# ACUTE TOXICITY OF SODIUM SELENITE AND SODIUM SELENATE TO TILAPIA, *Oreochromis niloticus*, FINGERLINGS

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**ABSTRACT**

Selenium is an essential nutrient for many organisms, including fish. It can be released in the water by natural processes of dissolving rocks and minerals, and by the wastewater from industries and agricultural activities, which can increase its concentration in the environment, leading to toxic effects to the aquatic biota. Median Lethal Concentrations ($LC_{50\text{-}96h}$) of two forms of selenium were estimated to fingerlings of Nile tilapia *Oreochromis niloticus,* focusing on estimating indicators for future environmental risk assessments in aquatic ecosystems contaminated with those elements, particularly for evaluate sources of water quality suitable for rearing tilapia. The results were: $LC_{50\text{-}96h}$ of sodium selenite ($Na_2SeO_3$) = 4.42 mg $Se^{4+}$ $L^{-1}$, and $LC_{50\text{-}96h}$ of sodium selenate ($Na_2SeO_4$ ) = 14,67 mg $Se^{6+}$ $L^{-1}$. According to those data, it was possible to classify sodium selenite as highly toxic and sodium selenate as moderately toxic to fingerlings of tilapia.

**Key words**: Selenium; aquatic toxicology; fish; aquaculture

# TOXICIDADE AGUDA DE SELENITO E SELENATO DE SÓDIO PARA ALEVINOS DE TILÁPIA, *Oreochromis niloticus*

**RESUMO**

O selênio é um nutriente essencial para muitos organismos. Este sal pode ser liberado na água por processos naturais, pela dissociação de rochas e minerais ou por restos de processos industriais e de atividades agrícolas, aumentando desta maneira sua concentração no ambiente, levando a efeitos tóxicos para a biota aquática. Concentrações Letais Medianas ($CL_{50\text{-}96h}$) de duas formas de selênio foram estimadas para alevinos de tilápia do Nilo, *Oreochromis niloticus*, com o intuito de estimar indicadores para futuras avaliações de risco ecotoxicológico em recursos hídricos contaminados com estes elementos, especialmente para avaliar as fontes de qualidade de água apropriadas para a tilapicultra. Os resultados obtidos foram: $CL_{50\text{-}96h}$ de selenito de sódio ($Na_2SeO_3$) = 4,42 mg $Se^{4+}$ $L^{-1}$, e $CL_{50\text{-}96h}$ de selenato de sódio – ($Na_2SeO_4$ ) = 14,67 mg $Se^{6+}$ $L^{-1}$. A partir desses valores foi possível classificar o selenito de sódio como altamente tóxico e o selenato de sódio como moderadamente tóxico para alevinos de tilápia.

**Palavras chave**: Selênio; toxicologia aquática; peixe; aquicultura

**Nota Científica**: Recebida em 26/07/2010 – Aprovada em 11/05/2011

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## INTRODUCTION

Selenium in nature is released into water by the dissolving or oxidation of rocks and minerals in soluble salts, and it is an essential nutrient for many animal species, including fish. Selenium is a component of the enzyme glutathione peroxidase which protects cells and membranes (WATANABE *et al.*, 1997, MONTEIRO, 2007). Industry utilizes selenium in the manufacture of pigments used in paints, dyes, glass, photoelectric cells, rectifier components, semi-conductors, medicines and insecticides (GUIMARÃES, 2010; MSPC, 2011). SWEE *et al.* (2002) comment that the increased levels of selenium in water due to industrial and agricultural runoff results in the contamination of aquatic ecosystems, which has noxious effects in fish. The most significant effect of excess Se in fish is the accumulation of Se in eggs and subsequent larval deformities (LEMLY, 1997; LEMLY, 2002; HOLM *et al.*, 2005; MUSCATELLO *et al.*, 2006). Other documented effects in fish include skin lesions, cataracts, swollen gill filament lamellae, myocarditis, and liver and kidney necrosis (LEMLY, 1997; LEMLY, 2002; LOHNER *et al.*, 2001).

Since aquaculture depends on water resources, originating from phreatic aquifers, rivers and lakes, the presence of toxic quantities of selenium can cause harm to this commercial activity. In general, selenium is considered one of the most toxic mineral elements for fish, when present at high concentrations. According to SORENSEN (1991) the mortality induced by selenium varies with species, temperature, source of contamination, concentration, exposure time and chemical form. TAKAYANAGI (2001) and BIRUNGI *et al.* (2007) pointed out that different oxidation states of each chemical element can cause various effects in toxicity tests carried out with fish.

Acute toxicity tests are generally carried out in order to determine the Median Lethal Concentrations ($LC_{50-96h}$) of xenobiotics, focusing on estimating indicators for environmental risk assessments in aquatic ecosystems (LOMBARDI, 2004).

The aim of the present study was to determine the median lethal concentration ($LC_{50-96h}$) of selenium in the form of sodium selenite and sodium selenate to tilapia fingerlings, *Oreochromis niloticus*, as a contribution for future environmental risk assessments, and particularly for evaluate sources of water quality suitable for rearing tilapia.

## MATERIALS AND METHODS

The experiments were conducted in the Laboratory of Pathology of Aquatic Organisms, Fisheries Institute, São Paulo, Brazil, in a climatized environment (constant temperature of 25.8 ± 0.21 $^{0}$C). Fingerlings of tilapia were purchased from commercial fish farms (mean weight of 0.41 ± 0.12 g and mean length of 2.46 ± 0.21 cm). They were acclimated initially for a period of 96 h, in aquaria of 60 L, at a density of 0.8 g fish $L^{-1}$, in accordance with toxicity tests (APHA *et al.*, 2005). During this period, fish were examined for possible diseases, presence of parasites or physical damage. Afterwards, they were kept for more 48 h in other aquaria, under the same laboratory conditions, where the tests would be conducted. During these periods, food was supplied in the form of commercial pellets with 38% crude protein.

Each assay was run in aquaria containing 5 L of dechlrorinated tap water, equipped with artificial aeration and covered internally with a transparent plastic bag. Ten fingerlings of tilapia were randomly distributed into each aquarium. The system utilized was the renewal test, where all test solutions, in their entire volume, were replaced every 24 h. The experiments were carried out for 96 hours of exposure time (APHA *et al.*, 2005) at selenium (Se) concentrations of 1.0, 2.0, 4.0, 8.0 and 16.0 mg $L^{-1}$ prepared from a stock solution of 1000 mg $L^{-1}$ Se. There were three replicates for each concentration. Two forms of Se: sodium selenite $Na_2SeO_3$ ($Se^{4+}$) and sodium selenate $Na_2SeO_4$ ($Se^{6+}$) were tested separately in the same concentration range cited above. The control groups consisted of aquaria containing only dillution water, with no addition of selenium. The range of concentrations employed in this definitive test was previously tested through preliminary and explanatory tests, carried out with the same methodology described for this definitive assay. The physical and chemical variables of the water, including temperature (ºC), electrical conductivity (µS.cm$^{-1}$), pH and dissolved oxygen (mg L$^{-1}$), were monitored two hours after the test beginning and every 24 h. At the end of the 96-h period, water samples were collected from the aquaria and analyzed for total hardness (mg $CaCO_3$ L$^{-1}$) and total ammonia (mg $NH_4$ L$^{-1}$), transformed to unionized ammonia (mg $NH_3$ L$^{-1}$) through the method recommended by Bower and Bidwell (1978) and adapted for this study. The analysis of water quality mentioned above were carried out according to standard methods (APHA *et al.*, 2005), and it was performed only for the lower, higher, and intermediate concentrations and control group as well.

After periods of 24, 48, 72 and 96 h, mortality was recorded and dead individuals were removed from the tanks. The $LC_{50-96h}$ was determined by the Trimmed Spearman Karber statistical method, and data set was adjusted for control mortality using Abbott's correction (HAMILTON *et al.*, 1977).

## RESULTS AND DISCUSSION

No alterations were observed in the physical and chemical variables of the water utilized in the acute toxicity tests with tilapia fingerlings (Tables 1 and 2) that could have interfered in the determination of the $LC_{50}$. The water characteristics were all at levels within the range considered as ideal for fish maintenance (BOYD, 1982). Even the concentrations of unionized ammonia (0.031 to 0.076 mg L$^{-1}$) were very below the level of 0.26 mg L$^{-1}$ considered by EL-SHAFAI *et al.* (2004) as lethal for tilapia.

**Table 1.** Physical and chemical variables of water in tests for acute toxicity of sodium selenite in *Oreochromis niloticus* fingerlings (Mean ± Standard Deviation)

| Concentration (mg $Se^{4+}$ L$^{-1}$) | Temperature (ºC) | Dissolved Oxygen (mg L$^{-1}$) | pH | Conductivity (µS cm$^{-1}$) | Unionized Ammonia (mg $NH_3$ L$^{-1}$) | Hardness (mg $CaCO_3$ L$^{-1}$) |
|---|---|---|---|---|---|---|
| **Control** | 26.0 ± 0.1 | 6.3 ± 0.2 | 7.4 ± 0.03 | 65.9 ± 2.5 | 0.031 ± 0.019 | 22.2 ± 0.9 |
| **1.0** | 26.0 ± 0.1 | 6.5 ± 0.2 | 7.5 ± 0.02 | 68.6 ± 1.2 | 0.032 ± 0.011 | 22.9 ± 0.9 |
| **4.0** | 26.0 ± 0.1 | 6.4 ± 0.1 | 7.6 ± 0.03 | 79.9 ± 0.9 | 0.051 ± 0.007 | 24.2 ± 2.3 |
| **16.0** | 26.0 ± 0.1 | 6.6 ± 0.1 | 7.8 ± 0.10 | 103.6 ± 3.5 | 0.076 ± 0.007 | 22.2 ± 1.1 |

**Table 2.** Physical and chemical variables of water in tests for acute toxicity of sodium selenate in *Oreochromis niloticus* fingerlings (Mean ± Standard Deviation)

| Concentration (mg $Se^{6+}$ L$^{-1}$) | Temperature (ºC) | Dissolved Oxygen (mg L$^{-1}$) | pH | Conductivity (µS cm$^{-1}$) | Unionized Ammonia (mg $NH_3$ L$^{-1}$) | Hardness (mg $CaCO_3$ L$^{-1}$) |
|---|---|---|---|---|---|---|
| **Control** | 26.1 ± 0.1 | 6.4 ± 0.2 | 7.3 ± 0.01 | 65.6 ± 1.1 | 0.036 ± 0.006 | 22.2 ± 0.9 |
| **1.0** | 25.6 ± 0.1 | 6.5 ± 0.0 | 7.4 ± 0.01 | 69.8 ± 0.9 | 0.033 ± 0.002 | 22.2 ± 0.9 |
| **4.0** | 25.7 ± 0.1 | 6.5 ± 0.1 | 7.7 ± 0.02 | 81.2 ± 1.4 | 0.038 ± 0.007 | 22.2 ± 1.1 |
| **16.0** | 25.6 ± 0.0 | 6.6 ± 0.0 | 7.7 ± 0.01 | 119.7 ± 2.5 | 0.047 ± 0.004 | 23.5 ± 0.4 |

Since the beginning of the experiment it was observed that sodium selenite ($Se^{4+}$) showed to be more toxic than sodium selenate ($Se^{6+}$) (Tables 3 and 4). Some clinical signs of intoxication were observed in the fish in the first hours of exposure, mainly at the highest $Se^{4+}$ concentration (16.0 mg L$^{-1}$), in which the animals showed rapid opercular movement, hyperactivity, darkening of the skin (melanosis), dyspnea and death. Similarly to the present study, MAUK and BROWN (1999) reported that in the first hours of exposure to sodium selenite, the pike-like freshwater perches *Stizostedion vitreum* juveniles showed a slight discoloration and spiraling and erratic swimming. GAIKWAD (1989) also observed rapid opercular movement in juveniles of the Cichlidae *Etroplus maculatus* submitted to 96h of acute toxicity, test with selenium.

In the major part of the experiments, the mortality rate of fingerlings increased with selenium concentration in the water (Tables 3 and 4). $Se^{4+}$ at 16.0 mg $L^{-1}$ caused 100% mortality at 72 h. Similar effects of acute toxicity with tilapia were observed by FRANÇA *et al.* (2007) employing the same concentration of selenium from the present study, confirming the aspects of test-reproductivety and low interference of genetic variability over the sensitivity of this species for this compound.

**Table 3**. Cumulative mortality (%) of *Oreochromis niloticus* fingerlings in the acute toxicity test with sodium selenite ($Se^{4+}$)

| Concentration (mg $Se^{4+}$ $L^{-1}$) | Cumulative Mortality (%) | | | | | | | | | | | | Means (96h) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time (hours) | | | | | | | | | | | | |
| | 24 | | | 48 | | | 72 | | | 96 | | | |
| **Replicates** | **A** | **B** | **C** | **A** | **B** | **C** | **A** | **B** | **C** | **A** | **B** | **C** | |
| **control** | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 3,3 |
| **1.0** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 10 | 13,3 |
| **2.0** | 0 | 0 | 0 | 0 | 20 | 0 | 0 | 20 | 0 | 0 | 20 | 0 | 6,6 |
| **4.0** | 0 | 0 | 0 | 10 | 0 | 0 | 30 | 20 | 0 | 60 | 40 | 40 | 46,6 |
| **8.0** | 0 | 0 | 0 | 80 | 30 | 0 | 90 | 80 | 50 | 90 | 90 | 70 | 83,3 |
| **16.0** | 50 | 50 | 70 | 70 | 90 | 90 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

**Table 4**. Cumulative mortality (%) of *Oreochromis niloticus* fingerlings in the acute toxicity test with sodium selenate ($Se^{6+}$)

| Concentration (mg $Se^{6+}$ $L^{-1}$) | Cumulative Mortality (%) | | | | | | | | | | | | Means (96h) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time (hours) | | | | | | | | | | | | |
| | 24 | | | 48 | | | 72 | | | 96 | | | |
| **Replicates** | **A** | **B** | **C** | **A** | **B** | **C** | **A** | **B** | **C** | **A** | **B** | **C** | |
| **control** | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 20 | 0 | 0 | 6.6 |
| **1.0** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 3.3 |
| **2.0** | 0 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 10 | 6.6 |
| **4.0** | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 10 | 0 | 10 | 30 | 10 | 16.6 |
| **8.0** | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 10 | 0 | 20 | 10 | 0 | 10.0 |
| **16.0** | 10 | 0 | 0 | 20 | 20 | 40 | 30 | 50 | 60 | 30 | 90 | 60 | 60.0 |

The Median Lethal Concentrations ($LC_{50-96h}$) estimated for $Se^{4+}$ and $Se^{6+}$ were, respectively, 4.42 mg $L^{-1}$ (with 3.71 – 5.26 mg $L^{-1}$ as 95% of confident limit), and 14.67 mg $L^{-1}$ (with 11.94 - 18.03 mg $L^{-1}$ as 95% of confident limits). These values are within the range of 1 to 35 mg $L^{-1}$ $Se^{4+}$ determined by NIIMI and LAHAM (1976) to the zebra fish (*Danio rerio*). MILLER *et al.* (2007) reported that sodium selenite ($Se^{4+}$) were more toxic than sodium selenate ($Se^{6+}$) in an experiment carried out with rainbow trout, *Oncorhynchus mykiss*. The same aspect was observed in the present study for tilapia. The $LC_{50-96h}$ values were lower than 70.0 mg $L^{-1}$ $Se^{4+}$ and similar to 10.6 mg $L^{-1}$ $Se^{6+}$ found to *Pargus major* (TAKAYANAGI, 2001). Higher value of $LC_{50-96h}$ to $Se^{4+}$ (11.7 mg $L^{-1}$) was reported by

MAUK and BROWN (1999) to juveniles of *Stizostedion vitreum*. Nevertheless, it may be attributed to the high hardness of the water used in that study (380 mg $CaCO_3$ $L^{-1}$) compared to the lower mean level of 22.86 mg $L^{-1}$ $CaCO_3$ observed in the present study. In the same way, the findings of different $LC_{50}$ values by other authors (Table 5) may be related to the different forms of selenium utilized in the respective studies, as well as the water quality, and the different species and organism sizes employed in the tests (TAKAYANAGI, 2001).

**Table 5**. $LC_{50\text{-}96h}$ values for different species of fish exposed to sodium selenate ($Na_2SeO_4$) and sodium selenite ($Na_2SeO_3$)

| References | Species | $Na_2SeO_3$ ($Se^{4+}$) | $Na_2SeO_4$ ($Se^{6+}$) |
|---|---|---|---|
| Present study | *Orechromis niloticus (fingerling)* | 4.4 mg $L^{-1}$ | 14.7 mg $L^{-1}$ |
| TAKAYANAGI (2001) | *Pargus major* (juvenile) | 70.0 mg $L^{-1}$ | 10.6 mg $L^{-1}$ |
| MAUK and BROWN (1999) | *Stizostedion vitreum* | 11.7 mg $L^{-1}$ | |
| HAMILTON and BUHL (1990) | *Oncorhynchus kisutch* | 39.0 mg $L^{-1}$ | 32.5 mg $L^{-1}$ |
| US EPA (1987) | *Morone saxatilis* (juvenile) | 85.8 mg $L^{-1}$ | |
| US EPA (1987) | *Cyrinodan variegatus* (juvenile) | 8.3 mg $L^{-1}$ | |
| CARDWELL *et al.* (1976) | *Pimefales promelares* | 2.9 mg $L^{-1}$ | |
| NIIMI and LAHAM (1976) | *Danio rerio* | 1 to 35 mg $L^{-1}$ | |
| PALAWSKI *et al.* (1985) | *Morone saxatilis* (larvae) | 9.8 mg $L^{-1}$ | 1.6 mg $L^{-1}$ |

According to the Ecotoxicological categories stated by ZUCKER (1985), $Se^{4+}$ would be classified as highly toxic, since its $LC_{50\text{-}96h}$ (4.42 mg $L^{-1}$) was within the range of 0.1 - 1.0 mg $L^{-1}$. At the same way, $Se^{6+}$ would be classified as moderately toxic, since its $LC_{50\text{-}96h}$ (14.67 mg $L^{-1}$) was within the range of > 1.0 < 10.0 mg $L^{-1}$.

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