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CHOLINERGIC AND GENOTOXIC CHANGES IN FISH (*Poecilia reticulata*) EXPOSED TO COMMERCIAL FORMULATION OF METHAMIDOPHOS, GLYPHOSATE, AND MANCOZEB

ALTERAÇÕES COLINÉRGICAS E GENOTÓXICAS EM PEIXES (*Poecília reticulata)* EXPOSTOS À FORMULAÇÃO COMERCIAL DE METAMIDOFÓS, GLIFOSATO E MANCOZEBE

Walter DIAS-JÚNIOR ✉*[1] • Kayo Rodrigo dos Santos BORGES*[1] •Anna Kharolyna Aciole CORREIA*[1] •Daniel Ramalho SANTOS*[1] •Denilson Rodrigues VIEIRA*[1] •Carlos Filipe CAMILO-COTRIM*[2] Luciana Souza ONDEI*[2]

Abstract

The growing concern over the rising demand for food, both in quality and quantity, highlights the need for studies evaluating the concentrations and consequences of pesticides. Monitoring the use of these substances is necessary to develop actions that minimize contamination and its consequences and to regulate them according to the maximum allowed concentrations. Our study aimed to evaluate the effects of prolonged exposure to methamidophos, mancozeb, and glyphosate on biochemical and genotoxic parameters of fish. The toxicity of the commercial formulations of methamidophos, glyphosate, and mancozeb was tested in a laboratory for 7, 15, and 30 days at concentrations allowed by the Brazilian Potable Water legislation (Ordinance No. 2.914/2011 of the Ministry of Health). The study evaluated the three main classes of pesticides: insecticides, herbicides, and fungicides. The parameters assessed in *guppy* (*Poecilia reticulata*) included the frequency of abnormalities (micronucleus, notched, lobed, broken eggs, blebbed, binucleate, and vacuoles) and plasma acetylcholinesterase activity. Pesticides, even at non-lethal doses, may alter the frequency of nuclear abnormalities and acetylcholinesterase activity. On the other hand, no changes were observed in the biometric parameters of the animals or in the physicochemical properties of the water.
Keywords: Pesticide; Acetylcholinesterase; Genotoxicity; Toxicity.

Resumo

A crescente preocupação com demanda maior de alimentos, tanto na qualidade quanto na quantidade, trouxe consigo a necessidade de estudos que avaliassem as concentrações de pesticidas utilizados e suas consequências. Assim, é extremamente necessário o monitoramento dessas substâncias, tendo como objetivo a criação de ações que minimizem a contaminação e suas consequências, além de regulamentar as concentrações permitidas. Esse trabalho teve como objetivo avaliar os efeitos da exposição prolongada ao metamidofós, mancozebe e glifosato sobre parâmetros bioquímicos e genotóxicos em peixes. A toxicidade da formulação comercial de metamidofós, glifosato e mancozebe foi testada em condições laboratoriais durante 7, 15 e 30dias, tendo como referência as concentrações permitidas pela legislação brasileira para potabilidade da água (Portaria nº.2.914/2011 Ministério da Saúde). O estudo avaliou cada uma das três classes principais de pesticidas, um inseticida, um herbicida e um fungicida. Foram utilizados como parâmetros avaliativos a frequência de anormalidades (micronúcleo, noched, lobed, broken eggs, blebbed, binucleadas e vacúolos) e a atividade da colinesterase plasmática em guppy (*Poecilia reticulata*). Foi constatado que os agrotóxicos, mesmo em doses não letais, causam alterações na frequência de anomalias nucleares e na atividade da acetilcolinesterase. Por outro lado, não houve alterações nos parâmetros biométricos dos animais e nem nos físico-químicos da água.

Palavras-chave: Agrotóxico; Colinesterase; Genotoxicidade; Toxicidade

✉ *Walter Dias Júnior, walterdiasjr@hotmail.com*

Endereço
*[1]: Universidade Estadual de Goiás – Unidade Universitária de Ceres-GO. Curso de Enfermagem
*[2]: Universidade Estadual de Goiás – Campus Central Anápolis-GO. Curso de Ciências Biológicas
ORCID:
Walter Dias Júnior: 0000-0003-3019-7089
Kayo Rodrigo dos Santos Borges: 0000-0002-7784-0441
Anna Kharolyna Aciole Correa: 0009-0007-6766-3609
Daniel Ramalho Santos: 0009-0009-1158-0075
Denilson Rodrigues Vieira: 0009-0003-6089-2485
Carlos Filipe Camilo-Contrim: 0000-0001-8173-5110
Luciana de Souza Ondei: 0000-0001-7608-689X

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Introduction

In recent decades, significant population growth driven by advances in health and improvements in living conditions has led to increased food demand. Consequently, it promoted adaptations and changes in the agricultural environment, making it increasingly dependent on inputs, particularly pesticides. Along with fertilizers, pesticides are widely used to increase productivity and food quality (SAATH e FACHINELLO, 2018; DA SILVA et al., 2017); however, if improperly handled, they can cause severe damage, such as the intoxication of non-target organisms (DE ALMEIDA NETO, 2017; BELTRAME et al., 2024).

Insecticides, fungicides, and herbicides contain toxic active ingredients that can contribute to water pollution, cause adverse environmental impacts, and affect the functioning and quality of ecosystems and organisms within them (STEFFEN et al., 2011).

Pesticides with potentially genotoxic properties are increasingly present in ecosystems where they should not be introduced; thus, the risk of poisoning is increasing, especially in fish and other aquatic animals, including amphibians (MUGNI et al., 2013). In this sense, pesticides may be indirectly consumed by humans through contaminated water and food, or through accumulation in the food chain (MESHKINI et al., 2019).

Herbicides are among the most used pesticides in agriculture, and the leading example of this class is the organophosphate Roundup®, which contains glyphosate as the active ingredient. Turek (2018) showed that the commercial formulation of glyphosate induces genotoxicity in lambari (Astyanax altiparanae) erythrocytes.

Fungicides prevent or eliminate fungal infections in plants. Among them, ethylenebis(dithiocarbamates) (e.g., mancozeb, maneb, zineb, and metiram) have been widely used since the 1940s. Mancozeb was introduced in 1962 and is currently among the most widely used worldwide, especially in Brazil and India (GULLINO et al., 2010). Although classified as low toxicity, studies indicated that it may cause adverse health effects (DING et al., 2012; GUPTA, 2018; YAHIA et al., 2015; PARO et al., 2012).

Research showed that mancozeb affects mitochondrial enzymes and increases the production of reactive oxygen species, leading to cell damage. Its exposure has also been linked to neurotoxic effects, Parkinson's-like symptoms, thyroid dysfunctions, liver, kidney, and brain lesions, neural tube defects, and neoplastic changes in mice (GOLDNER et al., 2010; DOMICO et al., 2006; LEIPHON et al., 2007).

Mancozeb may also cause liver necrosis, alter serum enzymes (e.g., alkaline phosphatase and acetylcholinesterase), and inhibit microsomal oxygenase, resulting in liver damage (KACKAR et al., 1997; ZHOU et al., 2004; NORDBY et al., 2005; CALVIELLO et al., 2006; TYAGI et al., 2011).

Methamidophos, an insecticidal and acaricidal organophosphate widely used in Brazilian agriculture and for managing endemic diseases, is one of the leading causes of high poisoning and death rates in the country. According to ANVISA, the acceptable daily intake is 0.004 mg/kg body weight, and higher doses may be harmful (LIMA et al., 2001).

Studies showed that methamidophos may interfere with spermatogenesis by affecting Sertoli cells, leading to decreased fertility in humans and animals. These effects strengthen the association between pesticide exposure, especially organophosphates, and reproductive problems, and are considered potential endocrine disruptors (YEN, LINK, and WANG, 2000; OKAMURA et al., 2009; BRETVELD et al., 2007).

Although methamidophos is forbidden in Brazil and its registration has been canceled (OLIVEIRA e LUCCHESE, 2013), acephate (a substitute for methamidophos) is still widely used in agriculture (MOTA et al., 2023; VALENTIM et al., 2023) since supervision and guidance on the safe management are limited. Thus, control is restricted to their commercialization (SILVA et al., 2005).

These pesticides were chosen based on a survey of the most cultivated crops in the state of Goiás (Goiânia – Ceres). Glyphosate is the main molecule used to control weeds in transgenic soybean farming, a practice widely documented in studies and technical bulletins in Brazil and in the state of Goiás (GAZZIERO et al., 2006; GAZZIERO et al., 2007; ADEGAS et al., 2022). Methamidophos (an acephate substitute) has historically been one of the main insecticides used in tomato cultivation, as shown in inventories and studies on pesticide use in Goiás and tomato residues (REIS FILHO et al., 2009; TREVISZAN E BAPTISTA, 2003; ZAVATTI e BLOTTARLI, 1999). On the other hand, mancozeb is a widely used fungicide for managing leaf diseases in watermelon (one of the primary crops in the city of Uruana, Goiás) and other vegetables, and is frequently mentioned in technical and scientific publications on watermelon disease control in Brazil (SANTOS et al., 2005; TERAO et al., 2019).

The presence of nuclear abnormalities (NA) resulting from a deficiency in the cell division process has been used in studies as a standard for genotoxicity assessments (SCHMID, 2015; HEDDLE et al., 1983; MATOZO et al., 2015; SAMPAIO et al., 2024).

Acetylcholinesterase (AChE) can also be considered one of the main biomarkers in species commonly exposed to intoxicating agents, as its levels are regulated by the interaction of acetylcholine (ACh) with its receptors; AChE levels increase when a strong interaction occurs between the tested substance and ACh. In addition, changes in enzyme activity indicate changes in ACh availability and in the expression level of its receptors (FERNANDEZ et al., 1992; COLOVIC et al., 2013; KLEMZ, 2015; GONÇALVES et al., 2024).

Therefore, this study aimed to evaluate whether doses of commercial formulations of pesticides containing methamidophos, glyphosate, and mancozeb at the maximum value allowed (MVA) for water potability, according to the Brazilian legislation, change the frequency of nuclear abnormalities in erythrocytes and AChE activity in fishes.

Material and Methods

Adult guppy (*Poecilia reticulata*), females, about 180 days old, weighing 200 mg, and obtained from the colony of the Laboratory of Physiology and Toxicological Biochemistry at the UEG Campus Ceres were kept in aquariums of the laboratory at natural temperature and photoperiod. The study was conducted according to ethical principles and approved by the Ethics Committee on Animal Use of the State University of Goiás (protocol No. 004/2.019).

Ten animals from an aquarium at a storage density of 0.8 g of fish per liter were used, as recommended by OECD No. 203 (1993). Four treatments (control, methamidophos, glyphosate, and mancozeb) were applied over three periods of daily exposure (7, 15, and 30 consecutive days), resulting in 12 groups and 120 animals. Exposures were conducted at different times of the year, and only the type of pesticide varied across exposures. Three commercial pesticide formulations representing the three main classes of pesticides were used at the following doses: methamidophos 12 µg/L (Tamaron® BR-BAYER), mancozeb 180 µg/L (Dithane® NT-Dow Agroscience), and glyphosate 500 µg/L (Glis® 480SL-Dow Agroscience); the control group consisted of colony aquarium water. The pesticides were obtained through donations from rural producers (Tamaron®BR-BAYER and Glis® 480SL-Dow Agroscience) and from commercial stores of agricultural products (Dithane®NT-Dow Agroscience).

The concentrations used were in accordance with the current MVA in the Ordinance No. 2,914, of December 12, 2011, of the Ministry of Health (BRASIL, 2012), which establishes the procedures for controlling and monitoring the quality of drinking water and its potability standards.

The experimental aquariums were made of glass and had a total volume of 2.5 liters. Before each exposure, the animals were transferred from the colony to the experimental aquariums and underwent a 15-day acclimatization period. All animals were fed daily with extruded Acqua Line Supra (Alisul Alimentos S/A, RS, Brasil) feed (42% crude protein) at approximately 10% of body weight/day. Each aquarium had an individual and continuous aeration system. The exposure aquariums were also cleaned every three days (siphoning technique) to remove bottom residues.

The doses were administered continuously by diluting pesticide into the water of the experimental aquariums. Serum equipment was used to control the flow rate of the renewal (333 mL/hr) and exchange approximately twice the total volume of the aquarium once a day; the total exchange (4 liters) occurred after approximately 12 hr of dripping to avoid stress from a sudden change in environment.

After each exposure, the animals were anesthetized with ice water (1 to 4º C) and euthanized by decerebration. Blood (erythrocytes) was collected by amputating the caudal fin of each guppy. Then, a smear (i.e., thin layer of blood arranged on a microscope slide) was prepared from the drained blood for NA analysis.

The smear was dried for approximately 24 hr, fixed, and subjected to the Feulgen reaction, which is a two-step procedure. The first step is the acid hydrolysis, in which the material is immersed in 10% HCl for 11 minutes at 60ºC. In the second step, the slides were immersed in Shiff reagent (dye) for two hours (protected from light) and washed three times.

The occurrence of cells with micronuclei (MN) and NA in erythrocytes of guppy was evaluated by observing 3,000 cells/slide, and the identification of MN and NA followed the recommendations of Jindal and Verma (2015), Gonçalves (2015), Fenech (2000), and Carrasco et al. (1990).

After blood collection for the smear, the body of the animals was crushed in phosphate buffer (pH 7.5), and the supernatant (after centrifugation at 3,000 rpm for 5 minutes) was used for enzymatic acetylcholinesterasedosing with a specific Labtest kit.

The physicochemical parameters (temperature, dissolved oxygen, pH, and ammonia) of the water from the experimental aquariums were quantified with specific commercial kits, a pH meter, and a thermometer.

Body parameters, including body weight (BW), total length (TL), standard length (SL), and height (H), were measured using an analytical scale and caliper after anesthesia at the beginning and end of each exposure. After that, the condition factor (CF%) was calculated to determine the healthiness of the animals before and after exposure to pesticides (LIMA, et al., 2017; SAMPAIO et al., 2024) using the following formula: CF% = [(BW-SL3)*100].

The experiment and analyses were conducted at the Laboratory of Physiology and Toxicological Biochemistry at UEG Campus Ceres. Analysis of variance (ANOVA) and Student's t-test were applied using 5% as the significance level.

Results

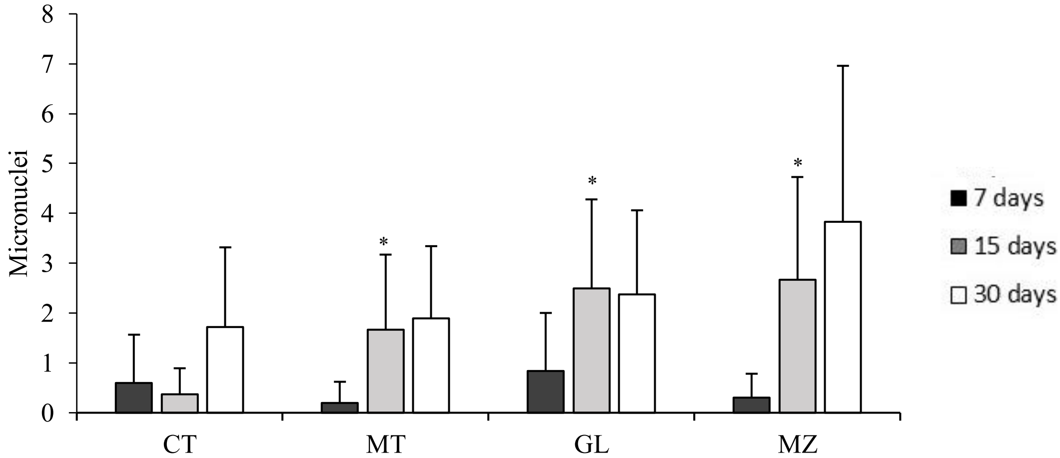
The frequency of MN, NA, and AChE activity is presented in Table 1 and Figures 1, 2, and 3. None of the pesticides tested changed ($p > 0.05$) the number of MN in the erythrocytes of the guppy treated for 7 or 30 days (0.5 ± 0.3 MN/3,000 cells). However, pesticide exposure for 15 days increased the occurrence of MN by 325%, 525%, and 575% for methamidophos (Tamaron), glyphosate (Glis480SL), and mancozeb (Dithane), respectively ($p < 0.05$).

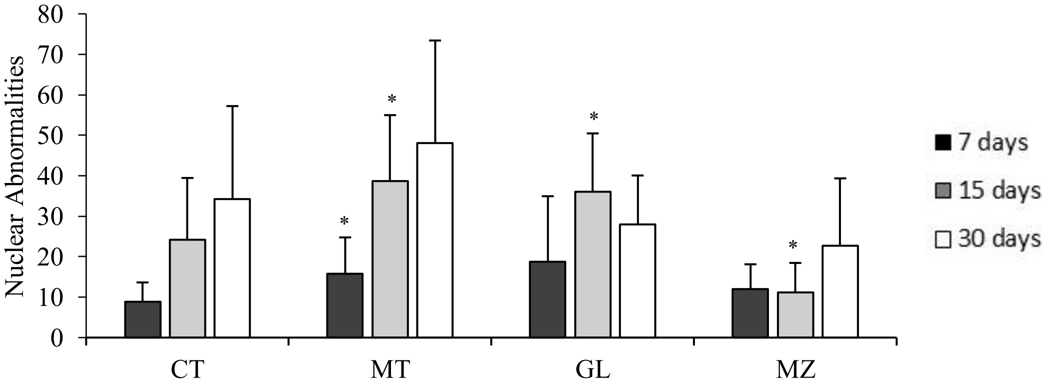
Table 1: Frequency of micronuclei (MN), erythrocytic nuclear alterations (NA), and plasma acetylcholinesterase (AChE UI/L) activity of guppies (*Poecilia reticulata*) exposed during 7, 15, and 30 days with commercial formulations of metamidophos (Tamaron®), glyphosate (Glis480SL®), and mancozeb (Dithane®NT) at doses of 12 µg/L, 500 µg/L, and 180 µg/L, respectively, according Maximum Value Allowed (MVA)/Ministry of Health (Ordinance No. 2914/2011). Values represent mean ± standard deviation per group and exposure time.

Days of exposition	Research variables	Control	Methamidophos	Glyphosate	Mancozeb
07 days	MN	0,6±1,0	0,2±0,4	0,8±1,2	0,3±0,5
	NA	8,9±4,7	15,8±9,0*	18,7±16,3	12,0±6,1
	AChE	355,8±124,9	241,3±70,3*	632,9±258,1*	346,5±209,3
15 days	MN	0,4±0,5	1,7±1,5*	2,5±1,8*	2,7±2,1*
	NA	24,1±15,3	38,6±16,3*	36,0±14,5*	11,2±7,2*
	AChE	1416,3±407,4	3002,4±1066,4*	3608,0±1386,5*	1941,1±1125,4
30 days	MN	1,7±1,6	1,9±1,5	2,4±1,7	3,8±3,1
	NA	34,1±23,1	48,0±25,4	27,9±12,2	22,7±16,6
	AChE	1027,7±450,8	1057,7±841,6	1301,4±513,9	1052,8±381,6

* (p<0,05) Treated versus control. MN: micronuclei; AChE: AcetilcNA: mean frequency of nuclear abnormalities (notched, lobed, broken-eggs, blebbed, binucleated cells, and vacuoles) for each treatment. MVA: Maximum Value Allowed.

Figure 1: Frequency of cells with Micronuclei (MN) and Nuclear Abnormalities (NA) per 3,000 cells observed in erythrocytes of *Poecilia reticulata* exposed for 7, 15, and 30 days to commercial formulations of metamidophos-MT (Tamaron®), glyphosate-GL (Glis®), and mancozeb-MZ (Dithane®NT) at concentrations of 12 µg/L, 500 µg/L, and 180 µg/L, respectively. These concentrations correspond to the Maximum Permitted Value established by the Ministry of Health under Ordinance No. 2914. (*p < 0.05, treated vs. control).





The following types of NA were observed in the nuclei of the erythrocytes of the guppy: notched nucleus, lobed nucleus, broken-egg nucleus, blebbed nucleus, binucleated cell, and presence of vacuoles (Figure 2). Regarding the effect of pesticides on total NA in fish, a 77.5% increase ($p < 0.05$) was observed in animals treated with methamidophos for seven days (Table 1 and Figure 1). This effect was also observed in animals treated with methamidophos and glyphosate for 15 days, with increases of 60.2% and 49.4%, respectively ($p < 0.05$). On the other hand, at the same exposure time, the fungicide mancozeb reduced NA by 53.3% ($p < 0.05$).

No effects of pesticides were observed on the number of MN or NA ($p > 0.05$) in animals exposed for an extended period (30 days). Plasma AChE (AChE – IU/L) activity in methamidophos-treated guppy decreased ($p < 0.05$) by 32.2% after seven days of exposure and increased ($p < 0.05$) by 112% after 15 days (Table 1 and Figure 3).

The herbicide glyphosate also caused a significant increase of 77.8% and 154.7% ($p < 0.05$) in AChE activity after seven and 15 days of exposure, respectively.

No changes in acetylcholinesteraseactivity ($p > 0.05$) were observed in animals exposed to the fungicide mancozeb at any time point tested.

None of the commercial pesticide formulations (Tamaron-methamidophos, Glis480SL-glyphosate, and Dithane-mancozeb) affected AChE activity, NA, or the frequency of MN ($p > 0.05$) in animals exposed for 30 consecutive days (Table 1 and Figures 1 and 2).

Figure 2: Frequency of micronuclei and nuclear abnormalities per 3,000 erythrocytes in guppies (*Poecilia reticulata*) following 7, 15, and 30 days of exposure to the commercial formulations of metamidophos (Tamaron®; 12 µg/L), glyphosate (Glis®480SL; 500 µg/L), and mancozeb (Dithane®NT; 180 µg/L), at concentrations corresponding to the Maximum Value Allowed established by the Brazilian Ministry of Health under Ordinance No. 2914/2011. Nuclear alterations observed include: A–C, notched nuclei; D, micronucleus; E, nuclear vacuole; F, binucleated cell; G, blebbed nucleus; H, lobed nucleus; I, broken-eggs nucleus. Photomicrographs obtained at 1,000× magnification.

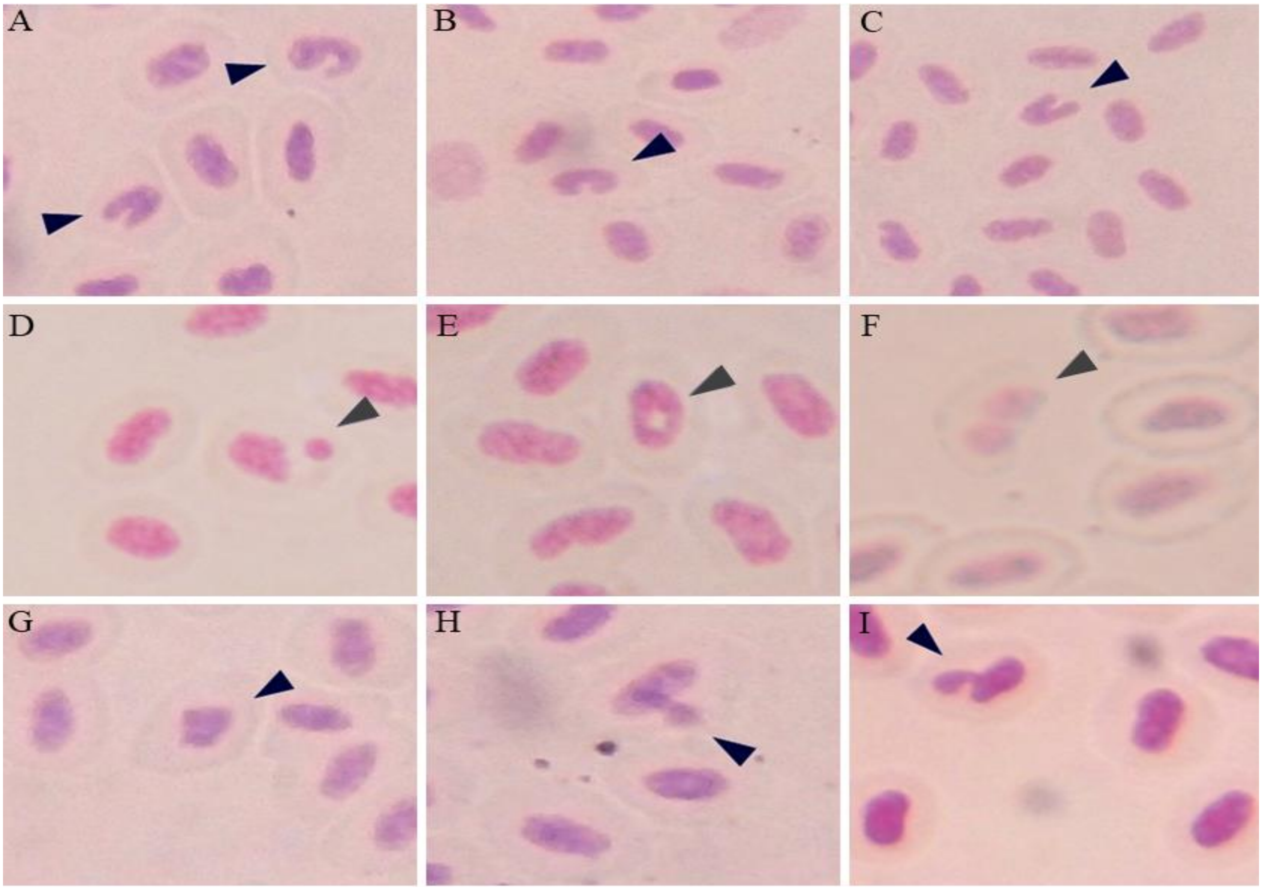
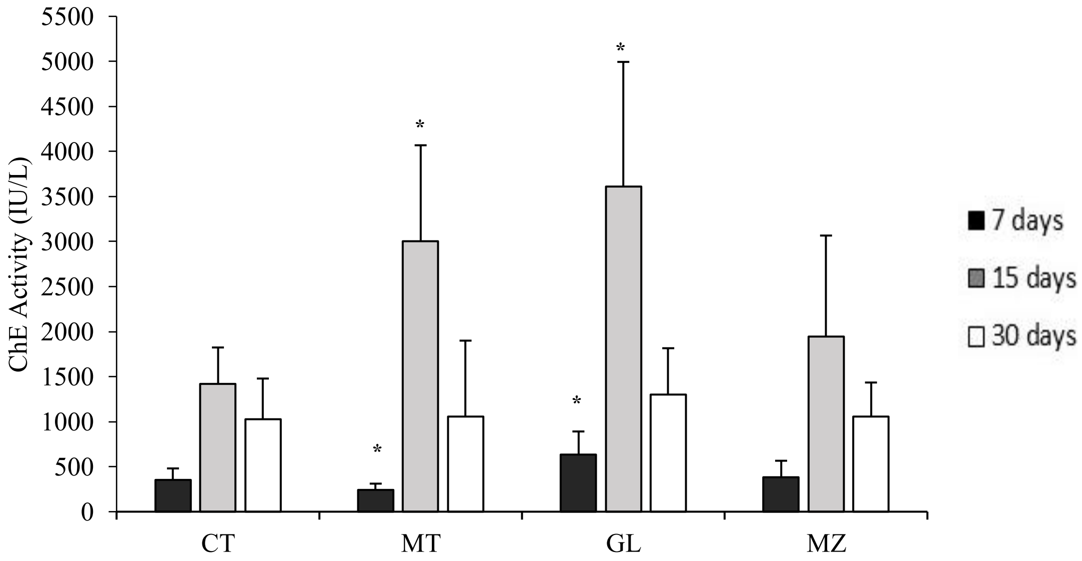


Figure 3: Plasma acetylcholinesterase (AChE; UI/L) activity in guppies (*Poecilia reticulata*) exposed for 7, 15, and 30 days to commercial formulations of metamidophos-MT (Tamaron®), glyphosate-GL (Glis480SL®), and mancozeb-MZ (Dithane®NT) at concentrations of 12 µg/L, 500 µg/L, and 180 µg/L, respectively. These concentrations correspond to the Maximum Permitted Value established by the Ministry of Health under Ordinance No. 2914/2011. Data are expressed as mean ± standard deviation for each group and exposure period. *(* p < 0.05, treated vs. control).*



The physicochemical parameters of water (hydrogen potential, dissolved oxygen, ammonia, nitrite, hardness, and temperature) (Figure 4) also showed no changes (p > 0.05) due to the addition of pesticides or exposure time. This indicates that the experimental environment in the aquariums was uniform and the water quality was not altered after adding pesticide solutions.

Results regarding the biometric parameters of guppies are presented in Table 2. No changes (p > 0.05) were observed in body parameters of the groups exposed to the commercial formulations of methamidophos, glyphosate, and mancozeb for 7, 15, and 30 days (Table 2). This demonstrates that the group of animals was homogeneous and maintained good physical integrity throughout the exposure time and across all pesticides tested, as evidenced by the CF% homogeneity (Table 2).

Figure 4. Physicochemical parameters of the water in exposure aquaria containing guppies (*Poecilia reticulata*) subjected for 7, 15, and 30 days to commercial formulations of metamidophos-MT (Tamaron®), glyphosate-GL (Glis®480SL), and mancozeb-MZ (Dithane®NT) at concentrations of 12 µg/L, 500 µg/L, and 180 µg/L, respectively. These concentrations correspond to the Maximum Permitted Value established by the Ministry of Health under Ordinance No. 2914/2011.

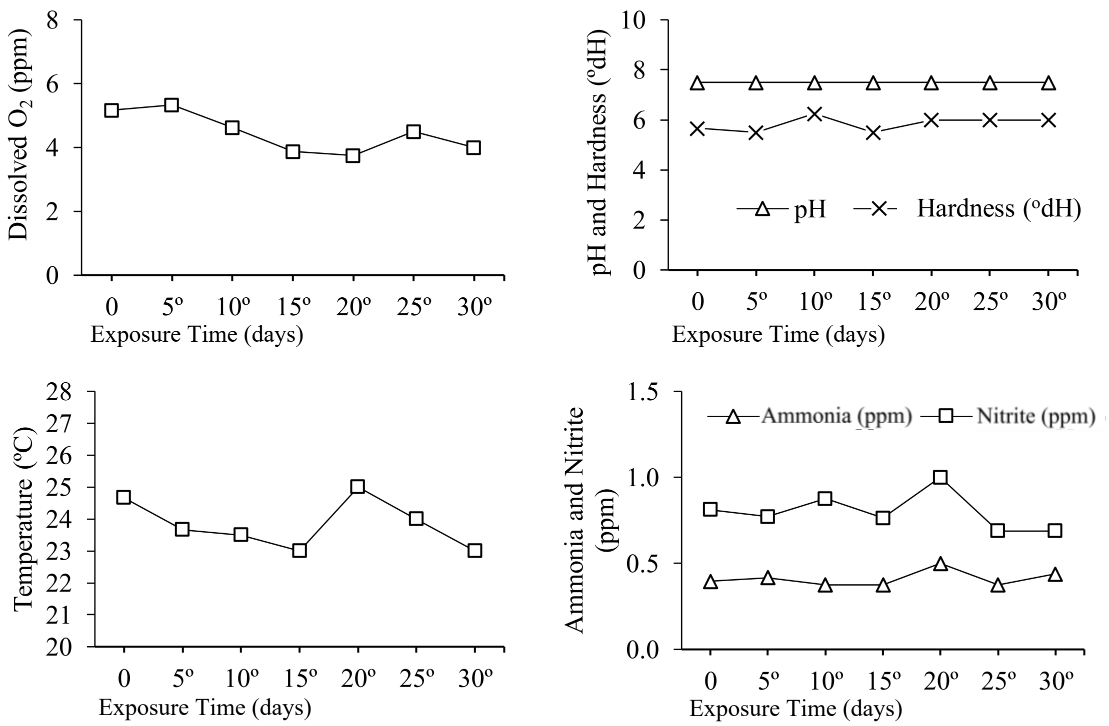


Table 2. Initial biometric parameters (first day of treatment) and final biometric parameters (last day of treatment) of guppies (*Poecilia reticulata*) exposed for 7, 15, and 30 days to the commercial formulations of metamidophos (Tamaron®; 12 µg/L), glyphosate (Glis®480SL; 500 µg/L), and mancozeb (Dithane®NT; 180 µg/L), at concentrations corresponding to the Maximum Permitted Value established by the Brazilian Ministry of Health under Ordinance No. 2,914.

Days of exposition	Biometric Parameters	Control		Methamidophos		Glyphosate		Mancozeb	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final
7 days	BW (g)	0,13±0,1	0,14±0,1	0,14±0,1	0,16±0,1	0,16±0,1	0,15±0,1	0,14±0,1	0,11±0,1
	SL (cm)	1,96±0,3	1,75±0,1	1,92±0,3	1,96±0,4	2,07±0,4	1,76±0,5	1,89±0,4	1,68±0,4
	TL (cm)	2,44±0,4	2,48±0,3	2,39±0,4	2,48±0,4	2,50±0,4	2,30±0,5	2,46±0,4	2,29±0,3
	H (cm)	0,55±0,1	0,47±0,1	0,49±0,1	0,51±0,1	0,57±0,1	0,54±0,1	0,48±0,1	0,48±0,1
	CF%	1,62±0,3	1,89±0,4	1,96±0,4	1,97±0,4	1,74±0,2	2,17±0,3	1,93±0,4	2,12±0,4
15 days	BW (g)	0,19±0,1	0,19±0,0	0,18±0,1	0,20±0,1	0,16±0,0	0,16±0,1	0,19±0,1	0,18±0,1
	SL (cm)	2,09±0,1	2,16±0,1	2,17±0,2	2,22±0,2	2,10±0,2	1,96±0,2	2,14±0,2	2,10±0,2
	TL (cm)	2,61±0,1	2,66±0,2	2,72±0,2	2,70±0,2	2,55±0,2	2,48±0,3	2,65±0,2	2,58±0,3
	H (cm)	0,53±0,1	0,57±0,1	0,54±0,1	0,55±0,1	0,53±0,1	0,50±0,0	0,55±0,1	0,53±0,1
	CF%	2,01±0,4	1,89±0,2	1,79±0,3	1,78±0,2	1,70±0,4	2,04±0,3	1,90±0,2	1,95±0,3
30 days	BW (g)	0,23±0,1	0,22±0,2	0,25±0,2	0,19±0,2	0,19±0,1	0,17±0,1	0,14±0,1	0,11±0,0
	SL (cm)	2,30±0,4	2,07±0,5	2,32±0,4	2,02±0,5	2,09±0,5	1,91±0,4	1,94±0,3	1,60±0,2
	TL (cm)	2,85±0,5	2,62±0,6	2,81±0,5	2,59±0,6	2,65±5,3	2,47±0,6	2,46±0,4	2,30±2,7
	H (cm)	0,53±0,1	0,53±0,1	0,56±0,1	0,51±0,1	0,54±0,1	0,47±0,1	0,47±0,1	0,40±0,0
	CF%	1,71±0,2	2,25±0,6	1,78±0,3	2,00±0,3	2,02±0,3	2,32±0,3	1,80±0,2	2,15±0,6

BW: Body weight (g); SL: Standard length (cm); TL: Total length (cm); H: Height (cm) and CF%: Condition factor (%) [$CF\%=(BW/SL^3).100$]. Values are expressed as mean ± standard deviation for each group and exposure period.

Discussion

Teleost fish are widely used as biomarkers for environmental contamination due to their sensitivity in detecting genotoxic effects of xenobiotics and the ability to mimic the toxicity of substances in humans (CORT and GHISI, 2014; LORENSI et al., 2018; AMAEZE et al., 2020). Castro (2018) evaluated the toxicity of the fungicide tebuconazole in zebrafish (Danio rerio) by exposing them for 24, 72, and 96 hr at 175 to 200 µg/L, the same concentrations used in our study (180 µg/L). Castro (2018) used the micronucleus test as a standard of evaluation and found an increase in the frequency of this cellular alteration. Similarly, the mancozeb-based fungicide tested in our study increased the frequency of MN by 575% after 15 days of treatment and decreased the occurrence of other alterations (notched nucleus, lobed nucleus, broken-egg nucleus, blebbed nucleus, binucleated cell, and vacuoles) by 53.3%. However, this fungicide did not affect MN frequency or caused other changes after 7 or 30 days of exposure, indicating adaptation in the animals.

Herbicides are the most commonly used types of pesticide, with glyphosate being the most common example of this class. Recent studies have already shown that glyphosate can cause genotoxicity. Turek (2018) demonstrated that Roundup® WG (glyphosate-based herbicide) induced genotoxicity in lambaris after exposure for 120 hr at concentrations of 1,250 µg/L and 5,000 µg/L. Our study also showed that, even at a 60% lower dose (500 µg/L) and with a 15-day exposure, the commercial formulation of glyphosate (Glis®480SL) caused a 49.4% increase in the number of NA and a 525% increase in MN frequency (Table 1 and Figure 1). Therefore, the effects of Glis®480SL after 15 days were confirmed, whereas 7 days were insufficient to cause significant damage.

The half-life of pesticides varies widely (e.g., 11 to 17 days in some soils and > 100 days in cold/sandy soils or specific conditions), while aminomethylphosphonic acid tends to persist longer (higher DT50 in some cases). The half-life of glyphosate ranges from 11 to more than 100 days, depending on soil and climate conditions (MEFTAUL et al., 2023). Tomlin (2006) observed that the pesticide remained in the water after 30 days of exposure, suggesting adaptation in guppy, as no changes in the frequency of MN, NA, or AChE activity were observed during this extended exposure time.

Our results showed that the MVA of the pesticides Tamaron® (methamidophos 12 µg/L), Glis®480SL (glyphosate 500 µg/L), and Dithane® (mancozeb 180 µg/L) in drinking water present genotoxic effects and alter the AChE activity in guppy fish within 15 days of exposure. On the other hand, a longer exposure time may allow the species to adapt to these levels of pesticides since no effects were observed on the variables studied.

Previous studies have shown that exposure to insecticides for 30 days does not have important effects. Jablonski (2018) tested the insecticide triflumuron in zebrafish (Danio rerio) and found no changes in the frequency of MN or new NA in erythrocytes of teleost fish after 30 days.

In our study, the herbicide Glis®480SL increased AChE activity only in fish exposed for 7 (77.8%) and 15 days (154.7%), whereas no changes were observed in animals treated for 30 days. Meshkini et al. (2019) investigated the LC50 of the herbicide Roundup® WG by assessing fish mortality at 96 hr (SDS Probit test). They found that herbicides may alter cerebral and hepatic AChE after 28 days of treatment in rainbow trout. AChE inhibition increases ACh in the synaptic cleft, which may cause behavioral changes, tremors, loss of balance, and possible fish death (ÜNER et al., 2016).

Zhu et al. (2014) conducted a study with five widely used fungicides (myclobutanil, fluconazole, flusilazole, trimizole, and epoxiconazole) and observed decreased ATPase and AChE levels in Chinese cyprinids (Gobiocypris rarus) after 72 hr of exposure at concentrations ranging from 1,000 to 15,000 µg/L. Moreover, this reduction was greater than 80% for fluconazole and myclobutanil fungicides. In contrast, our study found no changes in AChE activity after 7, 15, and 30 days of exposure to the commercial formulation of mancozeb. This difference may be related to the lower dose used in our study (180 µg/L, approximately 5 times lower) and differences in exposure time and specimen used.

The insecticide methamidophos acts as an AChE inhibitor and causes significant effects on biota that are not its target, including humans, other vertebrates, and invertebrates (DIAZ, 2012). Sandoval-Herrera (2019) observed more than 50% inhibition of AChE activity in the brains of lambari exposed to 14 µg/L of the insecticide Etoprofos for 48 hr, supporting our findings that insecticides disrupt AChE activity in fish after 7 and 15 days of exposure to methamidophos. Additionally, Sandoval-Herrera (2019) found associations between AChE inhibition and decreased fish escape ability using behavioral analysis. Abdelnour et al. (2024) presented possible explanations for the effects observed in our study. They stated that environmental stressors, such as pesticides, petroleum, and heavy metals, may cause epigenetic modifications in fish. Some of these changes are temporary, while others can be integrated into the genome, promoting transgenerational adaptation. These changes affect DNA and histone methylation, which may lead to organ dysfunction, oxidative stress, and reduced reproduction. On the other hand, these same epigenetic changes induced by pesticides may also lead to an adaptation of species to adverse environments (ABDELNOUR et al., 2024).

Regarding the physicochemical parameters of the water, the pesticides neither caused changes ($p > 0.05$) in the values of pH, dissolved oxygen, ammonia, nitrite, hardness, and temperature, nor affected the biometric parameters of the fish (BW, SL, TL, H, and CF%). The CF% is a quantitative indicator of the fish healthiness or well-being in the environment, reflecting recent dietary conditions (URBANSKI et al., 2023; LIMA et al., 2023). Since our study did not find significant differences in this parameter, we concluded that the fish remained healthy (i.e., good diet) throughout the exposure time.

In this sense, changes in NA and AChE activity were solely due to exposure to pesticides, without environmental interference.

Conclusion

We observed that the MVA indicated by the Ministry of Health for drinking water quality and the standards for potability of methamidophos pesticides (12 µg/L), glyphosate (500 µg/L), and mancozeb (180 µg/L), tested in their commercial formulations, Tamaron®BR, Glis®480SL, and Dithane®NT, respectively, changes the frequency of NA (micronucleus, notched nucleus, lobed nucleus, broken-eggs nucleus, blebbed nucleus, binucleated cell, and presence of vacuoles) and AChE activity in animals treated for 7 and 15 days. Regarding the 15 days of exposure, all groups changed the frequency of NA and AChE activity, except fishes treated with mancozeb.

Changes were not found in the biometric parameters of guppy treated for 30 days or in the physicochemical parameters of the water from the exposure aquariums.

The frequency of MN and NA and AChE activity change even at very low doses of pesticides, such as those defined by the MVA in Ordinance No. 2,914, of December 12, 2011, of the Ministry of Health for water potability. This suggests that these parameters are sensitive biomarkers of pesticide toxicity.

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