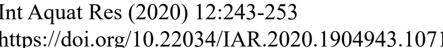
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ORIGINAL RESEARCH

Physiological and biochemical responses of Nile tilapia (*Oreochromis niloticus*) to acute trichlorfon exposure

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Abstract Trichlorfon [TCF] is an organophosphate compound used to eliminate or control a variety of parasites in farmed fish. The physiological and biochemical responses of juvenile Nile tilapia were studied after 96 hours of exposure to TCF. The experiment was conducted randomly with two treatments: control and TCF (0.5 mg L^{-1}). No mortality or changes in hematological profile were recorded for the fish. On the other hand, we found that TCF exposure caused behavioral, metabolic and hormonal changes that modified the response patterns of the immune and enzymatic defense system in muscle and liver tissues. Increased plasma cortisol and glucose combined with the reduction of acetylcholinesterase activity in muscle and liver tissue after 96-h of TCF exposure indicates chemical stress. In response to this harmful situation, we observed changes in the immune system that lead to an increased number of leukocytes, thrombocytes, monocytes, lysozyme and leukocyte respiratory burst activity. Furthermore, we associate the increased activity of glutathione-S-transferase in liver tissue as a strategy fish use to combat the effects of TCF. Thus, we conclude that TCF affects the physiologic parameters of Nile tilapia, which can generate irreversible situations of recovery of the clinical condition. Such fact highlights the need to search for new compounds that could replace TCF, that have no or few adverse effects on fish species.

Keywords Aquaculture . Toxicology . Chemotherapy . Trichlorfon

Introduction

For aquaculture, chemical products have become strategic for fish management and are considered an important factor for the success of such activity (Faruk et al. 2010), as they help reduce economic losses associated with disease outbreaks (Guimarães et al. 2007; Sinha et al. 2010; Reverter et al. 2014). However, the inadequate use of chemical products in fish farms can negatively affect fish by decreasing their immunity and productivity, as well as negatively impact the environment, economy and indirectly affect human health.

Trichlorfon [TCF] is an organophosphate compound used to eliminate or control a variety of parasites that are found in farmed fish species, e.g., *Lernae* sp. *Argulus* sp. *Ergasilus* sp. *Lernea* sp. *Dactylogyrus* sp. and *Trichodina* sp. (Heo and Shin 2009; Burridge et al. 2010; Coelho et al. 2011). In fish farms, TCF is generally used in immersion baths at concentrations of 0.1 to 0.5 mg L^{-1} to treat parasite infestations (Pavanelli et al. 2002; Chandrasekara and Pathiratne 2005; Hispano et al. 2013). For Nile tilapia (*Oreochromis niloticus*), the company that manufactures this pesticide recommends that it be used 0.5 mg L^{-1} to treat different parasites. Although TCF helps reduce economic losses caused by parasites in fish, this chemical is toxicologically classified by the Environmental Protection Agency (EPA) as a General Use Pesticide (GUP) that is Moderately Toxic (Class II; EPA 1991). In many developed countries, it is illegal to

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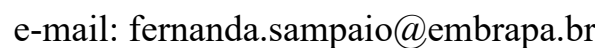
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use such compounds but is still commercialized in Brazil and other developing countries.

The primary mode of action for organophosphorus pesticides like TCF is cholinesterase (AChE) inhibition (WHO 1992; Sinha et al. 2010). In fish, TCF inhibition in AChE activity was reported in the brain or muscle of tambaqui (*Colossoma macropomum*) by Ducan et al. (2020), in silver catfish (*Rhamdia quelen*) by Baldissera et al. (2019), in pacu (*Piaractus mesopotamicus*) by Venturine et al. (2014) and pangas catfish (*Pangassius pangassius*) by Tham et al. (2014). Furthermore, TCF has also been associated with severe toxicity for many fish species, e.g., immunotoxicity and hematotoxicity for common carp (*Cyprinios carpio*) (Chandrasekara and Pathiratne 2005; Woo et al. 2018), hepatotoxicity for prussiana carp (*Carassius auratus gibelo*) (Xu et al. 2009) and pacu (*Piaractus mesopotamicus*) (Venturine et al. 2014), and embryotoxicity for zebrafish (*Danio rerio*) (Coelho et al. 2011). It is essential to highlight that little research has been done to elucidate the impact of TCF on Nile tilapia, a widely used aquaculture species (Costa-Pierce 2003).

Nile tilapia production is widespread on all continents, including 135 countries (FAO – Food and Agriculture Organization of the United Nations 2014). In Brazil, the Nile tilapia is the most common cultivated species in fish farms. The production of this species in 2018 was over 311 thousand tons, representing more than half (55.4%) of the total Brazilian fish production (IBGE – Brazilian Institute of Geography and Statistic 2018). However, Nile tilapia production is affected by several parasites that cause economic losses (Zago et al. 2014), demonstrating the importance of studies focused on comprehending how TCF affects the physiology of Nile tilapia. Knowing that TCF is potentially toxic for fish treated under production and for non-target organisms, monitoring the hematology, immune system, metabolism and enzymatic biomarkers of the nervous system and oxidative stress is essential to determine how fish deal with exposure.

In Brazil, TCF has not restricted use and its products are widely marketed for use in agriculture and livestock as insecticide and vermicide (ANVISA – National Health Monitoring Agency). However, TCF is not included on the list of compounds allowed in Brazilian surface waters (CONAMA – National Environment Council 2005). Thus, the TCF compound is neither regulated nor monitored by responsible agencies, and there are no regulations regarding the quantity used in animal production. Therefore, there are no records of the amount of TCF used in Brazilian fish farms (Duncan et al. 2019). Furthermore, it is possible that products containing TCF as active components are indiscriminately used in Brazilian fish farms, in high concentrations, and without specialized technical orientation (Guimarães et al. 2008).

Considering that there are few studies about the effects TCF has on physiological and biochemical responses of the Nile tilapia, this study could provide valuable information to fish producers about the fish health risks associated with using TCF. Thus, this study aimed to assess if TCF treatment at a concentration of 0.5 mg L^{-1} alters the hematological, immunological, metabolic and biochemical profiles of Nile tilapia after 96-h of exposure.

Materials and methods

Fish supply and acclimation

Juveniles of *O. niloticus* (42.23 ± 1.09 g) were obtained from a commercial fish farm (Polettini, Mogi Mirim, SP, Brazil). Fish were acclimated in laboratory conditions for three weeks before experimentation. Fish were maintained in a recirculation water system (4 L min^{-1} per aquarium) with physical and biological filter (1500 L) and a continuous supply of well-aerated, dechlorinated water and constant physicochemical parameters. Temperature (27.6 ± 0.68 ºC), dissolved oxygen (6.7 ± 1.24 mg L^{-1}) and pH (7.3 ± 0.43) were measured daily with a multiparameter (U-50, Horiba, Minami-ku, Kyoto, Japan) and ammonia (0.03 ± 0.49 mg L^{-1}) with a commercial kit (Hach, Loveland, CO, USA).

During acclimation, fish were fed *ad libitum* with commercial food ratio (Guabi® specific for tilapia: 4-5 mm; 10 % moisture; 32 % protein; 6.5 % fat), consuming an average of 4% of their body weight.

Trichlorfon

The commercial product utilized was Masoten Bayer®, which contains 80% trichlorfon (dimethyl

2,2,2-trichloro-1-hydroxyethyl phosphate). The concentration (0.5 mg. L^{-1}) used was recommended for fish farms to eliminate parasite infestations (Pavanelli et al. 2002; Chandrasekara and Pathiratne 2005).

Experimental protocol

All procedures involving animals in this study were performed following ethical principles in animal experimentation and approved by the Committee for Ethics in Animal Experimentation at the Embrapa Meio Ambiente (protocol nº 005/2017)

The experimental design was completely randomized with two treatments: group exposed to 0.5 mg. L^{-1} TCF for 96-h (TCF_{96h}; n = 12) and control group (Ctrl; n = 12) with three repetitions and 36 fish per treatment. Fish were placed in a glass aquarium (volume 150 L) containing bio filtered freshwater. During the experimental period, fish behavior was observed and recorded.

After 96 h of exposure, four fishes per experimental unit (12 fishes per treatment/24 fish total) were anesthetized with benzocaine (65 mg L^{-1}) for blood sampling and slaughtered for white muscle and liver excision. Then the muscle and livers were washed with saline solution (0.9 % NaCl), dried with filter paper, identified, and stored at -80 °C for further biochemical analysis.

The blood was collected through caudal puncture using syringes bathed with EDTA (3%) for the hematological analysis, heparin to determine the respiratory burst activity of leukocytes and with GLISTAB (EDTA 6 g/dL and KF 12 g/dL, Labtest, São Paulo, Brazil; code 29) to collect plasma to determine stress parameters. Anticoagulant was not used to obtain the serum used to evaluate the lysozyme concentrations. Aside from lysozyme and cortisol analyzes, the parameters were analyzed shortly after blood collection, as described below.

Hematological parameters

Blood samples were collected by caudal puncture using a syringe bathed with EDTA (3%) for the hematological analyses. To determine hemoglobin concentration (Hb) a commercial kit (Labtest, São Paulo, Brazil) was used. Erythrocytes were counted using a Neubauer camera with an optical microscope (Leica DM750 w) 40 x.

The hematocrit was determined for the microhematocrit in capillary tubes heparinized in a centrifuge (NI 1807 Nova Instruments, Piracicaba, SP, Brazil) for 5 min at 10,000 rpm. The hematimetric indices were determined as follow:

$$VCM\ (fL) = Ht*10/RBC \text{ e } CHCM\ (\%) = [Hb]*100/Ht.$$

Leukocytes and thrombocytes were determined using blood extension slides stained with May-Grunwald-Giemsa-Wright and counted under a microscope (Leica DM750 w) using an oil immersion objective (100 x).

Stress parameters

The blood was centrifuged, and the plasma was used to determine the glucose concentration as measured by glucose oxidase (Labtest, Sao Paulo, Brazil, code 84), and cortisol concentrations were determined by an immunoenzymatic assay from a commercial kit (DRG International, Inc., USA; Cortisol ELISA - EIA - 1887).

Immunological parameters

The leukocyte respiratory burst activity (RAL) was determined according to Biller-Takahashi et al. (2013). This method is based on a colorimetric determination of the reactive oxygen species (ROS), which are produced by the leukocytes respiratory burst, and promotes the reduction of nitrobluetetrazolium (NBT, Sigma, St. Louis, MO, USA) into dark blue precipitate inside the phagocyte called formazan granules (Soares et al. 2018). The optical density (OD) of the final solution was measured at 540 nm.

Lysozyme activity was measured using a turbidimetric assay (Ellis et al. 1990), with partial modifications

(Abreu et al. 2009), which lysed a suspension of *Micrococcus luteus* (Sigma-Aldrich M3770). Results were expressed in a concentration of serum lysozyme (ng/dL). One unit is defined as the amount of sample that triggers a reduction in the absorbance of 0.001 min^{-1} at 450 nm compared to the control (*M. luteus* suspension without serum).

Acetylcholinesterase (AChE)

AChE activity was determined using the colorimetric technique (Ellman et al. 1961) and adapted for microplate (Silva de Assisi 1998). The white muscle and liver samples were homogenized in 50 mM phosphate buffer pH 7.0, and centrifuged at (10,000 rpm, for 10 min, 4 ºC) and the supernatant was used for the analysis. To measure AChE activity, acetylthiocholine iodide (ATC) 9 mM was used as a substrate and 5.50-dithio-bis (2-nitrobenzoic) (DTNB) acid as a color reagent. The optical density at 412 nm was measured every minute for 10 min using an ELISA plate reader (BIO-RAD Benchmark). The AChE activity was expressed per mg of protein. The protein concentrations were measured at 595 nm by the Bradford method (1976) using bovine serum albumin (Sigma) as standard.

Hepatic oxidative stress biomarkers

To analyze lipid peroxidation (LPO), catalase activity (CAT) and glutathione-S-transferase (GST), liver samples were weighed and homogenized at 12000 rpm in phosphate buffer for 20 minutes. Centrifugation was carried out in a Hermle-Z323K (Hermle Labortechnik, Germany) refrigerated centrifuge and the supernatant used.

Liver protein was determined by the Bradford method (Kruger 1976), adapted for Dynex MRXTC 250 microplate reader (1994). Lipid peroxidation was quantified using the ferrous oxidation-xylenol orange (FOX method) (Jiang et al. 1992). CAT activity (Aebi et al. 1983) continuously evaluated the decrease in hydrogen peroxide (H_2O_2) concentration at $\lambda = 240$ nm. The GST activity was measured according to Habig et al. (1974), using l-chloro-2.4-dinitrobenzene (CDNB) as the substrate.

Spectrophotometric readings were recorded with a SpectronicGenesys 5 (Milton Roy Company, PA, USA) spectrophotometer, and the microplate readings were taken with a Tecan-SNR reader (Sunrise-Basic Tecan, NS 1105003419 Groding, Austria).

Statistical analysis

The data of this study was submitted to the normality test (Shapiro-Wilk) and homogeneity Levene Test. To compare the means of the Ctrl and TCF_{96h} groups, a Student *t*-test was performed with a significance level of $P < 0.05$ in the R studio software (v. 1.3). The results are presented as mean ± SD.

Results

In this study, the concentration of 0.5 mg L^{-1} of TCF did not cause mortality of the Nile tilapia. However, TCF exposure caused abnormal behavior such as increased gill movements, and abnormal swimming varying from erratic to lethargic and finally becoming immobile at the bottom of the aquaria.

Hematology

The values of Hb (%), Ht (%), CHCM (%), RBC (10^6 μL^{-1}) and TPP (mg dL^{-1}) from Ctrl and TCF_{96h} presented similar means throughout the experiment. On the other hand, glucose and cortisol levels in the plasma of the TCF_{96h} group were significantly higher than the Ctrl (Table 1).

Immune system

Exposure to TCF promoted an increase in the total leukocytes (25.4%), thrombocytes (42.3%) and monocytes (43.4%) when compared to the Ctrl (Figures 1A, 1B and 1C). On the other hand, compared to

the Ctrl there was a decrease of total lymphocytes (37.1%) in the TCF_{96} treatment (Figure 1D).

For leukocyte respiratory burst activity (RAL), there was an increase of 18.2% in the TCF_{96h} compared with Ctrl group, leading to a possible relationship between increased leukocytes and increased RAL (Figure 2A). For lysozyme activity, there was an increase of 24.6% in the TCF_{96h} group compared to Ctrl group (Figure 2B).

Acetylcholinesterase (AChE)

The AChE activity decreased by 86.3% in the muscle and 59.2% in the liver in the TCF_{96h} group compared to the Ctrl group (Figure 3A and 3B).

Table 1 Hemoglobin (Hb; g/dL-1), hematocrit (Ht; %), mean cell hemoglobin concentration (CHCM; %), red blood cells (RBC; µL-¹), total plasma protein (TPP), plasma glucose concentrations (glucose) of Nile tilapia exposed to Trichlorfon at concentration 0.5 mg L-1 by 96h. Values are mean ± standard error. Means in the same line with superscripts (*) are significantly different (P <0.05)

Parameters	Control	TCF_{96h}
Hb (g dL^{-1})	9.18 ± 1.7	8.60 ± 1.5
Ht (%)	27.6 ± 2.3	25.7 ± 1.0
MCHC (%)	38.9 ± 4.8	36.5 ± 7.4
RBC (10^6 μL^{-1})	1.43 ± 0.2	1.55 ± 0.3
TPP (mg dL^{-1})	4.02 ± 0.1	3.98 ± 0.3
Glucose (mg L^{-1})	48.3 ± 8.7	67.9 ± 14.4*
Plasma Cortisol (g/mL)	23.6 ± 2.5	34.8 ± 9.1 *

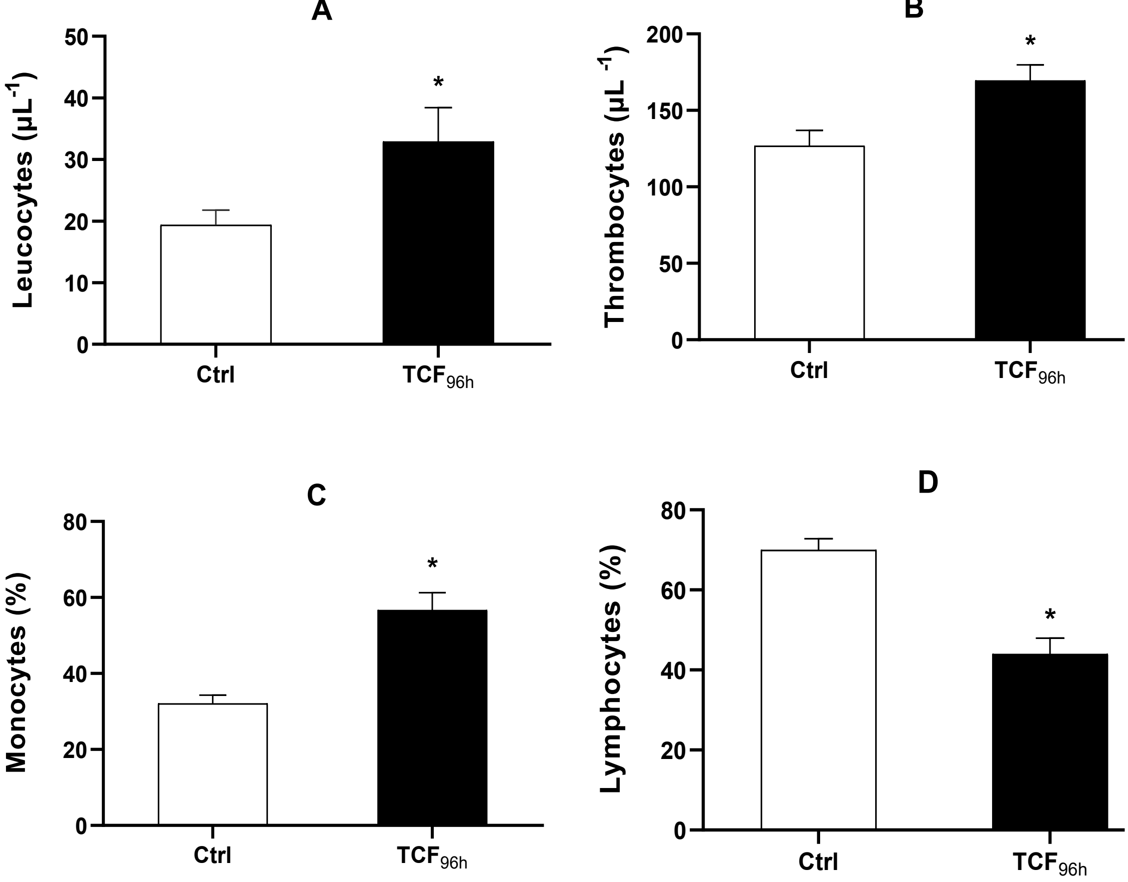

Fig. 1 Leukocytes (A), thrombocytes (B), monocytes (C) and lymphocytes (D) counts in Nile tilapia of the Control (Ctrl) and exposed to 0.5 mg L-1 trichlorfon by 96 h (TCF96h). Bars represent means ± standard error. * Differences between groups (P <0.05)

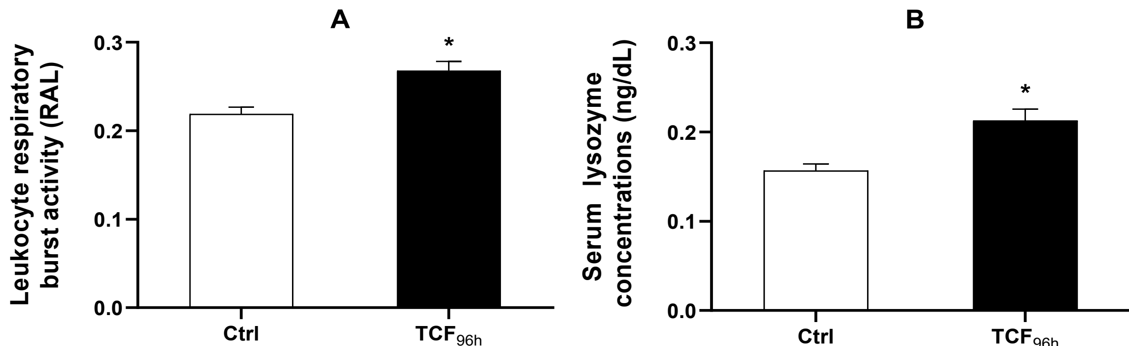

Fig. 2 Lysozyme activity (A) and leukocytes respiratory activity (B) of Nile tilapia of the Control (Ctrl) and exposed to 0.5 mg L-1 Trichlorfon by 96 h (TCF96h). Bars represent means ± standard error. * Differences between groups (P <0.05)

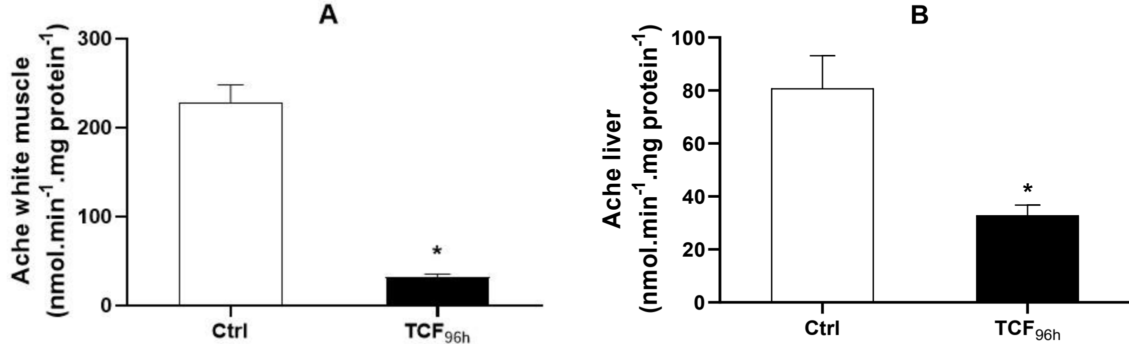

Fig. 3 White muscle and liver acetylcholinesterase activity (AChE; nmol. min mg protein-1) of the Nile tilapia of the Control (Ctrl) and exposed to 0.5 mg L-1 Trichlorfon for 96 h (TCF96h). Bars represent means ± standard error.
* Differences between groups (P <0.05)

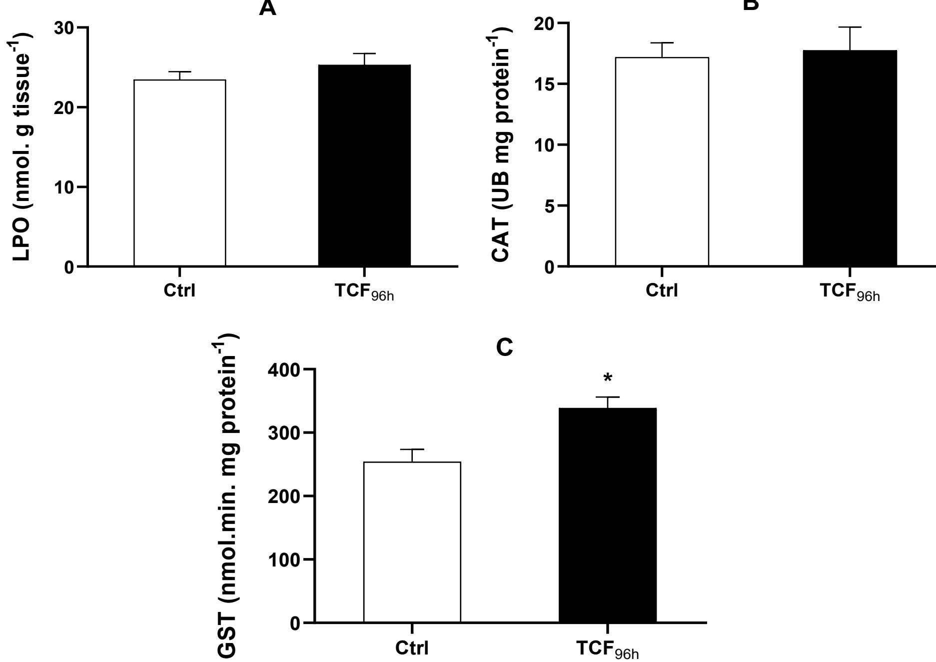

Fig. 4 Levels of lipid peroxidation (LPO; nmol g tissue-1), catalase activity (CAT; BU mg protein-1) and Glutathione S-transferase activity (GST; nmol mgPT-1) in the hepatic tissue of Nile tilapia of the Control and exposed to 0.5 mg L-1 Trichlorfon for 96 h (TCF96h). Bars represent means ± standard error. * Difference between groups (P <0.05).

Oxidative stress

The hepatic CAT activity and LPO were the same in both groups (Figure 4A and 4B). On the other hand, fish from TCF_{96h} presented an increase of 25.1% in the liver activity of GST enzyme compared to the Ctrl (Figure 4C).

Discussion

The Nile tilapia survived exposure to 0.5 mg L^{-1} of TCF after 96h. However, fish exhibited abnormal swimming behaviors and their physiological responses changed due to the stress condition. Although TCF was not lethal for the Nile tilapia, it could cause stress in fish, highlighting the need for the controlled and careful use of TCF in aquaculture.

Consistently, data available in the literature supports that plasma cortisol and glucose levels in fish indicate toxic effects of certain stressors (Aerts et al. 2015; Sadoul and Geffroy 2019). Increased cortisol levels in the blood are considered a primary response of fish under stress (Barton and Iwama 1991; Ray and Sinha 2014) and chemical exposure (Wendelaar Bonga 1997). When fish are submitted to stress conditions, a combined sequence of adaptive energy mobilization can be observed (Balasch and Tort 2019). The proposed model is that cortisol positively regulates the pathways involved in the mobilization of energetic substrates, including gluconeogenesis while regulating the pathways that require energy to deal with stress (Faught et al. 2016). Thus, the hyperglycemia observed after xenobiotic exposure appears beneficial as it provides the energy required to metabolize and eliminate the toxicant compound (Ahmad 2011). The Nile tilapia exposed to TCF_{96h} responded with hyperglycemia and increased their plasma cortisol levels, suggesting that fish were submitted to a stress condition. Hyperglycemia seems to be a striking response to TCF exposure in fish and has been reported by other authors: Venturini et al. (2014) observed hyperglycemia in pacu (*Piaractus mesopotamicus*) after exposure to 8.4 µg of TCF (Masoten®) and Woo et al. (2018) reported hyperglycemia in common carp (*Cyprinus carpio L.*) after exposure to TCF (Metriofonate®), in a range of 0.5 to 4.0 mg L^{-1}.

Additionally, the red blood cell profile of the Nile tilapia was not influenced by TCF_{96h} exposure. However, the total white blood cells increased under the TCF_{96h} with increased levels of monocytes and thrombocytes, and a decrease of lymphocytes. Among the leucocytes, the monocytes of exposed Nile tilapia doubled compared to unexposed fish. Leukocytes are known for their capacity to combat pathogens, increasing the production of ROS in a process known as leukocyte respiratory burst activity (RAL) (Biller-Takahashi et al. 2013). Furthermore, monocytes are considered leucocytes with a high ability to combat foreign agents by the RAL in the immune system (Girón-Pérez et al. 2009; Biller-Takahashi et al. 2018). The recorded increase of RAL observed for the TCF_{96h} can be understood as a strategy that Nile tilapia uses to deal with TCF exposure. Our findings corroborate the reports of Méndez et al. (2014), who observed increased RAL in silver pacu (*Piaractus brachypomus*) exposed to 0.029 mg L^{-1} of TCF and by Girón-Perez et al. (2009), who exposed Nile tilapia to 1.96 mg L^{-1} of another organophosphate, Diazinon.

The Nile tilapia activated the innate immune system when exposed to the TCF, while the adaptive immune system seems to be reduced. Such a reduction of lymphocytes indicates a deficiency in the adaptative immune system of Nile tilapia exposed to TCF. It has been suggested that OPs can modulate the lymphocytes through cholinergic receptors present in the non-neuronal cholinergic system (Diáz and Pérez 2015). According to Toledo-Ibarra et al. (2014), the spleen, an important lymphoid organ for fish, presents relevant AChE activity, indicating cholinergic activity in the tissue. Thus, the presence of the cholinergic extraneural structure makes fish susceptible to organophosphate action involving the AChE (Araújo et al. 2016). Girón-Pérez et al. (2008) observed that Nile tilapia exposed to 3.9 and 7.8 mg L^{-1} Diazinon for 96-h presented reduced AChE activity and lower acetylcholine in the spleen, leading to exhaustion of the lymphocytes proliferation capacity. Similar responses were registered by Toledo-Ibarra et al. (2016) after Nile tilapia was exposed to 0.97, 1.95 and 3.91 mg L^{-1} of Diazinon for 24 h.

The serum lysozyme, an important enzyme in the innate immune system of fish, increased in Nile tilapia in response to TCF_{96h} exposure. Lysozyme is an enzyme of the innate physiological mechanism of fish that has antiviral, antibacterial, and anti-inflammatory properties (Balfry and Iwama 2004). This enzyme is synthesized in both the liver and extra-hepatic sites as the neutrophils, monocytes and a small amount

in macrophages (Sauruabh and Sahoo et al. 2008). Lysozyme is frequently altered by organophosphates (Díaz-Resendiz and Girón-Pérez et al. 2015), and specific data about lysozyme activity in response to TCF exposure is scarce for Nile tilapia. Similar results were also registered for other fish species: Khoshbavar-Rostami et al. (2006) recorded an increase in the lysozyme content in the serum, kidney and spleen of the great sturgeon (*Huso huso*) exposed to 1.5 mg L^{-1} of Diazinon. Li et al. (2013) observed an increase in serum lysozyme of common carp (*Cyprinus carpio L.*) exposed to 15 µg L^{-1} of the pesticide chlorpyrifos.

Organophosphates, mainly TCF, have been highlighted as AChE inhibitors for different fish species (Sinha et al. 2010). For the Nile tilapia, TCF_{96h} exposure decreased muscle AChE activity, corroborating other authors findings and emphasizing this enzyme as a potential biomarker for TCF contamination. Decreased in AChE activity as a response to TCF exposure also was recorded by Chandrasekara and Pathiratne (2005), who submitted common carp to 0.25 and 0.5 mg L^{-1} of Dipterex®, Venturini et al. (2015) who exposed pacu to 8 µg L^{-1} of Masoten®, Lu et al. (2018) who exposed the Prussian carp (*Carassius auratus gibelio*) to 500, 1.000 and 2.000 mg L^{-1} of TCF (>90% pure), Duncan et al. (2019) who exposed the tambaqui (*Colossoma macropomum*) to 0.26 and 0.46 mg L^{-1} of Masoten® and Baldissera et al. (2019) who exposed the Silver catfish (*Rhamdia quelen*) to 11 and 22 mg L^{-1} of Masoten®.

Few studies have evaluated the AChE activity in the fish liver; however, some authors have reported that TCF changes the gene expression and AChE activity in fish liver (Sinha et al. 2010; Lu et al. 2010). Thus, the evaluation of AChE kinetics is an essential tool for assessing the degree of toxicity of TCF in extraneuronal tissues such as the liver. Also, as far as we know, this is the first study to demonstrate the effects of TCF on the kinetics of AChE in the liver of the Nile tilapia. Reduced AChE activity in the liver shows the presence of a non-neuronal cholinergic structure in Nile tilapia susceptible to structural and functional changes due to the presence of TCF. The susceptibility of the liver AChE means that the action of TCF in the cholinergic system is not limited to the central nervous system of fish. AChE is an enzyme that is present in the neuromuscular junction between the motor nerve and muscle. In this region, this enzyme has crucial neurophysiological functions such as propagating nerve impulses in the peripheral nervous system. In the liver, knowledge about the cellular organization of the cholinergic system and its biological significance is very limited.

The marked influence of TCF on a variety of species reaffirms the inhibitor role of TCF on AChE activity, highlighting the importance of using a safe quantity or limiting the use of this compound in aquaculture. The influence of TCF on AChE activity also reinforces the need for specific research to clarify the correlation between fish exposure and the abnormal swimming behaviors observed herein.

Observing the antioxidant defense system of the Nile tilapia exposed to TCF_{96h}, there was increased liver GST activity, while CAT activity or hepatic LPO formation remained at control levels. It is known that the GST enzyme plays an important role in the chemical biotransformation process and xenobiotic detoxification route in hepatic tissue (Huber et al. 2008). In fish, GST is involved with the conjugation mechanism of GSH with dichlorvos, a hepatic metabolite of TCF, allowing the detoxification of pesticides (Peña-Llopis et al. 2003). Coelho et al. (2011) also reported an increase in the liver GST activity in zebrafish (*Danio rerio*) exposed to 2.5 mg L^{-1} of TCF (Pestanal®). The positive regulation of GST activity in response to TCF emphasizes that the enzyme plays a crucial role in physiological detoxification for the Nile tilapia.

Increased liver GST activity in Nile tilapia seemed to be enough to prevent ROS production, while there was no increase in the hepatic LPO of fish exposed to TCF. The observed effect of TCF on the antioxidant defense system was also reported by Thomaz et al. (2009), who exposed Nile tilapia to 0.5 mg L^{-1} of Neguvon® for 96 h, and verified an increase in liver GST activity, while CAT activity and LPO did not change. Diazinon also increased the hepatic GST activity in Nile tilapia (Uner et al. 2007) and Mozambique tilapia (*O. mossambicus*) (Rao 2006), respectively. Thus, it seems that the induction of GST activity is a response to the detoxification process of organophosphates that prevents chemical injuries to fish bodies.

Conclusion

Despite the extremely efficient effect of TCF in combating fish parasites, the side effects can cause biochemical and physiological disorders in fish, leading to a stress condition. TCF led to neurotoxic actions on the central and peripheral nervous systems and caused abnormalities in the immune system, even at sub-lethal dosages. Such facts highlight the need to search for new compounds that have little to no adverse

effects on fish species and replace TCF, eliminating its use in fish production.

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Conflict of interest The authors declare that they have no conflict of interest.

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