Assessment of the distribution of AMG positive material in the brain of largemouth bass, *Micropterus salmoides*

DeOnna Johnson

Center for Environmental Biotechnology, Undergraduate in Biological Sciences, University of Tennessee-Knoxville

Abstract

The objective of this study was to evaluate the presence and distribution of mercury in the brain of fish collected from an aquatic system known to be contaminated with mercury from the Oak Ridge Reservation. The presence and distribution of mercury was evaluated by histochemistry based on the Autometallography staining technique that can distinguish the presence of AMG positive material believed to be mercury. This technique utilizes a method that encapsulates the mercury deposits into silver granules which can be visualized with light microscopy. Microscopic analysis has shown accumulations of AMG granules in neural cell bodies, capillaries, and the epithelial cells lining the ventricles of the brain.

Introduction

Accumulation of mercury is well documented in fish from many aquatic habitats. The main cause of this is the settling of elemental mercury in the sediment of lakes, rivers, and other bodies of water where it is changed into methyl mercury, absorbed by phytoplankton, and then consumed by fish. It has also been documented that the main source of human exposure to mercury is through consuming these fish[1].

Exposure to mercury is a concern because mercury is known to associate with nervous tissue resulting in neurological problems in humans. The effect of methyl mercury on the developing brain has an even larger impact. Problems emerge when women consume contaminated fish and methyl mercury crosses the placenta and accumulates in the fetal brain leading to conditions in babies ranging from developmental delays to severe brain damage[2].

Autometallography (AMG) is a technique that makes it possible to visualize mercury that has associated with sulfur or selenium in the tissue and accumulated in specific areas [3], [4]. There are several other types of mercury that could be in the tissue that cannot be visualized using this method [3].The technique works by creating silver clusters around the mercury compound which can then be visualized using standard light microscopy [5].

There have been a limited number of studies using autometallography to analyze the accumulation of mercury in fish tissues including the ovaries of crucian carp (Zarnesco, 2009)[6], the kidney and liver of rainbow trout (Baatrup et al., 1986 [7]; Baatrup and Danscher, 1987 [8]), and in the salmon olfactory system (Baatrup and Døving, 1990)[9]. There were no studies found that analyzed the accumulation of Mercury in the brain of fish.

Materials and Methods

Three large mouth bass were removed from the upper East Fork Poplar Creek at the Oak Ridge Reservation, known to be contaminated with Mercury. The bass skulls, with brains intact, were provided by Marshall Adams of the Oak Ridge National Laboratory. The skulls were preserved in 10% neutral buffered.

Upon receipt, these skulls were carefully dissected and the complete brain was extracted. For control purposes, three channel catfish were euthanized, dissected, and the entire brain was extracted. After removal, the brains were preserved in 10% neutral buffered formalin and were left intact to allow visualization of all areas of the brain. All preserved brains were processed for routine paraffin histology and histochemistry.

The processed samples embedded in paraffin were sequentially sectioned using a microtome. The prepared slides were subjected to an Autometallography (AMG) Staining Technique developed with reference to [4]. The slides were deparaffinized and the tissue was hydrated using ethanol. The slides were then coated with a thin film of gelatin, rinsed with deionized water, and placed in an AMG developer containing silver acetate, hydroquinone, citric acid, and trisodium citrate for 40 minutes. The development was stopped using a 10% sodium thiosulfate solution. The slides were then rinsed in running 40°C tap water to remove the gelatin and dipped into a 1% Farmer's Solution. Finally, the slides were counterstained using hematoxylin. Control brain tissues were stained concurrently with brain tissue expected to contain mercury. Concurrent staining eliminated any discrepancy that could occur from variation in staining procedures. The slides were photographed and the results were analyzed blindly.

Results

AMG granules were visualized in the brains of all fish collected from Oak Ridge. The largest concentration of granules was observed inside of nerve cell bodies (Figure 1), small capillaries, and epithelial cells lining the ventricles of the

brain. An oil immersion image of a capillary shows accumulation in the blood stream but not in the red blood cells (Figure 2). Heavy accumulation was also seen along the lining of the brain. In the control group, there a random distribution of AMG granules was seen (Figure 3), but there were no obvious areas of accumulation as seen in the mercury exposed fish.

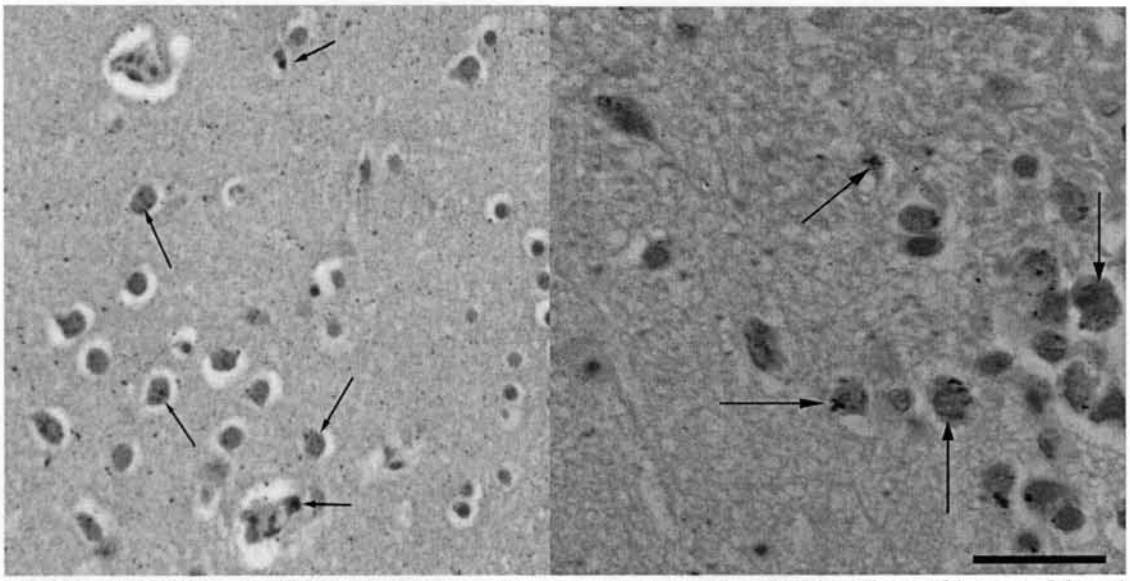


Figure 1: Right, AMG granules accumulated in nerve cell bodies of Large Mouth Bass; Left, oil immersion image if AMG granules in nerve cell bodies (bar=20 μm)

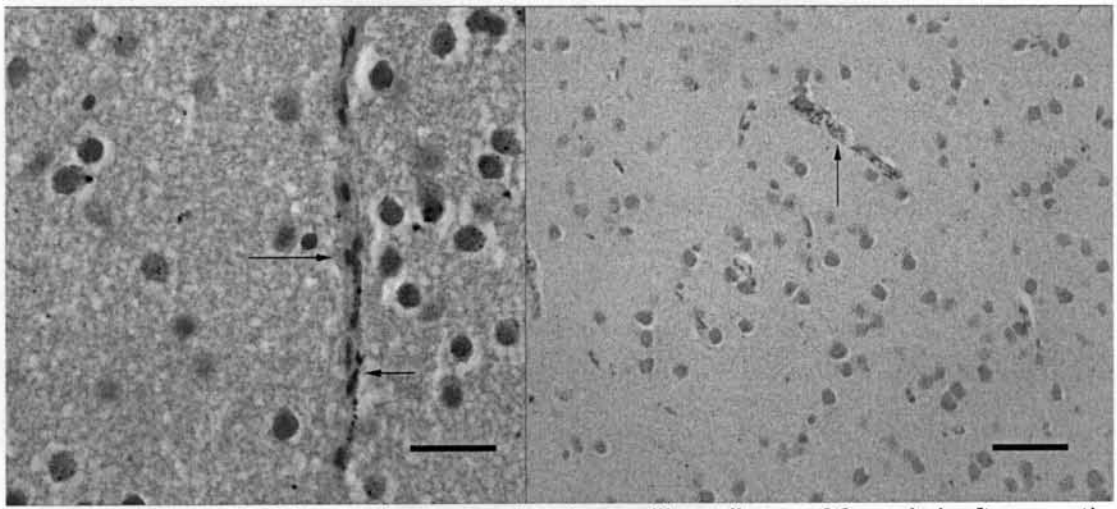


Figure 2: Right, AMG positive granules in capillary (bar = 20 μm); Left, negative control

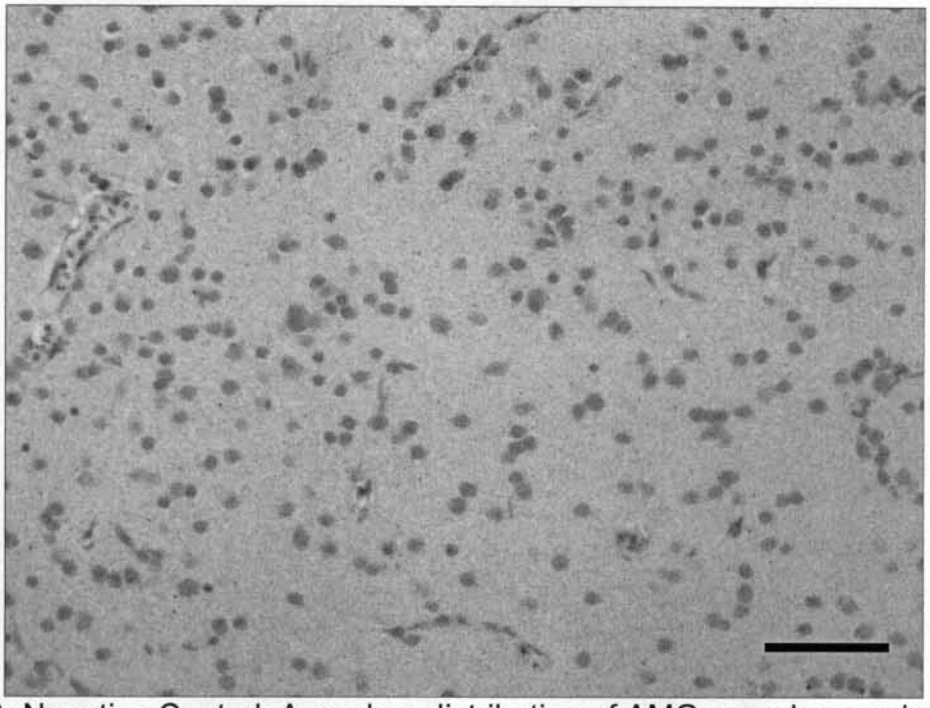


Figure 3: Negative Control; A random distribution of AMG granules can be visualized with no accumulation in specific areas. (bar = 50 μm)

Discussion

The fish used in the experiment, largemouth bass, were exposed to mercury through environmental contamination. Mercury deposits can differ depending on the method of uptake including consumption or absorption from the water. It is important to note that only mercury bound to sulfur or selenium can be visualized with the AMG technique meaning there could be other areas of mercury accumulation that were not visualized during this study. The AMG granules visualized in this study were mainly in neural cell bodies, capillaries, and epithelial cells in the brain. There is an indication travel through the blood

stream is a possible source of distribution of mercury in the brain because of visualization of AMG granules in the capillaries.

In conclusion, the results of this study demonstrate accumulation of AMG granules, believed to be mercury, in the capillaries, neural cell bodies, and epithelial linings of the ventricles in the brains of largemouth bass exposed to environmental mercury.

References

1. Organization, W.H. (2007) *Exposure to Mercury: A Major Public Health Concern.*

2. Clarkson, T.W., *The Three Modern Faces of Mercury.* Environmental Health Perspectives, 2002. **110**: p. 11-23.

3. Loumbourdis, N.S. and G. Danscher, *Autometallographic tracing of mercury in frog liver.* Environmental Pollution, 2004. **129**(2): p. 299-304.

4. Danscher, G. and M. Stoltenberg, *Autometallography (AMG) - Silver enhancement of quantum dots resulting from (1) metabolism of toxic metals in animals and humans, (2) in vivo, in vitro and immersion created zinc-sulphur/zinc-selenium nanocrystals, (3) metal ions liberated from metal implants and particles.* Progress in Histochemistry and Cytochemistry, 2006. **41**(2): p. 57-139.

5. Danscher, G. and B. Mollermadsen, *SILVER AMPLIFICATION OF MERCURY SULFIDE AND SELENIDE - A HISTOCHEMICAL METHOD FOR LIGHT AND ELECTRON-MICROSCOPIC LOCALIZATION OF MERCURY IN TISSUE.*
Journal of Histochemistry & Cytochemistry, 1985. **33**(3): p. 219-228.

6. Zarnescu, O., *Tracing the accumulation and effects of mercury uptake in the previtellogenic ovary of crucian carp, Carassius auratus gibelio by autometallography and caspase-3 immunohistochemistry.* Histology and Histopathology, 2009. **24**(2): p. 141-148.

7. Baatrup E. (1989). *Selenium-induced autometallographic demonstration of endogenous zinc in organs of the rainbow trout, Salmo gairdneri.* Histochemistry 90, 417-425.

8. Baatrup E. and Danscher G. (1987). *Cytochemical demonstration of mercury deposits in trout liver and kidney following methyl mercury intoxication: differentiation of two mercury pools by selenium.* Ecotoxicol. Environ. Saf. 14,129-141.

9. Baatrup E. and Døving K.B. (1990). *Histochemical demonstration of mercury in the olfactory system of salmon (Salmo salar L.) following treatments with dietary methylmercuric chloride and dissolved mercuric chloride.* Ecotoxicol. Environ. Saf . 20, 277-289.