

Triphenyltin hydroxide induces changes in the oxidative stress parameters of fish

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Abstract Among all organotin compounds, triphenyltin hydroxide (TPhTH) is widely used as fungicide and molluscicide in Brazil. However, the effects of TPhTH on the biochemical parameters of non-target organisms, such as fish, are little known. The aim of the present study is to assess the possible toxic effects of different concentrations of waterborne TPhTH on silver catfish belonging to species *Rhamdia quelen*. The fish were exposed to two different concentrations of TPhTH (1.08 and 1.70 µg/L as Sn) for 15 days and then compared to the control group (triplicate, $n = 3$). The antioxidant profile (catalase (CAT) and the glutathione *S*-transferase (GST)) and the oxidative stress parameters (TBARS—thiobarbituric acid-reactive substances and protein carbonyl (PC)) were set after the exposure to TPhTH. The TBARS level and the PC content increased in several organs of the *Rhamdia quelen* (brain, liver, muscle and gills) under the two concentrations of TPhTH in comparison to the control group. The CAT activity in the liver and gills has enhanced in all tested TPhTH concentrations. The GST activity increased in the brain, liver and muscle tissues under all the TPhTH

concentrations. The significant changes in the biomarkers indicated that the investigated pesticide could have harmful effect on fish, in the field. However, these biomarkers were measured after the fish received doses lower than the recommended for use in agriculture.

Keywords Oxidative stress · Silver catfish · Toxicity · TPhTH

Introduction

Triphenyltin hydroxide (TPhTH), also known as fentin hydroxide, is an organotin compound (OTC) used as agricultural fungicide and molluscicide (Grisolia, 2002). In Brazil, this compound is only used in cotton and bean cultures in the form of hydroxide, chloride or acetate to control fungi (Wensem et al. 1991). According to Ostrakhovitch (2015), triphenyltins are highly toxic to humans and animals and can be degraded into diphenyltin (DPhT) and into monophenyltin (MPhT). Such degradation occurs in the environment through the loss of aryl groups from the molecule structure. The order of phenyltin toxicity is TPhTH > DPhT > MPhT, whereas the inorganic tin (Sn) is often non-toxic (Antes et al. 2013).

Although organotin pesticides were banned from many crops, considerable amounts of them have been contaminating the soil and the water. The TPhTH is one of the most common organotin pesticides (Gui et al. 2016), but the contamination resulting from its use has encouraged several studies about aquatic organisms. Since the 1980s, some of these studies have been reporting damages caused by these organisms in *Nucella lapillus* (Gibbs and Bryan 1986),

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Thais clavigera (Leung et al. 2006) and *Xenopus tropicalis* (Yuan et al. 2011). Fish are suitable pollution indicators in the environment (exposure biomarkers) and the most feasible organisms for pollution monitoring in aquatic systems (Rüdel et al. 2007).

It is well-reported that toxic agents, such as pesticides, may trigger the development of reactive oxygen species (ROS) and generate oxidative stress. The antioxidant defense system of fish detoxifies their organism and eliminates reactive oxygen species (ROS). This system is formed by antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST), and by non-enzymatic defense systems such as glutathione (GSH), vitamin E and ascorbic acid (Trenzado et al. 2006; Clasen et al. 2012). The oxidative stress in fish results from environmental pollution.

Some studies have shown the deleterious effects organotin compounds have on animals and humans due to their widespread use (Dimitriou et al. 2003; Whalen et al. 2003; Leung et al. 2006; Rüdel et al. 2007). However, none of these studies have assessed the oxidative stress caused by these compounds in freshwater fish.

Therefore, the main aim of the present study is to proceed the investigation conducted by Antes et al. (2013) about the effects of TPhTH on *Rhamdia quelen*. The oxidative stress parameters response to the exposure to different concentrations of waterborne TPhTH was assessed in different tissues of the herein studied species, which is a native freshwater teleost fish of great economic importance in Southern Brazil (Baldisserotto 2004). The present study is the first record in the literature about the effects of the exposure to different concentrations of waterborne TPhTH on fish.

Materials and methods

Rhamdia quelen specimens (weigh, 107.27 ± 1.21 g; length, 22.21 ± 1.15 cm) were purchased in a commercial teleost fish farm (Rio Grande do Sul State, Brazil). The silver catfish were placed in tanks (250 L) under continuous aeration for 12 days, under natural photoperiod (12 h light/12 h dark), before the experiment. The water parameters throughout the acclimation period were: dissolved oxygen (7.1 ± 0.7 mg/L), temperature (20.5 ± 1.0 °C), pH (7.2 ± 0.2 units), total ammonia nitrogen (0.023 ± 0.008 mg/L), non-ionized ammonia (0.015 µg/L), water hardness (23.5 ± 2.1 mg CaCO₃/L) and alkalinity (32.1 ± 1.7 mg CaCO₃/L). There were no significant variations between the water quality parameters during the experimental period and the acclimation data.

Fish were fed with commercial feed for juveniles (Supra, 42% crude protein), once a day, during the acclimation and experimental periods. The commercial product used in the present investigation—Mertin 400® (Syngenta, Brazil)—contained 400 g/L of TPhTH. The TPhTH in the water was monitored every 3 days during the experiment. It was quantified through gas chromatography-inductively coupled plasma mass spectrometry (GC-ICP-MS), according to the method previously described by Antes et al. (2013). The concentration of solutions used in the experiment was calculated according to the pre-set concentrations of TPhTH—all values were expressed as Sn.

The *Rhamdia quelen* specimens were randomly separated in tanks (60 L)-five per tank, in triplicate ($n = 3$)—at the end of the acclimation period. Next, they were exposed to 0.0 (control, TPhTH-free), 1.08 and 1.70 µg/L Sn, respectively, for 15 days. The tested concentrations were set to each tank, before the experiment had started. Uneaten food and feces were siphoned on a daily basis. At least 30% of the water in the tanks was replaced by water containing previously adjusted concentrations of waterborne TPhTH in order to keep the desired concentration (1.08 and 1.70 µg/L Sn) during the experiment. According to Antes et al. (2013), the TPhTH LC₅₀ value for silver catfish juveniles was 9.73 µg/L (as Sn). It was verified that greater concentrations of TPhTH take fish to death after a few hours. Thus, the TPhTH concentrations 1.08 and 1.70 µg/L were used in the present study, although the concentration often used in rice crops is 0.48 mg/L.

After the exposure period, the fish were anesthetized and euthanized through spinal cord section. The organs (brain, liver, muscle and gills) were carefully removed and stored at -80 °C until the time for biochemical analyses. Lipid peroxidation was set through the TBARS levels, according to the method by Buege and Aust (1978). The PC assay was set according to Yan et al. (1995). The antioxidant enzyme 'CAT' followed the method described by Nelson and Kiesow (1972), and the GST activity described by Habig et al. (1974) was adopted. The protein in the samples was found through the methodology by Bradford (1976). The details about the biochemical analyses can be found in Clasen et al. (2012). Sample normality and homogeneity were tested through the Kolmogorov-Smirnov's and Levene tests, respectively. The statistical analysis was performed through one-way analysis of variance (ANOVA) and Tukey test as post-hoc comparison. The data were homogeneous and expressed as the mean \pm standard error of mean (S.E.M.) The p value was significant at ≤ 0.05 , in all comparisons. The statistical analysis was performed in the Statistica software, version 7.0 ($n = 3$, in all experimental groups). The methodology adopted in the present experiment was approved by the Ethics and Animal Welfare Committee of Federal University of Santa Maria, under protocol number 2007–24.

Results and discussion

The means of limit of quantification (LOQs) in the water were 0.54, 0.75, and 0.57 $\mu\text{g/L}$ Sn for MPHT, DPhT, and TPhTH, respectively. The TPhTH was the only pesticide detected in the water collected in the tanks during the experiment. It was detected through the Sn speciation analysis. All teleost fish have survived the 15-day exposure to different concentrations of TPhTH. Both the lipid peroxidation (LPO) and the protein oxidation in the present study were measured through oxidative damage biomarkers. The exposure to all tested concentrations of waterborne TPhTH has significantly increased the TBARS levels in all the assessed organs of the herein analyzed *Rhamdia quelen* specimens in comparison to the control group (Fig. 1a). The increased TBARS levels found in the present study clearly indicate LPO induced by TPhTH exposure. The structure of the proteins may be damaged by molecules from oxidation processes, such as LPO (as observed in the present study), or by direct ROS attacks (Ostrakhovitch 2015). This result

meets those by Mitra et al. (2013), who found increased TBARS levels in the brain of rats exposed to different tributyltin doses, for 3 and 7 days. The trace metal levels can be highly modified, so they may lead to the formation of excessive free radicals; and it results in increased lipid peroxidation and protein modification.

The PC values reported for *Rhamdia quelen* at 1.08 and 1.70 $\mu\text{g/L}$ of TPhTH showed significant increase in all analyzed organs (brain, liver, muscle and gills) (Fig. 1b). The damage in the proteins may have been caused by molecules resulting from oxidation processes, such as LPO, or by direct ROS attacks to the proteins' structure. The ROS formation in the present study was represented by changes in the enzyme activity. The LPO and protein oxidation results highlighted that TPhTH induces oxidative damage as a clear response to TPhTH poisoning. Fish often increase the TBARS and the protein carbonyl levels after they are exposed to pesticides.

The present results suggest that the oxygen radicals produced during the aforementioned process could cause structural defects in the cellular membrane because they increase the number of double bonds in the hydrocarbon chains, fact that leads to changes in membrane permeability (Langner et al. 1998). Hartl et al. (2001) have found that the consumption rates have significantly increased in groups of fish (*Platichthys flesus*) exposed to organotin pesticides. The results found by Langner et al. (1998) and by Hartl et al. (2001) explain the changes observed in the current study concerning the LPO membrane and protein oxidation levels. Therefore, increased lipid peroxidation and protein carbonylation can further affect the toxic effect of ROS; thus, lipid peroxidation and protein carbonylation allow the metal to be more catalytically active, thereby reaching higher ROS levels (Mitra et al. 2013). The adverse effect is clearly evident through the increased CAT and GST activity.

There was better CAT activity in the liver and gills of *Rhamdia quelen* after its exposure to the two tested concentrations in comparison to the control group (Fig. 2a). The CAT protects organisms from oxidative damage when the ROS is partially removed (Kochhann et al. 2009). The superoxide anion (O_2^-) is reduced to H_2O_2 through SOD, whereas H_2O_2 is converted into water and oxygen through CAT. The changes in the CAT activity in the present study could be a good indicative of the antioxidant system activation against TPhTH-induced toxicity.

The GST activity has significantly increased in all assessed organs and in both concentrations tested in the present study in comparison to the control group (Fig. 2b). This enzyme is involved in the detoxification of many xenobiotics and plays an important role in protecting the tissue from oxidative stress. The GST induction helps handling stress conditions. It was possible concluding that

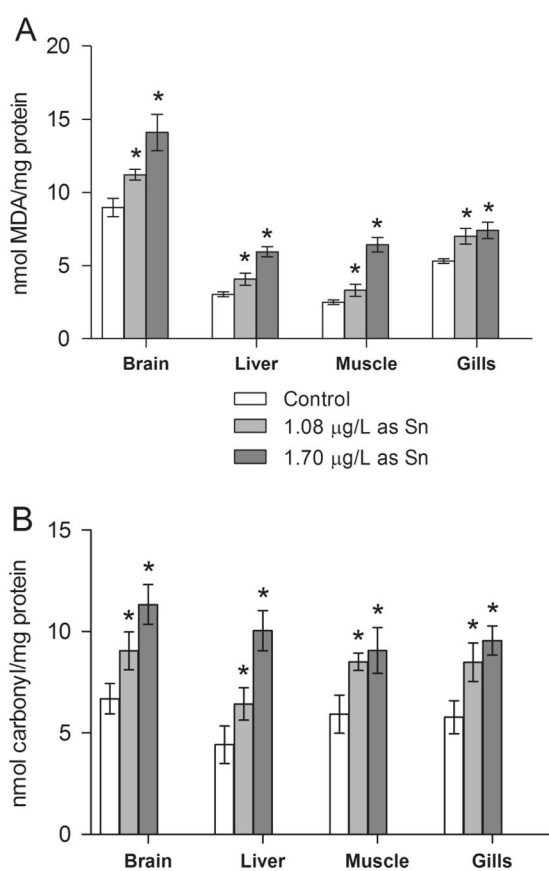


Fig. 1 TBARS levels (A) and PC content (B) in several organs of *R. quelen* after exposed to different waterborne TPhTH concentrations for 15 days. Data represent the mean \pm S.E.M. ($n=3$, for all experimental groups). Asterisks indicate significant difference from the control group at $p \leq 0.05$

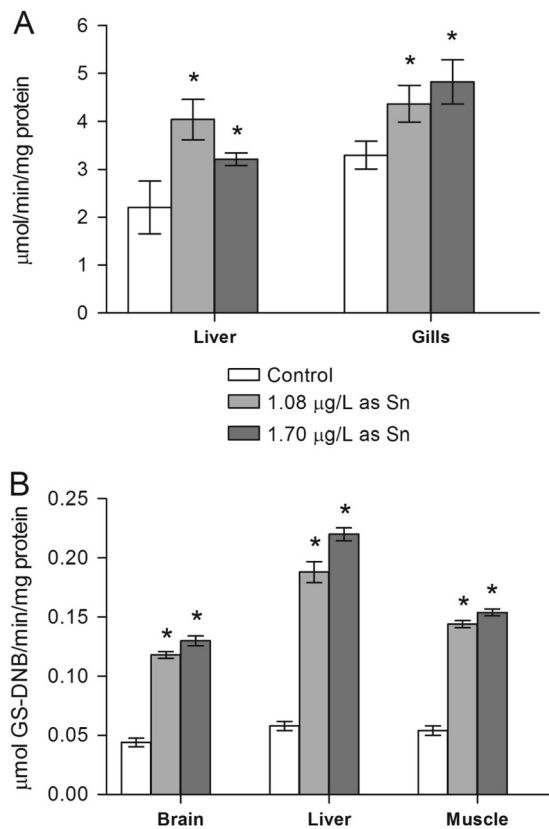


Fig. 2 CAT (A) and GST (B) activities in several organs of *R. quelen* after exposed to different waterborne TPhTH concentrations for 15 days. Data represent the mean \pm S.E.M. ($n = 3$, for all experimental groups). Asterisks indicate significant difference from the control group at $p \leq 0.05$

GST was an important tool to detoxify from TPhTH exposure. The present results indicate that all tested *Rhamdia quelen's* organs were highly affected by TPhTH. Early warnings about the toxic effects of pollutants, including oxidative stress manifestations and adaptive responses, mainly in polluted areas, can be ruled out through the adoption of the biomarker approach.

Conclusions

The oxidative stress was imposed to *Rhamdia quelen* due to its exposure to TPhTH. Such outcome was evidenced by the significant increase in LPO and in protein oxidation. It may explain the increased antioxidant enzyme activity. This increased activity has also indicated the short-time activation of enzymes that have resulted from the increased production of free radicals, which was caused by the TPhTH metabolism. The influence of TPhTH on the antioxidant system of *Rhamdia quelen* allowed understanding the ecotoxicological effect of TPhTH on the environment. Such effect helps setting the water quality standards suitable to

protect aquatic vertebrates. More experiments in future research should be performed in order to help better understanding the relationship between different biomarkers; however, it was clear that these pollutants represent a risk to aquatic ecosystems.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Human and animal rights and informed consent The current article does not contain any studies performed by any of the present authors involving human participants. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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