FULL ARTICLE

ISSN Online 1678-4456

Determination of acute median lethal concentration and sublethal effects on AChE activity of *Gymnotus carapo* (Teleostei: Gymnotidae) exposed to trichlorfon

Determinação da concentração letal média e efeitos subletais da atividade AchE de Gymnotus carapo (Teleostei: Gymnotidae) exposto ao triclorfon

Giovanni Henrique Ferri[1]; Israel Luz Cardoso[2]; Juliana Augusta Gil[1]; Claudio Martin Jonsson[3]; Francisco Tadeu Rantin[2]; Márcia Mayumi Ishikawa[1,3]

[1] Universidade Estadual de Campinas, Instituto de Biologia, Programa de Pós-graduação em Biologia Animal, Campinas – SP, Brazil
[2] Universidade Federal de São Carlos, Programa de Pós-graduação em Ciências Fisiológicas, São Carlos – SP, Brazil
[3] Embrapa Meio Ambiente, Jaguariúna – SP, Brazil

ABSTRACT

Trichlorfon (TRF) is a pesticide widely used in aquaculture to control fish ectoparasites. This pesticide is an inhibitor of acetylcholinesterase, an essential enzyme for termination of nerve impulses. High rates of TRF use generate risks to the environment and human health. In the environment, pesticides can affect the local fauna and generate an ecological breakdown. There are several studies performed with fish production; however, gaps are created for native fish with other commercial values. The tuvira (*Gymnotus carapo*) is a fish native to Brazilian fauna and has great commercial importance in sport fishing. The present study aimed to determine the lethal concentration of trichlorfon (Masoten) in *Gymnotus carapo* and its sublethal effects on the enzyme AChE. In this study, the acute toxicity (the concentrations to kill 50% of the fish LC_{50}) of TRF in tuviras (*Gymnotus carapo*) and acetylcholinesterase inhibition in liver and muscle tissue of tuviras submitted to sublethal concentrations were evaluated. For the acute assay, concentrations of 0.0, 5.0, 7.5, 15, 22.5, 30, 37.5 and 45 mg L^{-1} were used for a period of 96 h. After the acute exposure period, a LC_{50} of 6.38 mg L^{-1} was determined. In the sublethal assay, concentrations of 0.0, 0.238, 0.438 and 0.638 mg L^{-1} were used, based on 10% of the LC_{50}, over a period of 14 days. Two collections were performed: one at seven days and the other at the end (day 14). Inhibition of acetylcholinesterase in the liver was only shown ($p < 0.05$) for the treatment with 0.638 mg L^{-1} after 14 days of exposure. At seven days, muscle activity showed a significant difference only for the treatments 0.438 and 0.638 mg L^{-1}, compared with the treatment 0.238 mg L^{-1} and control. At 14 days of exposure, only the treatment 0.638 mg L^{-1} showed significant differences in relation to the other groups, thus showing that enzyme recovery had occurred. The value found in the acute test allowed the conclusion that TRF presents moderately toxic characteristics to *Gymnotus carapo*. The toxicity parameter values calculated in the present study assisted in estimation of maximum allowable limits in bodies of water when combined with test data from other non-target organisms.

Keywords: Organophosphate. Environment. Non-target organisms. Biomarkers. Tuviras.

RESUMO

O triclorfon (TRC) é um pesticida muito utilizado na aquicultura para o controle de ectoparasitos de peixes. Este pesticida é um inibidor da acetilcolinesterase, uma enzima essencial para a finalização de impulsos nervosos. As altas concentrações utilizadas de TRC geram riscos ao meio ambiente e à saúde humana. No ambiente, os pesticidas podem afetar a fauna local e gerar um colapso ecológico. Existem vários estudos com peixes de produção, no entanto, há lacunas para peixes nativos com outros valores comerciais. A tuvira (*Gymnotus carapo*) é um peixe nativo da fauna brasileira e possui grande importância comercial na pesca esportiva. O presente trabalho, delineado para determinar a concentração letal de triclorfon (Masoten®) em *Gymnotus carapo* e seus efeitos subletais na enzima AChE, avaliou a toxicidade aguda (concentrações para matar 50% dos peixes CL_{50}) do TRC em tuviras (*Gymnotus carapo*) e a inibição da acetilcolinesterase no fígado e tecido muscular de tuviras. Para o ensaio agudo, foram utilizadas concentrações de 0,0, 5,0, 7,5, 15, 22,5, 30, 37,5 e 45 mg L^{-1} por um período de 96 horas. Após o período de exposição aguda, foi determinado uma CL_{50} de 6,38 mg L^{-1}. No ensaio

subletal, foram utilizadas concentrações de 0,0, 0,238, 0,438 e 0,638 mg L^{-1}, com base em 10% do CL50, durante um período de catorze dias. Foram realizadas duas colheitas: uma aos sete dias e a outra ao final (décimo quarto dia). A inibição da acetilcolinesterase no fígado foi demonstrada apenas ($p < 0,05$) para o tratamento com 0,638 mg L^{-1} após catorze dias de exposição. Aos sete dias, a atividade muscular mostrou diferença significativa apenas para os tratamentos 0,438 e 0,638 mg L^{-1}, em comparação com o tratamento 0,238 mg L^{-1} e controle. Aos catorze dias de exposição, apenas o tratamento 0,638 mg L-1 apresentou diferenças significativas em relação aos demais grupos, demonstrando a recuperação enzimática. O valor encontrado no teste agudo permitiu concluir que o TRC apresenta características moderadamente tóxicas para *Gymnotus carapo*. Os valores dos parâmetros de toxicidade calculados no presente estudo permitiram o estabelecimento da estimativa dos limites máximos permitidos em corpos d'água quando combinados com dados de testes de outros organismos não-alvo.

Palavras-chave: Organofosforado. Meio-Ambiente. Organismos não-alvo. Biomarcadores. Tuviras.

Correspondence to:
Márcia Mayumi Ishikawa
Embrapa Meio Ambiente
Rodovia SP 340, Km 127,5, s/n
CEP: 13918-110, Jaguariúna – SP, Brazil
e-mail: marcia.ishikawa@embrapa.br

Received: May 03, 2020
Approved: July 28, 2020

How to cite: Ferri GH, Cardoso IL, Gil JA, Jonsson CM, Rantin FT, Ishikawa MM. Determination of acute median lethal concentration and sublethal effects on AChE activity of *Gymnotus carapo* (Teleostei: Gymnotidae) exposed to trichlorfon. Braz J Vet Res Anim Sci. 2020;57(3):e169354. https://doi.org/10.11606/issn.1678-4456.bjvras.2020.169354

Introduction

In aquaculture, as in all food production sectors, one of the inputs required for successful fish production is the use of chemicals. Chemical application, while having multiple negative impacts on the environment as well as on human health, is an alternative for reducing the economic losses associated with disease outbreaks in crop environments (Guimarães et al., 2007; Reverter et al., 2014).

A variety of chemicals have been used for health management in fish production. Trichlorfon (TRF) (dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphate) is a water-soluble organophosphate pesticide (OP) that is classified as a veterinary medicinal product and is widely used worldwide to eliminate or control fish parasites such as *Ergasilus* sp., *Lernaea* sp., *Dactylogyrus* sp., and *Trichodina* sp. (Coelho et al., 2011; Heo & Shin, 2009; Lopes et al., 2006; Trujillo-González et al., 2018). The toxicological classification of TRF is class II (highly toxic) and its active ingredient only has short persistence in the environment, since it undergoes rapid hydrolysis in air, water and soil (Brasil, 2009; Feng et al., 2008). The major decomposition product of TRF is dichlorvos, an organophosphate pesticide (OP) that has been widely used around the world for many years to control agricultural pests (Varó et al., 2008).

TRF is a specific acetylcholinesterase inhibitor (Gupta, 2011). Organophosphates like trichlorfon block the hydrolysis of the acetylcholine (ACh) neurotransmitter in the central and peripheral neuronal synapses, thereby leading to excessive accumulation of ACh in the synaptic cleft. Hence, this neurotransmitter tends to remain active for a longer period in the synaptic cleft, which increases cholinergic transmission (Araújo et al., 2016). Inhibition of acetylcholinesterase causes several side effects, such as decreased fish mobility, muscle spasms, epithelial lesions and aggressiveness towards other fish (Rodrigues et al., 1997). It is noteworthy that. according to CONAMA Resolution No. 430 (Brasil, 2011), there are no standards of maximum allowable limits for TRF use in Brazilian waters.

The genus *Gymnotus*, known as "tuviras", "carapo", "swordfish" or "knifefish", among other names, has wide distribution in Central and South America (Rotta, 2004). Thirty-nine species have been described and 24 of them occur in Brazilian territory. The Amazon basin has the highest diversity with 20 cataloged species (Campos da Paz & Buckup, 2007; Maxime & Albert, 2009). In Brazil, tuviras are of great commercial importance because they are widely known and used in fishing regions of the country as live bait for sport fishing (Ventura et al., 2018).

Tuviras are not a commercially produced fish. Thus, they are extracted from their natural habitat by fishermen who have been authorized to sell them in commercial establishments (Rotta, 2004).

In artificial environments, it is common to apply chemicals to control parasite infestation in fish without worrying about the correct dosage for each age or species of fish. The physiological responses of fish that are exposed to different drugs serve as tools for monitoring water quality.

The response of tuviras to treatment with TRF is unknown, and the purpose of the present study was to help expand the ecotoxicological database of native fish. Given the above, the aim of this study was to evaluate acute

toxicity by determining the mean lethal concentration of trichlorfon in *Gymnotus carapo*, and to assess its sublethal effects on hepatic acetylcholinesterase activity.

Materials and Methods

This experiment was conducted at the Embrapa Environment aquatic ecosystem laboratory, in Jaguariúna, SP, Brazil. The study was conducted in accordance with the experimental protocol that had been approved by the Embrapa Environment Animal Experimentation Ethics Committee (protocol no. 006/2017). Tuviras (*Gymnotus carapo*) specimens were obtained from a fish farm located in the region of Limeira, SP.

The commercial product used for fish exposure was Masoten® (Bayer, Brazil), which is composed of 80% trichlorfon (dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphate). This product was purchased legally from a veterinary store located in the state of Sao Paulo, Brazil.

The fish were acclimatized in an experimental system with water recirculation (4 L min^{-1} per aquarium) and individual control (water valve), physical and biological filtration (1,500 L), temperature control via a digital thermostat and electrical resistance (5,000 W) and supplemental aeration (746 W). The total volume of water in the recirculation system was 4,800 liters and the replacement rate was 80% per hour.

After the acclimatization period, the fish were subjected to an observation period that lasted for 15 days, in which their behavior and health were observed. During this period, the fish were fed a commercial diet (Tilapia-specific Guabi®: 4-5 mm; 10% moisture; 32% protein; 6.5% fat) for three weeks. They consumed an average of 4% of their body weight per day.

After the behavioral observation period, 96 fish were anesthetized with benzocaine (65 mg L^{-1}) for biometrics. These were then randomly distributed in a 32-aquarium system, in which each aquarium had a working volume of 300 L (3 fish per aquarium). These aquariums were provided with a supplemental aeration system by means of a 1.0 hp radial air compressor system, and constant temperature was maintained through a thermostat and electrical resistance.

The experimental design was completely randomized, with eight experimental groups, which received nominal concentrations of 0.0, 5.0, 7.5, 15, 22.5, 30, 37.5 and 45 mg L^{-1} with 4 replications. The 0.0 group was the control. The aquarium water recirculation system was closed prior to product inoculation. Each replication was formed by three fish, thus totaling 12 fish per experimental group. A stock solution was prepared to obtain the concentrations.

The fish were kept at the established concentrations over a 96-h period without feeding, as suggested by the OECD (Organisation for Economic Co-operation and Development, 2019). The photoperiod established consisted of 8 h of light and 16 h of darkness. This 16 h period of darkness was determined because these fish have nocturnal characteristics. During the hours of illumination, the light intensity remained at 184 lx. Throughout the experimental period, any dead fish were removed from the aquariums, and these occurrences were recorded to determine the mean lethal concentration during 24 - 96 h of exposure ($LC_{24\text{-}96h}$).

The following variables were measured daily in the morning: temperature (23.6 ± 0.71), dissolved oxygen (7.0 ± 0.14), pH (7.4 ± 0.04) and electrical conductivity (0.121 ± 0.01) with the aid of a multiparameter (U-50, Horiba, Minami-ku, Kyoto, Japan). Total ammonia (0.003 ± 0.01) was measured at baseline and at 24, 48 and 96 h by means of a commercial kit (Hach, Loveland, CO, USA). A water exchange was performed to maintain Masoten® concentration.

The sublethal dosage used for the acetylcholinesterase inhibition tests was 10% of the value obtained from the results found in the acute toxicity assessment assay. To perform the acetylcholinesterase inhibition test, 120 fish with average weight 68.6 g were used. These were obtained from the same batch of fish from which some had been directed to the acute toxicity test. Thus, all the laboratory procedures relating to acclimatization also applied to this group of fish in the sublethal experiment. The concentrations determined for the sublethal test were: 0.638; 0.438 and 0.238 mg L^{-1} (based on the LC50 value) and these were used along with a 0.0 control in an experimental design containing 4 replicates (10 fish per aquarium), thus totaling 40 fish per treatment. The fish were exposed for a period of 14 days, and liver and muscle enzyme quantifications were performed after seven days of exposure and again after 14 days of exposure to the material.

Liver and muscle proteins were determined by means of the Bradford (1976) method, adapted for use with a Dynex MRXTC 250 microplate reader, as described by Kruger (1994). AChE activity was determined by means of the colorimetric technique described by Ellman et al. (1961), adapted for microplates (Assis et al., 2011). The substrate used was 9 mM acetylthiocholine iodide (ATC) and 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB), which, in the presence of thiocholine, generated a colored compound. Its absorbance at 415 nm was measured every minute for 10 min using an ELISA plate reader (BIO-RAD Benchmark). The activity of AChE (nmoles min^{-1}) was expressed per mg of protein to calculate specific activity (nmoles min^{-1} mg^{-1}). Protein

concentrations were measured at 595 nm by means of the Bradford method (1976), using bovine serum albumin (Sigma) as the standard.

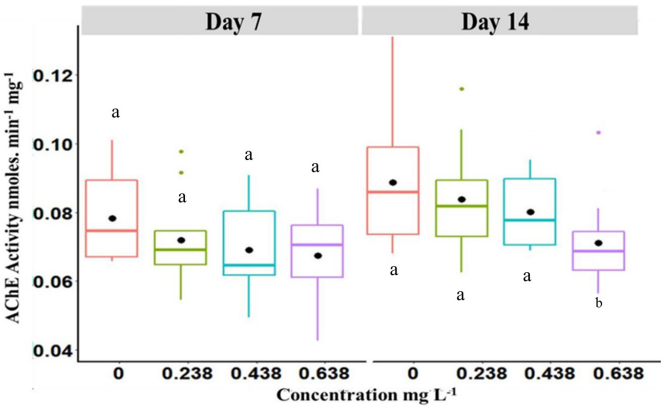
To determine $LC_{24\text{-}96h}$, the R software was used. The model fitted was Weibull type 2, with the lower limit at 0 and the upper limit at 1 (2 parms). The activity of the AChE enzyme was calculated using a parametric linear model. The physicochemical parameters were calculated by means of a general linear model with normal distribution, using one-way ANOVA followed by the Tukey test, at the 5% probability level.

Results

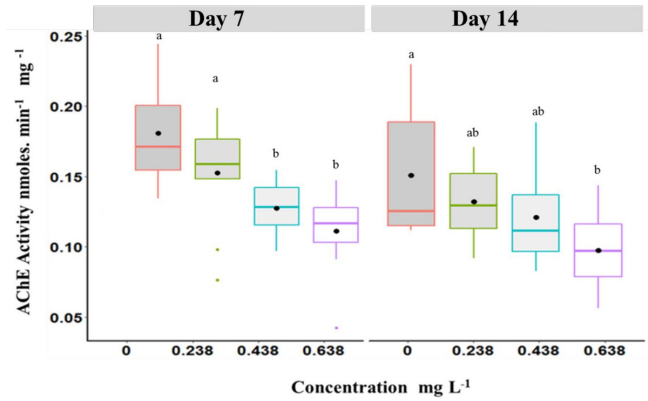
The acute toxicity of TRF (Masoten®) and the cumulative mortality after 24, 48, 72 and 96 h of exposure corresponded to the LC_{50} values, and their lower and upper limits with 95% confidence intervals are presented in Table 1. The values obtained after 24, 48, 72 and 96 h of exposure were respectively: 40.65, 19.95, 12.53 and 6.38 mg L^{-1}. Signs of intoxication could be observed after 2 h of exposure to the product, such that erratic swimming, muscle spasms, scale loss and agglomeration in aerated areas were identified at concentrations above 5 mg L^{-1}. The control group did not present any of the symptoms that were presented in the treated groups.

The results regarding altered AChE activity in the liver of *Gymnotus carapo* exposed to sublethal doses of trichlorfon (Masoten®) are shown in Graphic 1. There were significant differences in exposure between days 7 and 14 ($p = 0.0021$). At seven days of exposure, it was not possible to see any significant differences between the treatments, but this was no longer so after 14 days, when the treatment with 0.638 mg L^{-1} ($p = 0.0007$) showed a significant difference in relation to the other treatments, i.e., caused reduction in enzyme activity.

AChE muscle activity after 7 and 14 days of exposure to trichlorfon (Masoten®) is shown in Graphic 2. It was observed that exposure to the product for a period of seven days caused decays in enzyme activity in the 0.638 ($p = 0.0001$) and 0.438 mg L^{-1} ($p = 0.0025$) groups, in relation to the control group, but these two treatment groups did not differ from each other. However, the group with a concentration of 0.238 mg L^{-1} ($p = 0.2262$) did not show any significant



Graphic 1 – AChE activity (nmoles min^{-1}) of *Gymnotus carapo* liver exposed to different effects of trichlorfon (Masoten®) over a period of 7 and 14 days. Values are presented as the mean ± standard deviation (N = 10). Same letters indicate that the means are not statistically different by the Tukey method ($p < 0.05$).



Graphic 2 – AChE activity (nmoles min^{-1}) of *Gymnotus carapo* muscle exposed to different effects of trichlorfon (Masoten®) over a period of 7 and 14 days. Values are presented as the mean ± standard deviation (N = 10). Same letters indicate that the means are not statistically different by the Tukey method ($p < 0.05$).

Table 1 – Number of *Gymnotus carapo* mortality (%) in different concentrations of trichlorfon (Masoten®) for lethal concentration (LC_{50}) determination with confidence limits

Exposure time (h)	Mortality (%)								LC_{50} (mg L^{-1})
	Trichorfon concentration (mg L^{-1})								
	0	5.0	7.5	15.0	22.5	30.0	37.5	45.0	
24	0	0	0	16.7	50.0	50.0	58.3	25.0	40.7 (23.2-58.1)
48	0	8.3	0	33.3	66.7	83.3	100.0	100.0	20.0 (15.0-22.9)
72	0	8.3	16.7	83.3	91.7	91.7	100.0	100.0	12.5 (9.5-15.6)
96	0	41.7	50.0	100.0	91.7	91.7	100.0	100.0	6.4 (4.5-8.3)

difference in relation to the control. After 14 days of exposure, significant differences between the control group and the group treated with 0.638 mg L^{-1} ($p = 0.0035$), when reduction in enzyme activity could be seen, but there were no significant differences in relation to the other treatments.

Discussion

Acute fish toxicity tests are widely used to register and authorize use of chemicals. Acute exposure of fish needs to follow the guidelines set by the OECD, which encompass limits and specifications for doing this (Kluver et al., 2015). In this type of test, any response presented by the test organism is observed. These responses in aquatic animals comprise behavioral changes as well as the 50% lethality response of the population after exposure to the product for a fixed time period (Andrade et al., 2005).

Several studies have used TRF as an active ingredient among aquatic organisms. Hashimoto et al. (1982) determined an $LC_{50\text{-}96h}$ value of 15 mg L^{-1} for *Cyprinus carpio*, Flores-Nava & Vizcarra-Quiroz (1988) determined a value of 17.2 mg L^{-1} for *Cichlasoma urophthalmus* after 96 h of exposure; while the value found for Nile tilapia (*Oreochromis niloticus*) juveniles differed from the others: 21.7 mg L^{-1} after 96 h of exposure, according to Alkahem et al. (1998).

In the present study, *G. carapo* individuals had an LC_{50} of 6.38 mg L^{-1} after 96 h of exposure to TRF. In comparison with the species cited above, *G. carapo* was more sensitive to TRF contamination than were zebrafish embryos after 96 h of exposure (LC_{50} 25.4 mg L^{-1}) or after 72 h (45.13 mg L^{-1}) (Coelho et al., 2011). Therefore, according to the U.S. EPA classification (U.S. Environmental Protection Agency, 2019), TRF would be classified as "moderately toxic" for zebrafish . Thus, according to the calculated $LC_{50\text{-}96h}$ value for tuviras, the estimated unobserved lethality concentration (CENO) value would be approximately 0.6 mg L^{-1} ($CENO = LC_{50\text{-}96h}/10$) (Organisation for Economic Co-Operation and Development, 1995). The concentration at which there is absence of manifestation of acute effects can be estimated from the ratio $LC_{50\text{-}96h}/3$ (Gherardi-Goldstein et al., 1990; Poston & Purdy, 1986).

When a treatment is implemented using any type of medication, the effect desired is the death of the parasite without any damage to the host (Guimarães et al., 2007; Salte et al., 1987). However, host damage usually occurs, and this may include several symptoms (Chandrasekara & Pathiratne, 2005). In this context, as described in the Masoten® product package insert (Bayer), the highest dose for tank treatment is 1 g of the commercial product per 400 L of water. This recommended dosage for treating *Trichodina* sp. yields a TRF concentration of 2 mg L^{-1}. This is equivalent to a dose that would not show acute effects in tuviras, but it would be higher than CENO, which could cause lethal effects under prolonged exposure.

The enzyme acetylcholinesterase (AChE) is recognized as a biomarker for organophosphates (Payne et al., 1996). AChE is an enzyme for nerve conduction in biological systems that degrades acetylcholine in the synaptic cleft space, thereby terminating the excitatory effect of the neurotransmitter (Soreq & Seidman, 2001). Its inhibition can lead the animal to hyperactivity, loss of coordination, spasms and convulsions, among other effects (Kuhr & Dorough, 1976). Several studies have reported inhibition of AChE enzyme activity in different fish organs (Feng et al., 2008; Coelho et al., 2011). Thangnipon et al. (1995) evaluated AChE activity in the brain of tilapias that were subjected to monocrotophos and observed a marked reduction in its activity.

Kavitha & Venkateswara Rao (2007) showed that approximately 80% inhibition of AChE enzymatic activity occurred after exposure to monocrotophos. These results were not observed in the present study in the liver of tuviras, because only at the highest concentration (0.638 mg L^{-1}) was it possible to observe significant differences. This shows that the liver of tuviras has good capacity to metabolize intoxicants. Hai et al. (1997) evaluated AChE activity in the liver of *Cyprinus carpio* at two doses (1 and 5 mg L^{-1}) and observed that the highest inhibition occurred at 1 mg L^{-1}, which corroborates the results obtained in the present study, in which the concentration closest to 1 mg L^{-1} presented the highest enzymatic inhibition, thus showing that higher doses present saturation in hepatic metabolism.

Guimarães et al. (2007), investigating AChE muscle activity in *O. niloticus,* showed that it decays when exposed to low concentrations of trichlorfon (0.250 mg L^{-1}), after eight hours of exposure. Omar-Ali et al. (2017) kept *Atractosteus spatula* exposed to OF diazinon for 30 days, and observed 70% inhibition of plasma AChE at concentrations of 0.1 and 0.01 mg L^{-1}. These results corroborate those presented in the muscles of tuviras, where chronic exposure leads to exerts AChE inhibition even at low concentrations. Assis et al. (2011), after inhibition of AChE, found that it may remain inhibited for up to 30 days when exposed to a single dose. However, the pattern presented by tuviras (*G. carapo*) was different from what was seen in the study described above, because after 14 days of continuous exposure, the groups that received different concentrations of trichlorfon (Masoten®) did not present significant differences in enzyme

activity, but still remained with lower activity levels than the control group.

Recovery of tuviras enzyme activity may be associated with a biotic factor. This may be due to the higher enzyme activity of AChE, given that tuviras belong to a group of electric fish that are capable of generating pulses of about 5 V. These are produced in differentiated muscle cells that are known as the electric discharge organ (Albert & Crampton, 2001; Lovejoy et al., 2010). According to Bullock et al. (1979), electric discharge can be produced by differentiated muscle cells or specialized neurons. The electric organs derived from muscle cells are called myogenic. Because members of the Gymnotidae group present this ability, recovery of enzyme activity may, even after prolonged exposure at low concentrations, be associated with higher production of the AChE enzyme.

Conclusions

The results presented in this study allow us to state that tuviras have $LC_{50\text{-}96h}$ that is lower than that of the main species produced in Brazil (*O. niloticus*), as well as in relation to other fish species. Clinical signs caused by Masoten® poisoning could be observed in *G. carapo* from the concentration of 5 mg L^{-1} onwards in the acute test.

This acute sensitivity to trichlorfon was shown through inhibition of AChE at sublethal concentrations in a dose-dependent relationship. In association with this observation, the data point towards possible use of this enzyme as a biomarker for exposure to sublethal effects, at concentrations equivalent to or below 0.6 mg L^{-1} (CENO for lethal effects).

In the evaluation of exposure at sublethal concentrations, AChE enzyme activity was inhibited in a concentration-dependent relationship and was seen to be more sensitive in muscle than in liver tissue. The toxicity parameter values calculated in the present study assisted in estimation of maximum allowable limits in bodies of water when combined with test data from other non-target organisms.

Conflict of Interest

The authors declared no potential conflicts of interest.

Ethics Statement

The present investigation attended all of the rules proposed by Brazilian National Council of Animal Experimentation (CONCEA) for controlling the use of animals for teaching and research and was approved by the Ethic Commission of Animals Usage in Experiments (CEUA) from the Embrapa Meio Ambiente, Jaguariúna, Brazil (protocol no. 006/2017, amendment 005/2018).

Acknowledgements

The authors would like to thank Coordination for the Improvement of Higher Education Personnel (CAPES) for financial support. We are grateful to the BRS Aqua Project, a partnership between BNDES, FEA and Embrapa, with funding from BNDES, SAP/MAPA and Embrapa counterparts and support from CNPq.

References

Albert JS, Crampton WGR. Five new species of Gymnotus (Teleostei: Gymnotiformes) from an Upper Amazonian floodplain, with descriptions of electric organ discharges and ecology. Ichthyol Explor Freshwat. 2001;12(3):241-226.

Alkahem HF, Ahmed Z, Al-Akel AS, Shamsi IMKJ. Toxicity bioassay and changes in haematological parameters of *Oreochromis niloticus* induced by trichlorfon. Arab Gulf J Sci Res. 1998;(16):581-93.

Andrade RLB, Andrade LS, Boscolo WR, Soares CM. Comportamento, sobrevivência e desenvolvimento de lebistes, *Poecillia reticulata*, submetidos a agentes utilizados na profilaxia de doenças. Acta Sci Anim Sci. 2005;27(4):523-8.

Araújo RM, Santos VDA, Gonsalves AA. Acetilcolinesterase-AChE: uma enzima de interesse farmacológico. Revista Virtual de Química. 2016;8(6):1818-34. http://dx.doi.org/10.21577/1984-6835.20160122.

Assis CRD, Bezerra RS, Carvalho LB Jr. Fish cholinesterase as biomarkers of organophosphorus and carbamates pesticides. In Stoytcheva M, editor. Pesticides in modern world. Rijeka, Croatia: Intech; 2011. Book 5.

Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72(1-2):248-54. http://dx.doi.org/10.1016/0003-2697(76)90527-3. PMid:942051.

Brasil. Agência nacional de vigilância sanitária – ANVISA. Resolução da diretoria colegiada- RDC nº 8, de 27 de fevereiro de 2009. Dispõe sobre as medidas para redução da

ocorrência de infecções por Micobactérias de Crescimento Rápido - MCR em serviços de saúde. Diário Oficial da União; Brasília; 2009.

Brasil. Ministério do Meio Ambiente. Conselho Nacional do Meio Ambiente – CONAMA. Resolução n° 430, de 13 de maio de 2011. Dispõe sobre as condições e padrões de lançamento de efluentes, complementa e altera a Resolução no 357, de 17 de março de 2005, do Conselho Nacional do Meio Ambiente-CONAMA. Diário Oficial da União; Brasília; 2011 maio 16.

Bullock TH, Fernandes-Souza N, Graf W, Helligenberg W, Langner G, Meyer DL, Pimentel- Souza F, Scheich H, Viancour TA. Aspectos do uso da descarga do órgão elétrico e eletrorrecepção nos Gymnotoidei e outros peixes amazônicos. Acta Amazon. 1979;9(3):549-72. http://dx.doi.org/10.1590/1809-43921979093549.

Campos da Paz R, Buckup PA. Família Gymnotidae. In: Buckup PA, Menezes NA, Ghazzi MS, editores. Catálogo das espécies de peixes de água doce do Brasil. Rio de Janeiro: Museu Nacional do Rio de Janeiro; 2007. p. 120-2.

Chandrasekara HU, Pathiratne A. Influence of low concentrations of trichlorfon on haematological parameters and brain acetylcholinesterase activity in common carp, Cyprinus carpio L. Aquacult Res. 2005;36(2):144-9. http://dx.doi.org/10.1111/j.1365-2109.2004.01197.x.

Coelho S, Oliveira R, Pereira S, Musso C, Domingues I, Bhujel RC, Soares AM, Nogueira AJ. Assessing lethal and sub-lethal effects of trichlorfon on different trophic levels. Aquat Toxicol. 2011;103(3-4):191-8. http://dx.doi.org/10.1016/j.aquatox.2011.03.003. PMid:21473847.

Ellman GL, Courtney KD, Andres V Jr, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961;7(2):88-95. http://dx.doi.org/10.1016/0006-2952(61)90145-9. PMid:13726518.

Feng T, Li ZB, Guo XQ, Guo JP. Effects of trichlorfon and sodium dodecyl sulphate on antioxidant defense system and acetylcholinesterase of Tilapia nilotica in vitro. Pestic Biochem Physiol. 2008;92(3):107-13. http://dx.doi.org/10.1016/j.pestbp.2007.10.002.

Flores-Nava A, Vizcarra-Quiroz JJ. Acute toxicity of trichlorfon (Dipterex) to fry of Cichlasoma urophthalmus Günther. Aquacult Res. 1988;19(4):341-5. http://dx.doi.org/10.1111/j.1365-2109.1988.tb00583.x.

Gherardi-Goldstein E, Bertoletti E, Zagatto PA, Araujo RPA, Ramos MLLC. Procedimentos para a utilização de testes de toxicidade no controle de efluentes líquidos. São Paulo: Cetesb; 1990. 17 p.

Guimarães ATB, Assis HS, Boeger W. The effect of trichlorfon on acetylcholinesterase activity and histopathology of cultivated fish *Oreochromis niloticus*. Ecotoxicol Environ Saf. 2007;68(1):57-62. http://dx.doi.org/10.1016/j.ecoenv.2006.08.005. PMid:17055053.

Gupta RC. Toxicology of organophosphate and carbamate compounds. Cambridge: Academic Press; 2011.

Hai DQ, Varga SI, Matkovics B. Organophosphate effects on antioxidant system of carp (Cyprinus carpio) and catfish (Ictalurus nebulosus). Comp Biochem Physiol C Pharmacol Toxicol Endocrinol. 1997;117(1):83-8. http://dx.doi.org/10.1016/S0742-8413(96)00234-4. PMid:9206589.

Hashimoto Y, Okubo E, Ito T, Yamaguchi M, Tanaka S. Changes in susceptibility of carp to several pesticides with growth. J Pestic Sci. 1982;7(7):457-61. http://dx.doi.org/10.1584/jpestics.7.457.

Heo G, Shin G. Acute and subacute toxicity of trichlorfon in guppies (Poecilia reticulata). Korean J Vet Res. 2009;49(3):253-6.

Kavitha P, Venkateswara Rao J. Oxidative stress and locomotor behaviour response as biomarkers for assessing recovery status of mosquito fish, Gambusia affinis after lethal effect of an organophosphate pesticide, monocrotophos. Pestic Biochem Physiol. 2007;87(2):182-8. http://dx.doi.org/10.1016/j.pestbp.2006.07.008.

Kluver N, Konig M, Ortmann J, Massei R, Paschke A, Kuhne R, Scholz S. Fish embryo toxicity test: identification of compounds with weak toxicity and analysis of behavioral effects to improve prediction of acute toxicity for neurotoxic compounds. Environ Sci Technol. 2015;49(11):7002-11. http://dx.doi.org/10.1021/acs.est.5b01910. PMid:25939044.

Kruger NJ. The Bradford method for protein quantification. Methods Mol Biol. 1994;32:9-15. PMid:7951753.

Kuhr RJ, Dorough HW. Carbamate insecticides: chemistry, biochemistry, and toxicology. Boca Raton: CRC Press, Inc.; 1976.

Lopes RB, Paraiba LC, Ceccarelli PS, Tornisielo VL. Bioconcentration of trichlorfon insecticide in pacu

(Piaractus mesopotamicus). Chemosphere. 2006;64(1):56-62. http://dx.doi.org/10.1016/j.chemosphere.2005.11.029. PMid:16405959.

Lovejoy NR, Lester K, Crampton WGR, Marques FPL, Albert J. Phylogeny, biogeography, and electric signal evolution of Neotropical Knifefishes of the genus *Gymnotus* (Osteichthyes: gymnotidae). Mol Phylogenet Evol. 2010;54(1):278-90. http://dx.doi.org/10.1016/j.ympev.2009.09.017. PMid:19761855.

Maxime EL, Albert JS. A new species of *Gymnotus* (Gymnotiformes: Gymnotidae) from the Fitzcarrald Arch of Southeastern Peru. Neotrop Ichthyol. 2009;7(4):579-85. http://dx.doi.org/10.1590/S1679-62252009000400004.

Organisation for Economic Co-Operation and Development. Guidance document for aquatic effects assessment. Paris: OECD; 1995. OECD Environment Monographs 92.

Organisation for Economic Co-Operation and Development. OECD guideline for testing of chemicals. Paris: OECD; 2019. Section 2, n. 203; Fish, Acute Toxicity Test.

Omar-Ali A, Carr RL, Petrie-Hanson L. Inhibition of Plasma Cholinesterase Activity in Alligator Gar (*Atractosteus spatula*) Following Chronic Exposure to Diazinon. J Toxicol Pharmacol. 2017;1:14.

Payne JF, Mathieu A, Melvin W, Fancey LL. Acetylcholinesterase, an old biomarker with a new future? Field trials in association with two urban rivers and a paper mill in Newfoundland. Mar Pollut Bull. 1996;32(2):225-31. http://dx.doi.org/10.1016/0025-326X(95)00112-Z.

Poston TM, Purdy R. ASTM 921: aquatic toxicology and environmental fate (Vol. 9). Philadelphia: American Society for Testing and Materials; 1986. 530 p.

Reverter M, Bontemps N, Lecchini D, Banaigs B, Sasal P. Use of plant extracts in fish aquaculture as an alternative to chemotherapy: current status and future perspectives. Aquaculture. 2014;433:50-61. http://dx.doi.org/10.1016/j.aquaculture.2014.05.048.

Rodrigues EL, Eiras AC, Veiga ML, Pacheco FJ. Alterações Hematológicas em Curimbatá, Prochilodus scrofa STEINDACHNER, 1881, exposto ao Dipterex 500 (Trichlorfon). Boletim do Instituto de Pesca. 1997;24:187-96.

Rotta MA. Aspectos Biológicos e Reprodutivos para a Criação da Tuvira (*Gymnotus* sp.) em Cativeiro – I. Boletim de Pesquisa e Desenvolvimento. 2004;73:1-16.

Salte R, Syvertsen C, Kjonnoy M, Fonnum F. Fatal acetylcholinesterase inhibition in salmonids subjected to a routine organophosphate treatment. Aquaculture. 1987;61(3-4):173-9. http://dx.doi.org/10.1016/0044-8486(87)90146-3.

Soreq, H., Seidman, S. Acetylcholinesterasednew roles for an old actor. Nat Rev Neurosci. 2001;2(4):294-302.

Thangnipon W, Thangnipon W, Luangpaiboon P, Chinabut S. Effects of the organophosphate insecticide, monocrotophos, on acetylcholinesterase activity in the Nile tilapia fish (*Oreochromis niloticus*) brain. Neurochem Res. 1995;20(5):587-91. http://dx.doi.org/10.1007/BF01694540. PMid:7643964.

Trujillo-González A, Becker JA, Vaughan DB, Hutson KS. Monogenean parasites infect ornamental fish imported to Australia. Parasitol Res. 2018;117(4):995-1011. http://dx.doi.org/10.1007/s00436-018-5776-z. PMid:29427156.

U.S. Environmental Protection Agency. Takes next step in review process for herbicide glyphosate, reaffirms no risk to public health. Washington: EPA; 2019.

Varó I, Amat F, Navarro C. Acute toxicity of dichlorvos to Aphanius iberus (Cuvier & Valenciennes, 1846) and its anti-cholinesterase effects on this species. Aquat Toxicol. 2008;88(1):53-61. http://dx.doi.org/10.1016/j.aquatox.2008.03.004. PMid:18433895.

Ventura AS, Jerônimo GT, Ferri GH, Pádua SB, Martins ML, Ishikawa MM. Erythrocyte parameters and condition factor of *Gymnotus* spp. (Gymnotiformes: Gymnotidae) under culture conditions. Rev Bras Parasitol Vet. 2018;40(e20318):1-6.

Financial Support: BNDES, SAP/MAPA, Embrapa and CAPES.