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Endosulfan exposure inhibits brain AChE activity and impairs swimming performance in adult zebrafish (*Danio rerio*)

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ABSTRACT

Endosulfan is a broad spectrum organochlorine pesticide that is still widely in use in many developing countries. Following application, endosulfan can get to watercourses through surface runoff from agricultural fields and disturb the non-target aquatic animals including freshwater fish species. Given that the activity of the enzyme acetylcholinesterase (AChE) is one of the most recurrently used biomarkers of exposure to pesticides and there are controversial results concerning the effects of endosulfan exposure and AChE activity in fish, the aim of the present study was to evaluate the effects of endosulfan in brain AChE activity and its gene expression pattern using adult zebrafish (*Danio rerio*) as an animal model. Moreover, we have analyzed the effects of endosulfan exposure in different parameters of zebrafish swimming activity and in long-term memory formation. After 96 h of exposition, fish in the 2.4 μg endosulfan/L group presented a significant decrease in AChE activity (9.44 ± 1.038 μmol SCh h^{-1} mg $protein^{-1}$; $p = 0.0205$) when compared to the control group (15.87 ± 1.768 μmol SCh h^{-1} mg $protein^{-1}$; $p = 0.0205$) which corresponds to approximately 40%. The down-regulation of brain AChE activity is not directly related with the transcriptional control as demonstrated by the RT-qPCR analysis. Our results reinforce AChE activity inhibition as a pathway of endosulfan-induced toxicity in brain of fish species. In addition, exposure to 2.4 μg endosulfan/L during 96 h impaired all exploratory parameters evaluated: decreased line crossings (≈21%, 273.7 ± 28.12 number of line crossings compared to the control group 344.6 ± 21.30, $p = 0.0483$), traveled distance (≈20%, 23.44 ± 2.127 m compared to the control group 29.39 ± 1.585, $p = 0.0281$), mean speed (≈25%, 0.03 ± 0.003 m/s compared to the control group 0.04 ± 0.002, $p = 0.0275$) and body turn angle (≈21%, $69{,}940 \pm 4871$ absolute turn angle compared to the control group $88{,}010 \pm 4560$, $p = 0.0114$). These results suggest that endosulfan exposure significantly impairs animals' exploratory performance, and potentially compromises their ecological and interspecific interaction. Our results also showed that the same endosulfan exposure did not compromise animals' performance in the inhibitory avoidance apparatus. These findings provide further evidence of the deleterious effects of endosulfan exposure in the nervous system.

1. Introduction

Pesticides are chemicals that became indispensable in current agriculture to control pest populations. Three of the main classes of pesticides are organochlorines, organophosphorous and carbamates. Organochlorines are the most commonly found pesticides in the environment including water, sediments, atmospheric air and biotic environment (Chopra et al., 2011). Organochlorine pesticides consist of a variety of chemicals composed primarily of carbon, hydrogen and chlorine that include among others polychlorinated biphenyls (PCBs), polychlorinated dibenzofurans (PCDFs), dichlorodiphenyltrichloroethane (DDT), dieldrin, chlordane, heptachlor, toxaphenes, mirex, lindane, dicofol, hexachlorobenzene, chlordecone and endosulfan (for review see Van Dyk and Pletschke, 2011).

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Endosulfan is a broad spectrum insecticide–acaricide of the cyclodiene subgroup which consists of two biologically active isomers: alpha and beta, respectively in a 7:3 ratio (Wan et al., 2005) whereas endosulfan sulfate is the main environmental metabolite found in water, sediments and tissues (Rand et al., 2010). Following application, endosulfan can get to watercourses through surface runoff from agricultural fields (Miglioranza et al., 2002) and reach the groundwater through percolation (Chopra et al., 2011). According to previous studies, the toxicity of endosulfan and endosulfan sulfate to non-target aquatic animals including freshwater fish species seems to be similar (Wan et al., 2005; Carriger et al., 2011; Da Cuña et al., 2011).

Although the molecular mechanisms of endosulfan's toxicity are still mostly unknown, some aspects have already been addressed. The adverse effects caused by exposure to endosulfan include DNA damage and mutagenicity (Bajpayee et al., 2006), genotoxicity (Neuparth et al., 2006), neurotoxicity (Yavuz et al., 2007), oxidative stress in macrophages (Tellez-Bañuelos et al., 2009), histological alterations in gills and liver (Da Cuña et al., 2011) and decline in cortisol secretion and increase in glutathione S-transferase activity (Ezemonye and Ikpesu, 2011).

In cholinergic neurotransmission, the neurotransmitter acetylcholine (ACh) promotes the activation of muscarinic and nicotinic cholinergic receptors. Acetylcholine is a neurotransmitter critically involved in psychomotor control of movement and an important modulator of cognitive functions such as learning and memory (Hasselmo, 2006). The reaction responsible for the maintenance of levels of ACh in the extracellular space is catalyzed by acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), by the degradation of ACh into choline and acetate (Soreq and Seidman, 2001). The zebrafish (*Danio rerio*) is recognized as a consolidated experimental model for studying several biological events. More recently, zebrafish has also became an attractive model for environmental and toxicological studies (Hernández and Allende, 2008). It has been demonstrated that BuChE is not encoded in the zebrafish genome, but AChE is encoded by a single gene that has been functionally detected in zebrafish brain (Bertrand et al., 2001).

AChE activity has been extensively used as a bioindicator of environmental exposure. The inhibition of AChE as a biomarker for assessment of the exposure of organisms to organophosphate and carbamate pesticides is long well-known (for review see Van Dyk and Pletschke, 2011). The inhibition of AChE by organophosphates and carbamates occurs, respectively, as a result of the phosphorylation or carbamylation of the serine residue in the active site of the enzyme (Fukuto, 1990). Other toxic componds than organophosphate and carbamate pesticides either promote AChE inhibition and AChE activation in fish. For instance, the inhibition of zebrafish brain AChE activity by methanol (Rico et al., 2006) and the heavy metals mercury and lead (Richetti et al., 2011) was demonstrated, as well as the activation of zebrafish brain AChE by ethanol (Rico et al., 2007) and microcystin-LR exposure (Kist et al., 2012).

There are controversial results concerning the effects of organochlorine endosulfan exposure and AChE activity in fish. In one hand, it was demosntrated that AChE activity was not altered in the serum of *Clarias gariepinuss* (Ezemonye and Ikpesu, 2011) and in brain of *Jenynsia multidentata* (Ballesteros et al., 2009) and *Cichlasoma dimerus* (Da Cuña et al., 2011). On the other hand, AChE activity was inhibited in brain of *Lepomis macrochirus* (Dutta and Arends, 2003) and *Labeo rohita* (Kumar et al., 2011) and in muscle of *Jenynsia multidentata* (Ballesteros et al., 2009).

There is still less information concerning the effects of endosulfan exposure and behavior parameters in fish species. Endosulfan induces reduced feeding behaviors of *Thalassoma pavo* (Giusi et al., 2005) and changes in spontaneous swimming activity of *Jenynsia multidentata* (Ballesteros et al., 2009).

Therefore, considering that: (i) endosulfan is still widely in use in many developing countries, (ii) the neurotoxic effects of endosulfan exposure are far from being completely understood, (iii) measurement of AChE activity in organisms is used worldwide as a biomarker of environmental contamination, (iv) there are controversial results concerning the effects promoted by endosulfan exposure in AChE activity, and that (v) zebrafish is a well-established organism model for toxicological analysis, the aim of the present study was to evaluate the effects of endosulfan in brain AChE activity and its gene expression pattern. Furthermore, we have analyzed the effects of endosulfan exposure in distinct parameters of zebrafish swimming activity and in long-term memory formation.

2. Material and methods

2.1. Chemicals

Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,3,4-benzodioxathiepin-3-oxide), number 067-09-01350, was purchased from a commercial supplier (COTRIJUI, RS, Brazil). Trizma Base, ethylenedioxy-diethylene-dinitrilo-tetraacetic acid (EDTA), ethylene glycol bis(beta amino ethylether)-N,N,N′,N′-tetraacetic acid (EGTA), sodium citrate, Coomassie Blue G, bovine serum albumin, acetylthiocholine, and 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) were purchased from Sigma Chemical Co (St. Louis, MO, USA). TRIzol® reagent, Platinum® Taq DNA Polymerase and SYBR® Green I were purchased from Invitrogen (Carlsbad, CA, USA). ImProm-II™ Reverse Transcription System was purchased from Promega (Madison, USA). All other reagents used were of analytical grade.

2.2. Animals

Adult wild-type zebrafish (*Danio rerio*, Cyprinidae) of both sexes (6–9 months-old) were obtained from a specialized supplier (Redfish Agroloja, RS, Brazil). Animals were kept at a density of up to five animals per liter in 50 L housing tanks with tap water that was previously treated with Tetra's AquaSafe® (to neutralize chlorine, chloramines, and heavy metals present in the water that could be harmful to fish/supplier Redfih Agroloja) and continuously aerated (7.20 mg O_2/L) at 26 ± 2 °C, under a 14/10 h light/dark controlled photoperiod. Animals were acclimated for at least two weeks before the experiments and were fed three times a day with TetraMin Tropical Flake fish food® (supplier Redfih Agroloja). The fish were maintained healthy and free of any signs of disease and were used according to the "Guide for the Care and Use of Laboratory Animals" published by the US National Institutes of Health. All procedures in the present study were approved by the Animal Ethics Committee of the Pontifical Catholic University of Rio Grande do Sul (PUCRS), protocol number 10/00211-CEUA.

2.3. In vitro assay

In vitro assays were performed as previously described (Seibt et al., 2009; Siebel et al., 2010). Briefly, endosulfan was added to the reaction medium before the preincubation with the enzyme-containing lysate from zebrafish brain homogenate and maintained during the enzyme assays. Endosulfan was tested at a final concentration of 0.6, 1.2, 2.4, 5.0, 10.0 and 50.0 μg/L. For the control group, the enzyme assay was performed in the absence of endosulfan (*i.e.* no pesticide was added to the reaction medium).

2.4. In vivo assay

The *in vivo* exposures were performed in 2-L aquariums (10 fish per aquarium). Fish were exposed to different endosulfan concentrations (0.6, 0.9, 1.2 and 2.4 μg/L) dissolved in water for

Table 1
PCR primers design.

Proteins	Primer sequences (5′–3′)	Accession number (mRNA)
EF1α[a]	F-CTGGAGGCCAGCTCAAACAT R-ATCAAGAAGAGTAGTACCGCTAGCATTAC	NSDART00000023156
Rpl13α[a]	F-TCTGGAGGACTGTAAGAGGTATGC R-AGACGCACAATCTTGAGAGCAG	NM_212784
β-Actin[a]	F-CGAGCTGTCTTCCCATCCA R-TCACCAACGTAGCTGTCTTTCTG	ENSDART00000055194
AChE[b]	F-GCTAATGAGCAAAAGCATGTGGGCTTG R-TATCTGTGATGTTAAGCAGACGAGGCAGG	NP_571921

[a] According to Tang et al. (2007).
[b] Designed by authors.

24 and 96 h and immediately after the exposure, they were euthanized by decapitation. For the control group the animals were exposed only to water, after which they were euthanized. The endosulfan concentrations and the exposure periods were chosen based on a previous study using *Cichlasoma dimerus* (Da Cuña et al., 2011). The water/endosulfan of the aquarium was changed after each 24 h of exposure in order to circumvent the endosulfan's low persistence (half-time) in water.

2.5. Determination of AChE activity

Zebrafish were euthanized and whole brain tissue was dissected. The brain tissue (brains from two individuals were used for each sample) was homogenized with in 60 volumes (v/w) of Tris–citrate buffer (50 mM Tris, 2 mM EGTA, 2 mM EGTA, pH 7.4, adjusted with citric acid) in a glass Teflon homogenizer. The rate of acetylthiocholine hydrolysis (ACSCh, 0.88 mM) was assessed in a final volume of 300 μL with 11 mM phosphate buffer, pH 7.5, and 0.22 mM DTNB using a method previously described (Ellman et al., 1961). Before the addition of substrate, samples containing protein (5 μg) and the reaction medium described above were preincubated for 10 min at 25° C. The hydrolysis of substrate was monitored by the formation of thiolate dianion of DTNB at 412 nm for 2–3 min (intervals of 30 s) in a microplate reader. Controls without the homogenate preparation were performed in order to determine the non-enzymatic hydrolysis of the substrate. The linearity of absorbance against time and protein concentration was previously determined. AChE activity was expressed as micromoles of thiocholine (SCh) released per hour per milligram of protein. All enzyme assays were evaluated in triplicate and at least four independent experiments were performed.

2.6. Protein determination

Protein was measured by the Coomassie blue method (Bradford, 1976) using bovine serum albumin as standard.

2.7. Gene expression analysis by quantitative real time RT-PCR (RT-qPCR)

Immediately after 96 h of exposure to 2.4 μg endosulfan/L, the animals were euthanized by decapitation. For each sample, a pool of three zebrafish whole brains was used. Total RNA was isolated with Trizol® reagent (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's instructions. The total RNA was quantified by spectrophotometry and the cDNA was synthesized with ImProm-II™ Reverse Transcription System (Promega) from 1 μg total RNA, following the manufacturer's instructions. Quantitative PCR was performed using SYBR® Green I (Invitrogen) to detect double-strand cDNA synthesis. Reactions were done in a volume of 25 μL using 12.5 μL of diluted cDNA (1:100 for *EF1α*, *Rlp13α* and *β-actin*; 1:20 for *ache*), containing a final concentration of 0.2× SYBR® Green I (Invitrogen), 100 μM dNTP, 1× PCR Buffer, 3 mM $MgCl_2$, 0.25 U Platinum® Taq DNA Polymerase (Invitrogen) and 200 nM of each reverse and forward primers (Table 1). The PCR cycling conditions were: an initial polymerase activation step for 5 min at 95 °C, 40 cycles of 15 s at 95 °C for denaturation, 35 s at 60 °C for annealing and 15 s at 72 °C for elongation. At the end of cycling protocol, a melting-curve analysis was included and fluorescence measured from 60 to 99 °C. Relative expression levels were determined with 7500 Fast Real-Time System Sequence Detection Software v.2.0.5 (Applied Biosystems). The efficiency per sample was calculated using LinRegPCR 11.0 Software (http://LinRegPCR.nl) and the stability of the references genes, *EF1α*, *Rlp13α* and *β-actin* (*M-value*) and the optimal number of reference genes according to the pairwise variation (*V*) were analyzed by GeNorm 3.5 Software (http://medgen.ugent.be/genorm/). Relative RNA expression levels were determined using the $2^{-\Delta\Delta CT}$ method.

2.8. Behavioral assessment

Behavioral testing of locomotion was realized after 96 h of exposure to 2.4 μg endosulfan/L or water (control group) during the light period between 9:00 a.m. and 2:00 p.m. Animals were individually placed in the experimental tank (30 cm L × 15 cm H × 10 cm W), habituated to the tank for 30 s, as previously described (Gerlai et al., 2000). The animals' locomotor activity was recorded on video for 5 min after the habituation period. The tank was divided into equal sections with four vertical lines and one horizontal line, and the following behavior patterns were measured: number of line crossings (vertical and horizontal lines), distance traveled (m), mean speed (m/s) and absolute turn angle. The video was analyzed using ANY-Maze software (Stoelting. Co., Wood Dale, IL, USA).

2.9. Inhibitory avoidance

Long-term memory was evaluated by using the inhibitory avoidance (IA) protocol previously described in detail by Blank et al. (2009). After 96 h of treatment, control and 2.4 μg endosulfan/L group, were individually trained and tested in a white/dark compartment IA apparatus (18 cm L × 9 cm H × 7 cm W). On training session, animals were placed in the white side of the tank while the partition between compartments was closed. After 1 min of habituation to the new environment the partition was raised, allowing fish to cross to the dark side of the tank. When animals entered the dark side with their entire body, the sliding partition was closed and a pulsed electric shock (3 ± 0.2 V) was administered for 5 s. Fish were then removed from the apparatus and placed in the dedicated temporary tank. Animals were tested 24 h after training. The test session repeated the training protocol except that no shock was administered and animals immediately removed from the dark compartment. The latency to completely enter the dark compartment

was measured on both sessions and the test latencies used as an index of long-term memory retention.

2.10. Statistical analysis

AChE activity was expressed as the mean ± S.E.M. and analyzed by one-way analysis of variance (ANOVA). *Post hoc* comparisons were made using Tukey's test. Molecular and locomotion data was presented as the mean ± S.E.M. and analyzed by Student's *t*-test. Inhibitory avoidance memory data were presented as mean ± S.E.M. and latencies of groups were compared using Kruskal–Wallis followed by Mann–Whitney *U* tests. Significance was set at $p \leq 0.05$ in all assays.

3. Results

3.1. Brain AChE activity

The effect of different endosulfan concentrations (0.6, 1.2 and 2.4 μg/L) and times of exposure (24 and 96 h) on brain AChE activity was demonstrated by performing *in vivo* experiments using adult zebrafish. None of the concentrations tested altered AChE activity when zebrafish were exposed to endosulfan during 24 h (Fig. 1A). Nevertheless, after 96 h of exposition, fish in the 2.4 μg endosulfan/L group presented a significant decrease in AChE activity (9.44 ± 1.038 μmol SCh h^{-1} mg $protein^{-1}$; $p = 0.0205$) when compared to the control group (15.87 ± 1.768 μmol SCh h^{-1} mg $protein^{-1}$; $p = 0.0205$) which corresponds to approximately 40% (Fig. 1B).

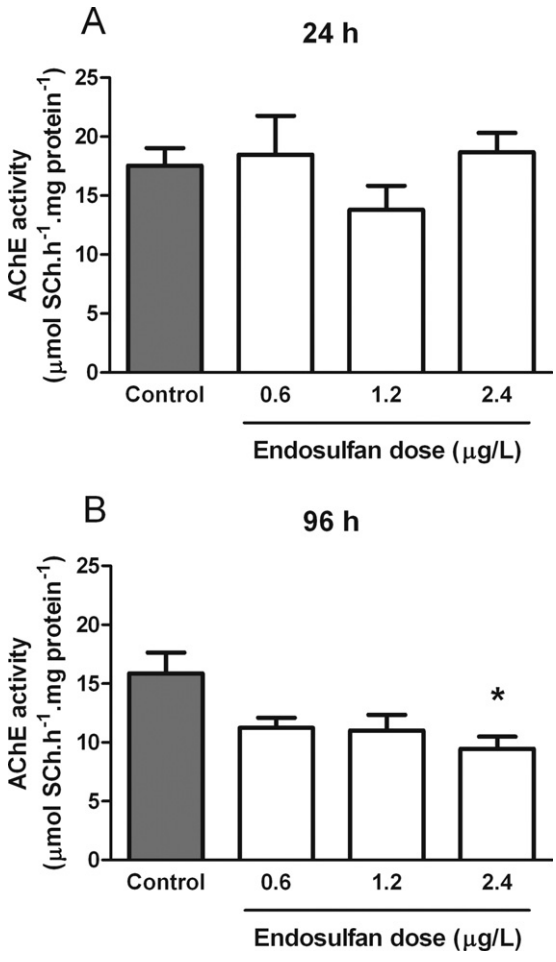

Fig. 1. *In vivo* AChE activity. *In vivo* AChE activity in zebrafish brain after 24 (A) and 96 h (B) of endosulfan exposure at distinct concentrations (0.6–2.4 μg/L). Bars represent the mean ± S.E.M. $n = 7$. The specific enzyme activity is reported as micromoles of thiocholine released per hour per milligram of protein. The asterisk (*) indicates a significant difference compared to control group ($p \leq 0.05$).

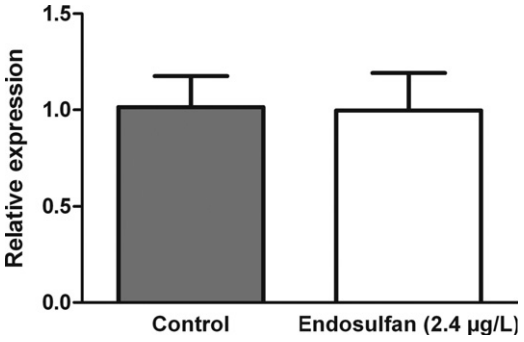

Fig. 2. RT-qPCR analysis. Relative *ache* expression profile after endosulfan exposure (2.4 μg/L for 96 h) on zebrafish brain. Bars represent the mean ± S.E.M. $n = 4$.

The down-regulation of AChE activity after exposure to 2.4 μg endosulfan/L could be a consequence of transcriptional control. In order to determine if transcriptional regulation of *ache* has occurred, a RT-qPCR analysis was carried out. The results have shown that *ache* transcript levels in the 2.4 μg endosulfan/L group were not decreased when compared to the control group ($p = 0.943$; Fig. 2) suggesting that the down-regulation of brain AChE is not directly related with the transcriptional control.

In order to evaluate if endosulfan could have a direct effect on the enzyme, we tested the *in vitro* effect of endosulfan on AChE activity in zebrafish brain. The results showed that none of the endosulfan concentrations tested (0.6, 1.2, 2.4, 5.0, 10.0 and 50.0 μg/L) effectively altered AChE activity ($p = 0.8383$; Fig. 3).

3.2. Swimming performance and memory

Considering that ACh is known to play a major role in the regulation of locomotor control, we evaluated four parameters of zebrafish swimming activity in the 5-min tank diving behavioral test. Exposure to 2.4 μg endosulfan/L during 96 h decreased line crossings (≈21%, 273.7 ± 28.12 number of line crossings compared to the control group 344.6 ± 21.30, $p = 0.0483$), traveled distance (≈20%, 23.44 ± 2.127 meters compared to the control group 29.39 ± 1.585, $p = 0.0281$), mean speed (≈25%, 0.03 ± 0.003 m/s compared to the control group 0.04 ± 0.002, $p = 0.0275$) and body turn angle (≈21%, 69,940 ± 4871 absolute turn angle compared to the control group 88,010 ± 4560, $p = 0.0114$) (Fig. 4).

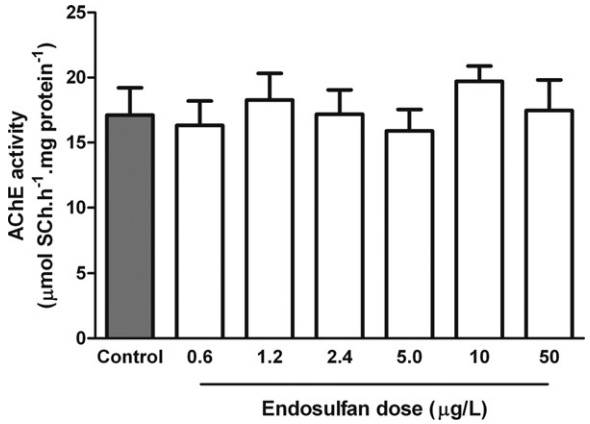

Fig. 3. *In vitro* AChE activity. *In vitro* effect of different concentrations of endosulfan (0.6–50 μg/L) on ACh hydrolysis in zebrafish brain. Bars represent the mean ± S.E.M. $n = 5$.

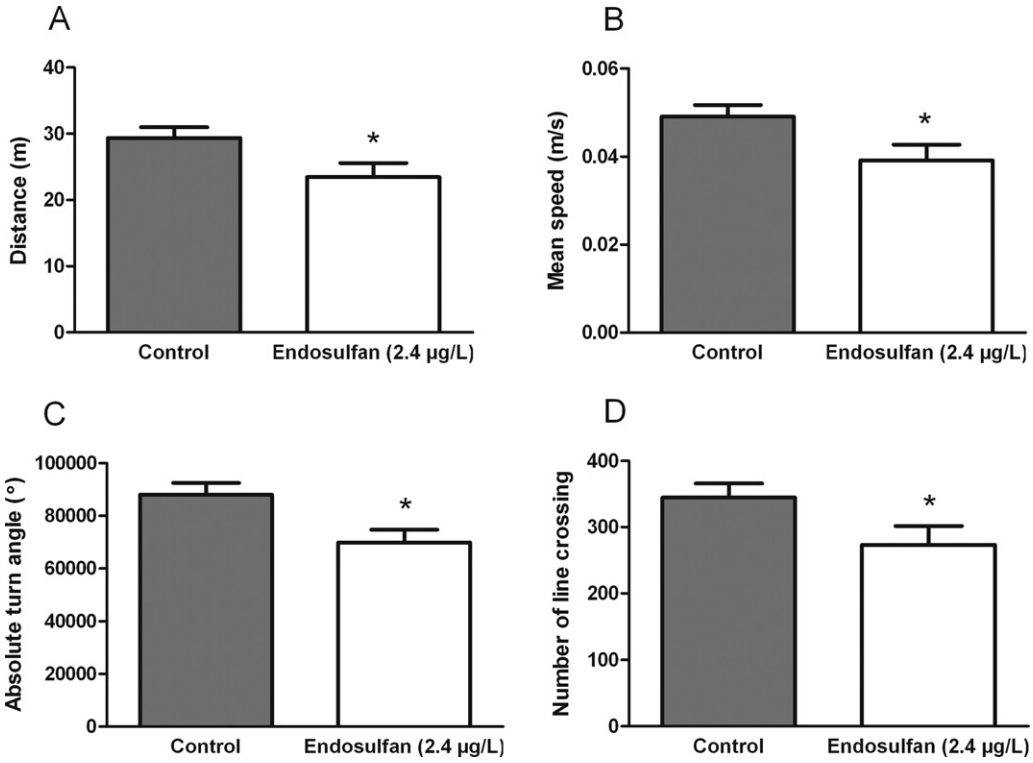

Fig. 4. Swimming performance. Effect of endosulfan exposure (2.4 μg/L for 96 h) on the distance traveled (A), mean speed (B), absolute turn angle (C) and number of line crossings (D) determined during 5 min of video recording in the tank-diving behavioral test. Bars represent the mean ± S.E.M. $n = 16$. The asterisk (*) indicates a significant difference compared to control group ($p \leq 0.05$).

In addition, taking into account cholinergic system's crucial modulatory role in higher cognitive functions, such as memory formation (Hasselmo, 2006), we have also evaluated the effects of the endosulfan exposure over long-term memory formation on the inhibitory avoidance paradigm. For that, two groups were established: control group (n= 22) and 2.4 μg endosulfan/L ($n = 23$). The 2.4 μg endosulfan/L exposure (during 96 h) neither altered training session ($p = 0.1679$) nor test session ($p = 0.7221$) (Fig. 5).

4. Discussion

In the present study, we have evaluated the effect of different endosulfan concentrations (0.6, 1.2 and 2.4 μg/L) and different exposure periods (24 and 96 h) on AChE activity and on *ache* expression in zebrafish brain. After 96 h of exposition, fish in the 2.4 μg endosulfan/L group presented a significant decrease in AChE activity. The RT-qPCR analysis demonstrated that endosulfan exposure did not alter *ache* mRNA levels in zebrafish brain. In addition, the *in vitro* assays did not reveal any significant changes in AChE activity. It is important to emphasize that *in vitro* experiments do not evaluate the influence of other mechanisms such as cell signaling pathways. Altogether, our results indicate that the effect of endosulfan on brain AChE activity is neither related to *ache* inhibition nor to the direct action of this pesticide on the protein, but probably involves a posttranscriptional or post-translational modulation of this enzymatic activity. Another possibility to explain our results is that endosulfan exposure may be causing destruction to cholinergic neurons, and therefore resulting in loss of AChE activity. The identification of some pathology of the brain or even the demonstration that the number of cholinergic neurons was decreased in selected brain regions after endosulfan exposure could give support to this supposition.

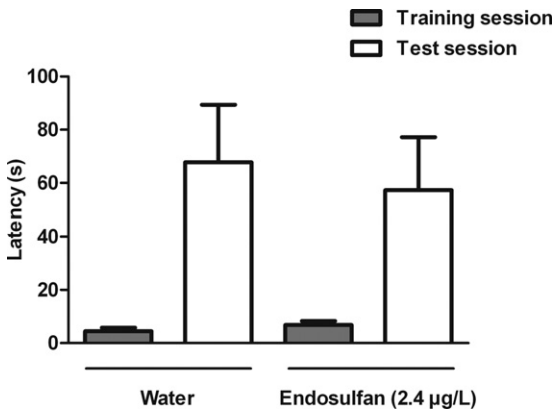

Fig. 5. Long-term memory. Effect of endosulfan exposure (2.4 μg/L for 96 h) on latency to cross to dark compartment in training and long-term memory test sessions in the inhibitory avoidance paradigm. Bars represent the mean ± S.E.M. $n = 22$.

There are inconclusive results regarding the effects of endosulfan exposure and AChE activity in fish. It was demosntrated that AChE activity was not altered in the serum of juveniles of *Clarias gariepinuss* when exposed (from seven to 28 days) to distinct concentrations of endosulfan (0.00, 0.0025, 0.005, 0.0075 and 0.01 μg/L) (Ezemonye and Ikpesu, 2011). In addition, brain AChE activity was not changed in the freshwater species *Cichlasoma dimerus* exposed to different concentrations of endosulfan (from 0.25 to 4.00 μg/L) (Da Cuña et al., 2011). In contrast, brain AChE activity inhibition in juvenile bluegill sunfish *Lepomis macrochirus* after endosulfan exposure (1.0 μg/L) occured in a time-related relationship (Dutta and Arends, 2003). Brain AChE activity was inhibited in *Labeo rohita* fingerlings exposed to low-dose endosulfan (Kumar et al., 2011). Even more interesting

were the results obtained by Ballesteros et al. (2009) where AChE activity significantly decreased in muscle of adult onesided livebearer (*Jenynsia multidentata*) after a sublethal endosulfan exposure while no significant changes were observed in brain. The results presented herein reinforce AChE activity inhibition as a pathway of endosulfan-induced toxicity in brain of fish species.

It is well-known that inhibition of AChE leads to a marked increase in the ACh accumulation in the brain causing an overstimulation of cholinergic receptors. As a result, an overall decline in neural and muscular control occurs. In addition, there is a large body of evidence in the literature associating changes in normal behavioral patterns with neurotoxic effects of exposure to pollutants. However, at present, only few studies dealing with the effects of endosulfan exposure and behavior parameters in fish species were published. Endosulfan exposures impaired startle response and escape from predation of *Oryzias latipes* (Carlson et al., 1998). The results obtained by Gormley and Teather (2003) suggested that short-term exposure to endosulfan (0.01, 0.1 and 1 μg/L) for 24 h beginning either 4–6 h postfertilization or 4–6 h posthatch have long-term effects on growth, behavior, and reproduction of the *Oryzias latipes*. Endosulfan exposure (1.3 μg/L) for one week induces reduced feeding behaviors of *Thalassoma pavo* (Giusi et al., 2005). Decline in the traveled distance after exposure to endosulfan (1 μg/L) for 240 h together with an increase in lipid peroxidation in brain of *Cyprinus carpio* was demonstrated by Rehman (2006). Ballesteros et al. (2009) reported that endosulfan exposure (from 0.072 and 1.4 μg/L) decresed the swimming activity of *Jenynsia multidentata*.

Our results showed that exposure to 2.4 μg endosulfan/L for 96 h, a condition that resulted in brain AChE inhibition, also impaired all exploratory parameters evaluated. Endosulfan exposed animals showed a general decreased exploratory ability, including reduced mean speed which resulted in lower traveled distance and line crossings in the evaluated period. Interestingly, it also affected animals swimming body turn angles, suggesting it significantly impairs animals' exploratory performance, and potentially compromises their ecological and interspecific interaction. Despite cholinergic modulation of learning and memory processes, no effect of endosulfan exposure was observed in the inhibitory avoidance performance. This result suggests that the decreased AChE activity was not sufficient to compromise long-term memory formation in this task, and can be attributed to the task rapid acquisition that does not depend on complex strategies, sustained attention, and intense exploration of the apparatus or endurance. These findings provide further evidence of the deleterious effects of endosulfan exposure in the nervous system and suggest that additional studies are needed to better understand its extent and ecological and physiological impacts.

Conflict of interest

The authors declare that they have no conflict of interest.

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