containing the visual pigment. Note the photoreceptor discs being eliminated (open arrow) to be phagocytized by retinal epithelial pigment cells. (D) Photoreceptor of exposed group (0.75 µg MeHg g^{-1}). Note the formation of vacuoles (filled arrow) at the extremities of the photoreceptor discs being eliminated to be phagocytized. (E) Photoreceptor of exposed group (0.075 µg MeHg g^{-1}). Removal of membranous discs at the sides of photoreceptor cells (filled arrow). (F) Photoreceptor of exposed group (0.75 µg MeHg g^{-1}). Removal of membranous discs at the side of photoreceptor cells (filled arrow) with the formation of vacuoles (✦). (G) Rod from control group. The disks are surrounded by a normal plasma membrane (open arrow). (H) Rod from exposed group (0.075 µg MeHg g^{-1}). Clear structural changes in plasma membrane (filled arrow). (I) Photoreceptor of exposed group (0.075 µg g^{-1}). Observe a discontinuity in the structure of lamellar discs in the rods (filled arrow).

studies have demonstrated accumulation of mercury in the plexiform layer of fish (Mela et al., 2010) and in plexiform layer of monkeys (Warfvinge and Bruun, 2000). The primary effects of MeHg on synaptic transmission is the increase, followed by decrease, of the spontaneous release of neurotransmitters, as well as decrease in the release of neurotransmitters activated by the nerve impulses (Juang and Yonemura, 1975; Shrivastav et al., 1976). Studies demonstrating the effect of mercury exposure on synaptic response of plexiform layers of fish retina have not yet been performed, however our group observed a dose dependent increased in the amplitude of the horizontal cell response, followed by a decrease, in the retina of *H. malabaricus* to the doses of 0.075 and 0.75 MeHg, respectively (Tanan et al., 2006). According to Yuan and Atchison (1993), MeHg completely blocks the synaptic transmission and its effects are irreversible or only partially reversible. Due to the lack of data concerning the effect of mercury in the plexiform layers, the hypothesis here is that the mercury deposits in these layers damage the retinal synaptic connections in *H. malabaricus* and impair the communication between the different retina cells. This implies that the action of MeHg on synaptic transmission may be responsible for, or partially contribute to, its neurotoxicity.

Because of the deleterious impacts of pollutants in aquatic ecosystems, the histopathological response of fish to mercury need to be determined and characterized (Rabitto et al., 2011; Mela et al., 2010). The retina of *H. malabaricus* is duplex in nature,

containing both rods and cone photoreceptors. The photoreceptor population is dominated by rods, but the cone population is substantial. A retina dominated to a large extent by rods is generally characteristic of animals whose eyes have adapted for life under dim light or scotopic conditions. *H. malabaricus* lives in muddy and murky water and its rod-dominated retina is well adapted to its behavior. The results of this study show that methylmercury disturbs the integrity of the photoreceptors in both doses used. Such high mercury toxicity in the retina is consistent with observations on other tissue (Oliveira Ribeiro et al., 1996, 2002, 2006; Rabitto et al., 2005; Mela et al., 2007) and shows that the mercury can produce toxic effects at very low concentrations.

The neurotoxic effect of mercury can be explained by damage caused to the cell membrane structure by mercury ions forming cross-linkages with membrane proteins, and by inhibition of certain associated enzymes (Barboni et al., 2008). Delnomdedieu et al. (1992) suggested that mercury reacts non-specifically with any sulfhydryl group in the cell membrane to form –S–Hg–S– bridges. These bridges would constitute a molecular stress which would cause a generalized breakdown of the cell membrane and subsequent release of cell contents. Mercury interferes initially with the permeability of the plasma membrane, and then moves into the cell where it interferes with important intracellular mechanisms (Miura and Imura, 1987), all leading to decreased integrity and function of cells. According to Baatrup (1991) mercury interferes with the structural integrity and the physical properties of the cell membrane leading to weakening of the architecture of the membranes and causing structural disorganization. Damage to the plasma membrane of photoreceptors can change ionic flow and consequently cause a change in membrane potential. Changes in juxtaposed membrane of the double cones may impair polarized vision in *H. malabaricus*, since the elliptical cross section of the inner segments act as a birefringent, polarization-sensitive and dielectric waveguide (Kusmic and Gualtieri, 2000).

Tessier-Lavigne et al. (1985) observed gross changes in the structure of rods. They suggested that the outer segment destruction of the rods was induced by mercury leading to the hypothesis that rod mediated vision was selectively impaired in humans and primates exposed to MeHg (Merigan, 1980). According to Wu et al. (2006) the photoreceptors are the most sensitive cells to environmental damage, and the rods cells are more sensitive than cones. Structural changes in the cells may be due to a direct interference of mercury with important intracellular processes, such as nucleic acid synthesis, protein synthesis and enzyme function (Sillman and Weidner, 1993) or even due to changes in the oxido-reductive state of cells (Filipak Neto et al., 2008) leaving to potential disturbances in proteins and other macromolecules. It is possible, therefore, that the structural changes in visual tissues reflect secondary damage due to failure of the intracellular mechanisms (Sillman and Weidner, 1993). Atypical outer segment morphology may change the phototransdution efficiency due to a decreased probability that a photon will be absorbed by a cone or rod (Farjo et al., 2007).

Similarly to most mature neurons, photoreceptors do not undergo cell division, but their outer segments are continually renewed. New discs are formed and old disks are discarded and removed by phagocytic activity of RPE cells (Mustafi et al., 2009). Because rods outer segments are cylindrically shaped and each rod disc becomes independent after its initial formation at the outer segment base, a continuous displacement of discs toward the outer segment tip is easy to visualize. In the cones, the process is more complex because the discs retain some connection, not only with the adjacent discs, but also with the outer plasma membrane (Anderson et al., 1978; Daniele et al., 2005; Mustafi et al., 2009). In the current study we sometimes found that photoreceptors exposed to MeHg eliminated their discs by the lateral region of outer segments rather than by the apical region. A possibility exists that these discs were not phagocyted by pigment epithelial cells and that this could affect photon absorption and regeneration by the visual pigments. The presence of a significant number of older discs within outer segment membranes should not be detrimental to photoreceptor function, because function in daylight efficiently signals the absorption of thousands to millions of photons despite a high level of noise (Kusmic and Gualtieri, 2000). Even so, the efficiency of outer segment renewal mechanisms may limit the sensitivity of a photoreceptor to light (Eckmiller, 1992). Furthermore, we observed the presence of vacuoles in older discs in the apical and lateral regions. Vacuolization had been previously described in several other cells of fish in different tissues after metal exposure (Paris-Palacios et al., 2000; Oliveira Ribeiro et al., 2002; Rabitto et al., 2005; Mela et al., 2007; Oliva et al., 2009; Liu et al., 2010) but not in fish photoreceptor. Sillman and Weidner (1993) related the vacuolization in the corneal endothelium of bullfrog to mercury exposure. The vacuolization in fish cells has been related with disturbs in cellular metabolism and is considered a useful histopathologic biomarker in freshwater fish exposed to different types of contaminants (Oliveira Ribeiro et al., 2002; Khan et al., 2004).

Deterioration of cells in retina has been reported in mammalians after metal exposure (Erie et al., 2005) and in rat retina after UV light exposure (Tokuda et al., 2007), however there are not studies of photoreceptor deterioration in the fish retina. The current results suggest that the shown deterioration in the photoreceptor layer after trophic exposure to methylmercury could be associated with cell death in the cones and rods, since a decrease of immunoreactivity of photoreceptor cells in *H. malabaricus* exposed to both methylmercury doses was observed (Mela, 2009). Bonci et al. (2006) described a significant reduction in the number of amacrine cells and ganglion cell in the retina of *H. malabaricus* after trophically exposure to methylmercury (0.075 mg Hg g^{-1}) interpreted according to the author by the induction of apoptosis. A decrease in the number of amacrine and bipolar cells in the retina of *H. malabaricus* was also described by the authors after intraperitoneal injection of 6 mg g^{-1} of methylmercury, or a reduction in the number of amacrine cells when exposed to 2 mg g^{-1} of methylmercury. In addition, there was a reduction of the intracellular response to light of horizontal cells in *H. malabaricus* after methylmercury exposure (Tanan et al., 2006). The hypothesis of deterioration by apoptosis can be enhanced by a loss of plasma membrane integrity, a feature of this type of cell death.

The results demonstrate that the morphological damage observed in the photoreceptors was dose-dependent. However, in previous data (Tanan et al., 2006) it was observed that after exposure to a low dose (0.075 mg/kg) of MeHg an increase of the amplitude of the horizontal cell response to light was found, followed by cessation of activity with high doses (0.75 mg/kg) of MeHg. According to Tanan et al. (2006), at low doses horizontal cells might have a physiological mechanism of protection against the toxic effects of MeHg. Although there is a direct link between horizontal cells and photoreceptors, we do not observe this same protection mechanism in photoreceptor cells. Therefore, it is possible that damage to photoreceptors outweighs the protective mechanisms of these cells and the increase of the dose leads the cells to lose the ability to protect from mercury effects.

Our data clearly demonstrate that exposure to methylmercury disrupts retinal morphology, and functional changes (*e.g.*, increase in predation time) could be an interesting question for future research. Further behavioral studies are needed to understand the mechanisms involved in decrease of the predation time, which could help understand mercury neurotoxicity in *H. malabaricus*.

This study did not conduct behavioral analysis; however, sporadic observations were performed. On the last days of the experiment we observed an increase in the predation time. This difference was small (s), but easily observed. Despite the evidences it is not possible with the present data affirm that this alteration is related to the visual changes or motor changes. In a previous study, Mela et al. (2007) observed a decrease in acetylcholinesterase activity in axial muscle of *H. malabaricus* exposed to MeHg 0.075 μg MeHg g^{-1}. When the AChE activity is inhibited in some way, there is blockage in the transmission of nerve impulses, paralyzing the vital functions, caused by the persistence of Na^+ channels open. The toxic effects involve the parasympathetic system, sympathetic, motor and central nervous systems. This decreased of AChE activity can affect swimming performance and compromise the ability of these species to capture their prey. Mela et al. (2010) described the localization of mercury in retina of *D. rerio* trophically exposed and the related physiological effects. A very interesting relation could be done helping the understanding of mercury effect concerns to visual disturbs. In the current study was showed that the ultrastructural damage may corroborate the localization and in consequence the potential disturbs to visual system.

The localization and distribution of mercury in the studies of neurotoxicity in retina may be used as a basis to understand the consequences to visual system. This study shows that the photoreceptor can be impaired by low levels of MeHg, typically in the range of those encountered by fish in their environments over long periods. Although our results cannot be extended to human beings, it should be recalled that human communities inhabiting some Amazonian areas are still exposed to fish containing comparable levels of MeHg. Further studies are needed to evaluate the characteristics of mercury accumulation in other retinal cell types and the impact of mercury intoxication upon retinal functions. This knowledge would help elucidate mercury intoxication in the visual system.

Conflict of interest statement

There are no conflicts of interest, sponsors or funding bodies, or financial disclosures in this work.

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References

Alvarez Y, Cederlund ML, Cottell DC, Bill BR, Ekker SC, Torres-Varquez J, et al. Genetic determinants of hyaloid and retinal vasculature in zebrafish. BMC Dev Biol 2007;114:2–17.

Amaral AF, Alvarado N, Marigomez I, Cunha R, Hylland K, Soto M. Autometallography and metallothionein immunohistochemistry in hepatocytes of turbot (*Scophthalmus maximus* L.) after exposure to cadmium and depuration treatment. Biomarkers 2002;7:491–500.

Anderson DH, Fisher SK, Steinberg RH. Mammalian cones: disc shedding phagocytosis and renewal. Invest Ophthalmol Vis Sci 1978;17:117–33.

Baatrup E. Structural and functional effects of heavy metals on the nervous system including sense organs of fish. Comp Biochem Physiol C 1991;100:253–7.

Barboni MTS, Costa MF, Moura ALA, Feistosa-Santana C, Gualtiere M, Lago M, et al. Visual field losses in workers exposed to mercury vapor. Environ Res 2008;107:124–31.

Barboni MTS, Feitosa-Santana C, Zachi EC, Lago M, Teixeira R, Taub A, et al. Preliminary findings on the effects of occupational exposure to mercury vapor below safety levels on visual and neuropsychological functions. J Occup Environ Med 2009;51:1403–12.

Bernet D, Schmidt H, Meier W, Burkhardt-holm P, Wahli T. Histopathology in fish: proposal for a protocol to assess aquatic pollution. J Fish Dis 1999;22:25–34.

Bok D. The retinal pigment epithelium: a versatile partner in vision. J Cell Sci 1993;17:189–95.

Bonci DMO, de Lima SMA, Grötzner SR, Oliveira Ribeiro CA, Hamassaki DE, Ventura DF. Losses of immunoreactive parvalbumin amacrine and immunoreactive α protein kinase C bipolar cells caused by methylmercury chloride intoxication in the retina of the tropical fish *Hoplias malabaricus*. Braz J Med Biol Res 2006;39:405–10.

Bridges CC, Battle JR, Zalups RK. Transport of thiol-conjugates of inorganic mercury in human retinal pigment cells. Toxicol Appl Pharmacol 2007;221:251–60.

Canto-Pereira LHM, Lago M, Costa MF, Rodrigues AR, Saito CA, Silveira LCL, et al. Visual impairment related to occupational exposure in dentists. Environ Toxicol Pharmacol 2005;19:517–22.

Chang JY. Methylmercury causes glial IL-6 release. Neurosci Lett 2007;416:217–20.

Costa GM, Anjos LM, Souza GS, Gomes BD, Saito CA, Pinheiro MCN, et al. Mercury toxicity in Amazon gold miners: visual dysfunction assessed by retinal and cortical electrophysiology. Environ Res 2008a;107:98–107.

Costa MF, Tomaz S, Souza JM, Silveira LCL, Ventura DF. Electrophysiological evidence for impairment of contrast sensitivity in mercury vapor occupational intoxication. Environ Res 2008b;107:132–8.

Daniele LL, Lillo C, Lyubarsky AL, Nikonov SS, Philp N, Mears AJ, et al. Cone-like morphological, molecular and electrophysiological features of the photoreceptors of the Nrl Knockout mouse. Invest Ophthalmol Vis Sci 2005;46:2156–67.

Danscher G, Möller-Madsen B. Silver amplification of mercury sulphide and selenide. A histochemical method for light and electron microscopic localization of mercury in tissue. J Histochem Cytochem 1985;33:219–28.

Danscher G. Autometallography. A new technique for light and electron microsocpic visualization of metals in biological tissues (gold, silver, metal sulphides and metal selenides). Histochemistry 1994;81:331–5.

Delnomdedieu M, Boudou A, Georgescauld D, Dulfourc EJ. Specific interactions of mercury chloride with membranes and other ligands as revealed by mercury-NMR. Chem Biol Interact 1992;21:179–84.

Eckmiller MS. Shifting distribution of autoradiographic label in cone outer segments and its implications for renewal. J Hirnforsch 1992;34:179–91.

Erie JC, Bitz JA, Good JA, Erie EA, Burritt MF, Cameron JD. Heavy metal concentrations in human eyes. Am J Ophthalmol 2005;39:888–93.

Evans HL, Garman RH. Scotopic vision as an indicator of neurotoxixity. In: Merigan WH, Weiss B, editors. Neurotoxicity of the visual system. New York: Raven Press; 1980, pp. 135–47.

Farjo R, Fliesler SJ, Naash MI. Effect of Rds abundance on cone outer segment morphogenesis, photoreceptor gene expression and outer limiting membrane integrity. J Comp Neurol 2007;504:619–30.

Feitosa-Santana C, Costa MF, Lago M, Ventura DF. Longterm loss of color vision after exposure to mercury vapor. Braz J Med Biol Res 2007;40:409–14.

Filipak Neto F, Zanata SM, Silva de Assis HC, Nakao LS, Randi MAF, Oliveira Ribeiro CA. Toxic effects of DDT and methyl mercury on the hepatocytes from *Hoplias malabaricus*. Toxicol In Vitro 2008;22:1705–13.

Finocchio DV, Luschei ES, Mottet NK, Body R. Effects of methylmercury on the visual systems of rhesus macaque (*Macaca mulatta*). Pharmacokinetics of chronic methylmercury related to changes in vision and behavior. In: Merigan WH, Weiss B, editors. Neurotoxicity of the visual system. New York: Raven Press; 1980, pp. 113–22.

Fox DA, Sillman AJ. Heavy metals affect rod, but not cone, photoreceptors. Science 1979;206:78–80.

Giari L, Somin E, Manera M, Dezfuli BS. Histo-cytological responses of *Dicentrarchus labrax* (L.) following. Ecotoxicol Environ Saf 2008;70:400–10.

Hawryshyn CW, MacKay WC, Nilsson TH. Methyl mercury induced visual deficits in rainbow-trout. Can J Zool 1982;60:3127–33.

Iwata K, Abe H. Neuroophthalmological and pathological studies of organic mercury poisoning Minamata disease in Japan. In: Tsubaki T, Takahashi H, editors. Recent adavances in Minamata disease studies. Methylmercury poisoning in Minamata and Nigata Japan. Tokyo: Kodansha; 1986, pp. 58–74.

Juang MS, Yonemura K. Increased sponataneous transmitter release from presynaptic nerve terminal by methylmercury chloride. Nature 1975;256:211–3.

Khan MS, Khan SA, Chaudhary ZI, Khan MN, Aslam A, Ashraf K, et al. Mercury intoxication in Grass Carp (*Ctenopharyngodon idella*). Pakistan Vet J 2004;24:33–8.

Kusmic C, Gualtieri P. Morphology and spectral sensitivities of retinal and extraretinal photoreceptors in freshwater teleosts. Micron 2000;31:183–200.

Liu XJ, Luo Z, Xiong BX, Liu X, Zhao YH, Hu GF, et al. Effect of waterborne copper exposure on growth, hepatic enzymatic activities and histology in *Synechogobius hasta*. Ecotoxicol Environ Saf 2010;73:1286–91.

Mela M, Randi MA, Ventura DF, Carvalho CE, Pelletier E, Oliveira Ribeiro CA. Effects of dietary methylmercury on liver and kidney histology in the neotropical fish *Hoplias malabaricus*. Ecotoxicol Environ Saf 2007;68:426–35.

Mela M. Avaliação dos efeitos tóxicos do metilmercúrio na retina de duas espécies de teleósteos: *Hoplias malabaricus* e *Danio rerio*, utilizando um conjunto de biomarcadores biológicos. Tese de doutorado em neurociência e comportamento. Universidade de São Paulo, Brasil; 2009, unpublished results.

Mela M, Cambier S, Mesmer-Dudons N, Legeay A, Grotzner SR, Oliveira Ribeiro CA, et al. Methylmercury localization in *Danio rerio* retina after trophic and subchronic exposure: a basis for neurotoxicology. Neurotoxicology 2010;31:448–53.

Merigan WH. Visual fields and flicker thresholds in methylmercury-poisoned monkeys. In: Merigan WH, Weiss B, editors. Neurotoxicity of the visual systems. New York: Raven Press; 1980, pp. 149–63.

Miura K, Imura N. Mechanism of methylmercury cytotoxicity. CRC Crit Rev Toxicol 1987;18:161–88.
Mustafi D, Engel AH, Palczewski K. Structure of cone photoreceptors. Prog Retin Eye Res 2009;28:289–302.
Oliva M, Garrido MC, Sales Marquez D, Gonzales de Canales ML. Sublethal and lethal toxicity in juvenile Senegal sole (*Solea senegalensis*) exposed to copper: a preliminary toxicity range-finding test. Exp Toxicol Pathol 2009;61:113–21.
Oliveira Ribeiro CA, Guimarães JR, Pffeiffer WC. Accumulation and distribution of inorganic mercury in a tropical fish (*Trichomycterus zonatus*). Ecotoxicol Environ Saf 1996;34:190–5.
Oliveira Ribeiro CA, Rouleau C, Pelletier E, Audet A, Thalve H. Distribution kinetics of dietary methylmercury in the Artic Charr (*Salvelinus alpinus*). Environ Sci Technol 1999;33:902–7.
Oliveira Ribeiro CA, Schatzmann M, Silva de Assis HC, Silva PH, Pelletier E. Evaluation of tributyltin subchronic effects in tropical freshwater fish (*Astyanax bimaculatus*, Linnaeus, 1758). Ecotoxicol Environ Saf 2002;51:61–167.
Oliveira Ribeiro CA, Filipack Neto F, Mela M, Silva PH, Randi MAF, Costa JRA, et al. Hematological findings in neotropical fish *Hoplias malabaricus* exposed to subchronic and dietary doses of methylmercury, inorganic lead and tributyltin chloride. Environ Res 2006;101:74–80.
Oliveira Ribeiro CA, Mesmer-Doudons N, Gonzalez P, Dominique Y, Bourdineaud J-P, Boudou A, et al. Effects of dietary methylmercury on zebrafish skeletal muscle fibres. Environ Toxicol Pharmacol 2008;25:304–9.
Paris-Palacios S, Biagianti-Risbourg S, Vernet T. Biochemical and (ultra) structural hepatic pertubations of *Brachydanio rerio* exposed to two sublethal concentrations of copper sulfate. Aquat Toxicol 2000;50:109–24.
Rabitto IS, Costa JRMA, Silva de Assis HC, Randi MAF, Akaishi FM, Pelletier E, et al. Dietary Pb(II) and TBT (tributyltin) exposures to neotropical fish *Hoplias malabaricus*: histopathological and biochemical findings. Ecotoxicol Environ Saf 2005;60:147–56.
Rabitto IS, Bastos WR, Almeida R, Anjos A, Barbosa de Holanda IB, Galvao RCF, et al. Mercury and ddt exposure risk to fsh-eating human poulaions in amazon. Environ Int 2011;37:56–65.
Rand MD, Dao JC, Clason TA. Methylmercury disruption of embryonic neural development in Drosophila. Neurotoxicology 2009;30:794–802.
Rice DC, Gilbert G. Early chronic low-level methylmercury poisoning in monkeys impairs spatial vision. Science 1982;216:759–61.
Saldana M, Collins CE, Gale R, Backhouse O. Diet-related mercury poisoning resulting in visual loss. Br J Ophthalmol 2006;90:1432–4.
Shaw CM, Mottet NK, Chen WJ. Effects of methylmercury on visual system of Rhesus macaque (*Macaca mulatta*). Neuropathological findings (with emphasis on vascular lesions in the brain). In: Merigan WH, Weiss B, editors. Neurotoxicity of the visual system. New York: Raven Press; 1980, pp. 123–34.
Shrivastav BB, Brodwick MS, Narahashi T. Methylmercury: effects on eletrical properties of squid axon membranes. Life Sci 1976;18:1077–82.
Sillman AJ, Weidner WJ. Low levels of inorganic mercury damage the corneal endothelium. Exp Eye Res 1993;57:549–55.
Soto M, Quincoces I, Marigómez I. Autometallography procedure for the localization of metal traces in molluscan tissues by light microscopy. J Histotechnol 1998;21:123–7.
Tanan CL, Ventura DF, de Souza JM, Grötzner SR, Mela M, Gouveia A Jr, et al. Effects of mercury intoxication on the response of horizontal cells of the retina of trahira fish (*Hoplias malabaricus*). Braz J Med Biol Res 2006;39:987–95.
Tessier-Lavigne M, Mobbs P, Attwell D. Lead and mercury toxicity and the rod light response. Invest Ophthalmol Vis Sci 1985;26:1117–23.
Tokuda K, Zorumski CF, Izumi Y. Effects of ascorbic acido n UV light-mediated photoreceptor damage in isolated rat retina. Exp Eye Res 2007;84:537–43.
Ventura DF, Costa MTV, Costa MF, Berezovsky A, Salomão SR, Canto-Pereira LHM, et al. Multifocal and full-field electroretinogram changes associated with color-vision loss in mercury vapor exposure. Vis Neurosci 2004;21:421–9.
Ventura DF, Simões AL, Tomaz S, Costa MF, Lago M, Costa MTV, et al. Colour vision and contrast sensitivity losses of mercury intoxicated industry workers in Brazil. Environ Toxicol Pharmacol 2005;19:523–9.
Ventura DF, Feitosa-Santana C, Barboni MTS, Oiwa N, Paramei N, Galina V, et al. Irreversible color vision losses in patients with chronic mercury vapor intoxication. Vis Neurosci 2008;25:487–91.
Warfvinge K, Bruun A. Mercury accumulation in the squirrel monkey eye after mercury vapor exposure. Toxicology 1996;107:189–200.
Warfvinge K, Bruun A. Mercury distribution in the squirrel monkey retina after in uterus exposure to Mercury vapor. Environ Res 2000;83:102–9.
Wu J, Seregard S, Algvere PV. Photochemical damage of the retina. Surv Ophthalmol 2006;51:461–81.
Yuan Y, Atchison WD. Disruption by methylmercury of membrane excitability and synaptic transmission of CA1 neurons in hippocampal slices of the rat. Toxicol Appl Pharmacol 1993;120:203–15.