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CLINICAL EVALUATION AND MORTALITY OF LAMBARIS SUBMITTED TO DIFFERENT RESIDUAL CONCENTRATIONS OF TRICLOSAN IN WATER

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Abstract: Triclosan is a broad-spectrum antimicrobial that has activities against gram positive and gram negative bacteria, fungi and viruses, being used in formulations of personal hygiene products such as toothpastes, shampoos and soaps. Studies demonstrate its presence in urban and industrial effluents and report the detection of residues in surface waters and sediments from freshwater environments. The objective of this study is to evaluate the effect of residual concentrations of triclosan in yellowtail lambaris (Astyanax altiparanae), through the physiological response, such as clinical signs and mortality. Two tests were carried out,the first in concentrations of 5mg/L, 10mg/L, 15mg/L, 25mg/L and two controls. In the second assay, concentrations of 4mg/L, 3mg/L, 2mg/L, 1mg/L, 0.5 mg/L and two controls were used. In both, each fish was considered as a sampling unit with three replications. In the first trial, all fish exposed to triclosan died within 30 minutes. In the second trial, the fish showed clinical signs of intoxication and mortalities between 4h and 24h at the highest concentrations and, at the concentration of 0.5 mg/L, they remained alive for 96 h without any clinical change. Thus, the range between 1mg/L and 0.5mg/L was established as a reference for sublethal experiments. The clinical signs observed wereneurological, such as erratic swimming and spinning, culminating in the death of fish; in addition to clinical signs of hemorrhage, mainly in the gills. With the results obtained, it is concluded that the lambaris resulting from acute exposure to triclosan showed compromised health with clinical signs of intoxication and high mortality.

Keywords: *Astyanax altiparanae*, Antimicrobial, Aquatic toxicology, Emerging pollutants, Animal health, Environmental health

INTRODUCTION

The study of contaminants present in aquatic ecosystems has aroused great scientific interest, mainly due to the increasing amount of pollutants from human activities, which are released into the air, soil or directly into aquatic environments, causing impacts resulting from synergistic or antagonists of the various contaminants present in the environment (CARACCIOLO et al., 2015; YOU et al., 2015; COSTA et al., 2008).

Recent studies on pollutants in aquatic ecosystems have reported water contamination by emerging contaminants, including pharmaceutical, cosmetic and personal care products (PPCPs) (PETRIE et al., 2015).

The occurrence of PPCPs has been reported in different countries and different environments such as soil, sediment, and in surface waters (MARTINS, 2018; MATAMOROS et al., 2009). Antimicrobials are among the most detected in evaluation and biomonitoring studies (BRAUSCH; RAND, 2011). Triclosan (TCS) (5-chloro-2-(2,4-dichlorophenoxyphenol) stands out among PPCPs, and has the ability to permeate the cell membrane and target multiple sites in the cytoplasm and membrane, including those related to RNA synthesis and production of macromolecules (SALEH et al. 2010), exerting toxic effects on aquatic organisms.

The toxic activity of TCS in non-target organisms is still not well understood, however it can cause narcosis in some organisms (LYNDALL et al., 2010) and the inhibition of type II enzyme system components (McMURRAY et al., 1998; LU; ARCHER, 2005).

In Brazil, there are few works available on the evaluation of the effects of TCS on aquatic organisms. Cortez (2011) observed anomalies in the embryo-larval development of the sea urchin Lytechinus variegatus through short-term chronic ecotoxicity assays and

evaluated the acute and chronic toxicity to Nitokra sp. (Crustacea), Lytechinus variegatus (Echinodermata) and Perna perna (Mollusca).

Some studies have used native fish species with bioindicator potential and their physiological biomarkers to assess the effects of contamination of the aquatic ecosystem (BERTIN et al., 2009). The use of bioindicator species of aquatic contamination is an efficient tool for biomonitoring both in natural environments in rivers, lakes and streams as well as in the commercial production environments of fish farms and other rural productions.

The yellow-tailed lambari (Astyanax altiparanae)(GARUTTI, 1995)is a native fishSouth Americawith wide geographic distribution, of ecological importance, economic value both as live bait or snack in cooking (FÁVARO, 2002); and fits the criteria described by Beeby (2001), as a sentinel species, in addition to having the ability to adapt to different types of environments, including animal facilities and aquariums.

The objective of this work was to evaluate the effect of residual concentrations of triclosan in yellowtail lambaris, through the physiological response, such as clinical signs and mortality.

MATERIAL AND METHODS

EXPERIMENTAL SITE

The experiment was carried out in the Laboratory of Aquatic Ecosystems (LEA) of Embrapa Meio Ambiente.

ACQUISITION OF ANIMALS, MAINTENANCE AND ACCLIMATIZATION

Fish of the species Astyanax altiparanae (yellow-tailed lambari) were used, which were acquired from a fish farm registered and registered in the CEUA of Embrapa Meio Ambiente. In total, 39 adult lambaris were used in both trials, 18 fish in the first trial and 21 fish in the second trial.

The essays were approved by the CEUA of Embrapa Meio Ambiente Protocol n°010/2019 and are part of the master's thesis of the student Juliana Augusta Gil under the supervision of the Researcher Márcia Mayumi Ishikawa and co-supervision of the Researcher Vera LúciaScherholzfrom Castro.

The fish were initially acclimatized in the laboratory in a net tank installed inside a 2,000L fiberglass tank with a water recirculation system until the start of the experiment. They were fed twice a day with commercial food and constant oxygenation.

TESTS AND EXPERIMENTAL DESIGN

After a period of seven days of acclimatization, the fish were distributed in glass aquariums with 100L of useful volume without water recirculation and constant oxygenation.

Triclosan is not soluble in water so initially it was diluted in common alcohol. The dilution in alcohol was determined in a previous test to obtain the smallest volume of alcohol for its complete dilution. The dilution was carried out in the proportion of 1mg/5mL of alcohol. Triclosan concentrations were determined according to what was found in the literature in a study with tilapia (Oreochromis niloticus). Three fish were used per treatment, each fish being considered as a sample unit, that is, three replications.

In the first trial, four concentrations of triclosan were used, being 5mg/L, 10mg/L, 15mg/L and 25mg/L and two controls, one with alcohol and the other without alcohol (Figure 1).

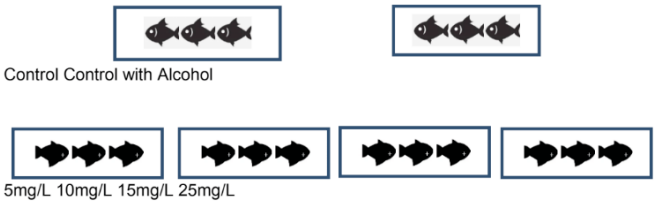

Figure 1. Representation of the design of the first trial

In the second trial, five concentrations of triclosan were used, being 4mg/L, 3mg/L, 2mg/L, 1mg/L and 0.5 mg/L and two controls, one with alcohol and the other without alcohol (Figure 2).

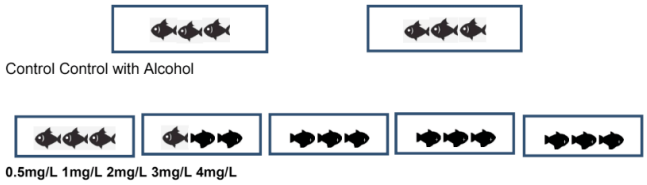

Figure 2. Representation of the design of the second trial

RESULTS AND DISCUSSION

In the first test, alteration of the fish was observed soon after the addition of Triclosan in the water. The fish showed agitation, breathing difficulties, muscle spasms, hemorrhages throughout the body, mainly in the gills, erratic swimming and whirling that culminated in the death of all fish in less than 30 minutes (Figure 3). The three fish from the control treatment without alcohol and the three from the control group with alcohol remained alive without showing any clinical signs throughout the experiment.

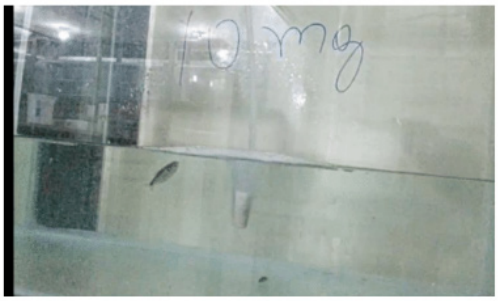

Figure 3.Lambari showing restlessness and erratic swimming in the 10mg/L treatment

In the second trial, the same clinical changes and the death of all fish were observed within 30 minutes in the treatments of 4 and 3mg/L. The fish in the 2mg/L treatment died within 4h, those in the 1mg/L treatment died within 24h with less intense clinical signs and the three fish in the 0.5mg/L treatment remained alive for 96h, that is, until the end of the experiment without showing any clinical signs of intoxication. Thus, it was possible to establish the concentration range from 0.5mg/L to 1mg/L for sublethal experiments with adult lambaris. However, the importance of more specific and detailed studies on the toxicology of triclosan in lambaris and especially its effects on the different stages of life of the lambari and in the aquatic ecosystem is observed.

According to Dube and Hosetti (2010), the respiratory activity of fish is one of the first physiological variables that is affected by aquatic contamination. Consequently, parameters closely associated with respiratory demand, such as oxygen consumption, ventilatory frequency and gill irritation/damage are suggestive indicators of intoxication in these animals and, consequently, of water contamination.

In a study conducted by Martins (2018), the effects of Triclosan on the cardiorespiratory response of Brycon amazonicus were evaluated, finding results similar to those observed in this work. The author found

temporary loss of balance, slow or confused movement and eyes with opaque coloration after approximately 8 hours of exposure to TCS at concentrations of 1.3 and 13 mg/L.

Priyatha and Chitra (2018) also described similar behavioral changes in Anabas testudineus at a concentration of 1.4 mg/L; and Çelebi and Gök (2018) observed that Danio rerio and Poecilia reticulata when exposed to TCS at different concentrations (0.01; 0.02; 0.05; 0.1; 0.2; 0.5 mg/L), also reported behavioral changes in the animal, such as rapid and uncontrolled swimming, erratic movement, often trying to escape the respirometer, loss of balance, difficulty breathing, and mucus production by the body.

CONCLUSION

High concentrations of triclosan residues in water causedchangesneurological disorders in lambaris such as erratic swimming and whirling, in addition to hemorrhages, mainly in the gills. The lambaris evaluated in this study showed compromised health with clinical signs of intoxication and high mortality.

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