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Ecotoxicological Assessment and Environmental Risk of the Insecticide Chlorpyrifos for Aquatic Neotropical Indicators

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Abstract Chlorpyrifos (CPF) is an organophosphorus insecticide detected in aquatic environments considered harmful to living beings. The aim of this research was to evaluate the ecotoxicity of CPF for neotropical aquatic organisms of distinct trophic levels (*Lemna minor*, *Azolla caroliniana,* and *Wolffia brasiliensis* macrophytes; *Pomacea canaliculata* snail; *Macrobrachium acanthurus* shrimp; *Xiphophorus maculatus* and *Hyphessobrycon eques* fish), to verify the risk of environmental poisoning for each organism, and to determine the best bioindicator species of aquatic contamination by the insecticide. Ecotoxicological assays were carried out with different concentrations of CPF under controlled laboratory conditions standardized for each species. IC50;7d, LC50;7d, EC50;48h, and LC50;48h values were calculated using the Trimmed Spearman Karber software with 95% confidence limits. The toxicity data were used to classify the CPF according to the ecotoxicity categories for aquatic organisms. The risk of CPF environmental poisoning was determined by the quotient method considering different environmental scenarios. The sensitivity order of neotropical aquatic organisms to chlorpyrifos was *Macrobrachium acanthurus* (0.002 mg L^{-1}) > *Xiphophorus maculatus* (0.07 mg L^{-1}) > *Hyphessobrycon eques* (1.65 mg L^{-1}) > *Pomacea canaliculata* (30.66 mg L^{-1}) > *Azolla caroliniana* (849.72 mg L^{-1}) > *Wolffia brasiliensis* (1271.63 mg L^{-1}) = *Lemna minor* (1299.60 mg L^{-1}). The risk of poisoning by chlorpyriphos may vary according to the environmental concentration of the insecticide and the exposed trophic level. The best bioindicator and with the greatest risk of environmental poisoning was shrimp. The difference in CPF ecotoxicity for distinct aquatic trophic levels shows the relevance of evaluating the effects of contaminants considering food chains and highlights the importance of studying these levels in environmental monitoring programs.

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1 Introduction

The degradation of environmental quality and equilibrium associated with the uncontrolled urbanization process and population growth has become a global concern. Contamination of environmental matrices (soil, water, and air) by toxic substances such as pesticides, metals, dyes, hormones, volatile organic compounds, pharmaceutical and personal hygiene products can cause direct and indirect adverse effects on human health and living beings (Bhat et al., 2021; Rasheed et al., 2020; Storto et al., 2021; Torres et al., 2021; Zeng et al., 2021).

Pesticides are among the main environmental contaminants because they are used in agricultural activities around the world. Most of these compounds are synthetic products with characteristics that are effective in eliminating or controlling of agricultural pests, but which can also affect non-target organisms due to their toxicity and persistence in the environment (Rajput et al., 2021). Aquatic ecosystems are the environmental compartments most impacted by pesticides. The occurrence of these contaminants has been detected in surface and underground waters in various regions of the world (Becker et al., 2021; Kalantary et al., 2022; Lee et al., 2019; Rousis et al., 2017; Wang et al., 2021).

Organophosphorus (OPs) insecticides are one of the main groups of pesticides used in agriculture, public health, and disease vector control. There is evidence that the use of these insecticides has declined in developed countries; however, in developing countries, their use is increasing (Moretto, 2014). The insecticide chlorpyrifos (CPF) is one of the most used OPs in the world (EPA, 2017; Huanga et al., 2020). CPF is included as an active ingredient in various formulations from emulsifiable concentrates, granular, and wettable powders to microencapsulated suspensions (Eaton et al., 2008).

In the current Pesticides Commercialization Report published by the Brazilian Institute for the Environment and Natural Resources (IBAMA), the CPF was among the 10 most commercialized active ingredients in Brazil (IBAMA, 2021) and the Brazillian National Council for the Environment (CONAMA) does not establish limits for CPF concentration on waters destined to the conservation of fauna and flora (Brasil, 2005).

The knowledge about a toxicant action on non-target organisms contributes to the conservation and monitoring of the ecosystems, which maintains the environmental balance (Brogan & Relyea, 2017; Silva-De-Sá et al., 2019). The use of different bioindicators that cover distinct levels of biological organization makes it possible to recognize the level of interaction of the substance with the organism and the level of susceptibility of the organisms to the substance. Research with bioindicators is essential to design effective strategies that promote the recovery of biodiversity in aquatic ecosystems (Arias et al., 2007).

In accordance with European Communities Regulations Directive 1488/94, the risk assessment of chemical compounds must be carried out in toxicity tests with organisms inserted in at least three different trophic levels. In these tests, the studied substance must not present a risk of environmental poisoning for organisms of the three trophic levels (EC, 2003).

Some studies have determined the ecotoxicity of chlorpyrifos for aquatic organisms such as microcrustaceans and mainly fish (Fan et al., 2021; Ferrario et al., 2018; Kunwar et al., 2021; Maggio et al., 2021). However, there is limited information about the ecotoxicity of this insecticide to aquatic organisms of different trophic levels and its potential risk of environmental poisoning.

Therefore, the aim of this research was to evaluate the ecotoxicity of CPF for neotropical aquatic organisms of distinct trophic levels (*Lemna minor*, *Azolla caroliniana,* and *Wolffia brasiliensis* macrophytes; *Pomacea canaliculata* snail; *Macrobrachium acanthurus* shrimp; *Xiphophorus maculatus* and *Hyphessobrycon eques* fish), to verify the risk of environmental poisoning for each organism, and to determine the best bioindicator species of aquatic contamination by the insecticide.

2 Materials and Methods

The present research was approved by the Ethics Committee on Animal Use (CEUA) from the Barretos

Educational Foundation (UNIFEB), Barretos – São Paulo, Brazil (Protocol n°02_2019).

The insecticide used in the toxicity assays was the organophosphorus CPF (CAS No. 2921-88-2) in the commercial formulation Capataz BR® (480 g L^{-1}). The commercial formulation was diluted in distilled water and natural water (the same used in the culture of each test organism) to obtain the dilutions for each toxicity assay based on the concentration of the active ingredient (Fig. 1). Toxicity assays were carried out with three species of macrophytes (*Lemna minor*, *Azolla caroliniana,* and *Wolffia brasiliensis*), one species of mollusk (*Pomacea canaliculata*), one species of crustacean (*Macrobrachium acanthurus*), and two species of fish (*Xiphophorus maculatus* and *Hyphessobrycon eques*).

2.1 Toxicity Assay for Aquatic Macrophytes

Aquatic macrophytes (*L. minor*, *A. caroliniana,* and *W. brasiliensis*) were cultivated in 2.5 L containers with dechlorinated water containing a substrate (dirt, sand, and organic matter, equal measures). After vegetative growth stadium, the macrophytes were acclimatized on *Hoagland's* solution (distilled water reconstituted with nutrients and pH 6.5 ± 0.2) during seven days in a bioassay room at 24.0 ± 1.0 °C, continuous air flow, 6.500 Lux light intensity, and photoperiod (12 h light:12 h dark).

The toxicity assays for macrophytes were performed according to the procedures of the Guideline for testing of chemicals—*Lemna minor* Growth Inhibition of the OECD (2002) with some adaptations for *A. caroliniana* and *W. brasiliensis.* In the assays, macrophytes with good sanitary aspect and homogeneous size were selected. The tests were conducted in an acclimatized room (same condition as the acclimatization) with duration of seven days.

The assay for *L. minor* was conducted with three replicates for each concentration tested. For each replicate, four colonics of *L. minor* with 3 fronds ("leaves") each were chosen, totaling 12 fronds per replicate. For *A. caroliniana*, three replicates were used for each concentration tested with five healthy

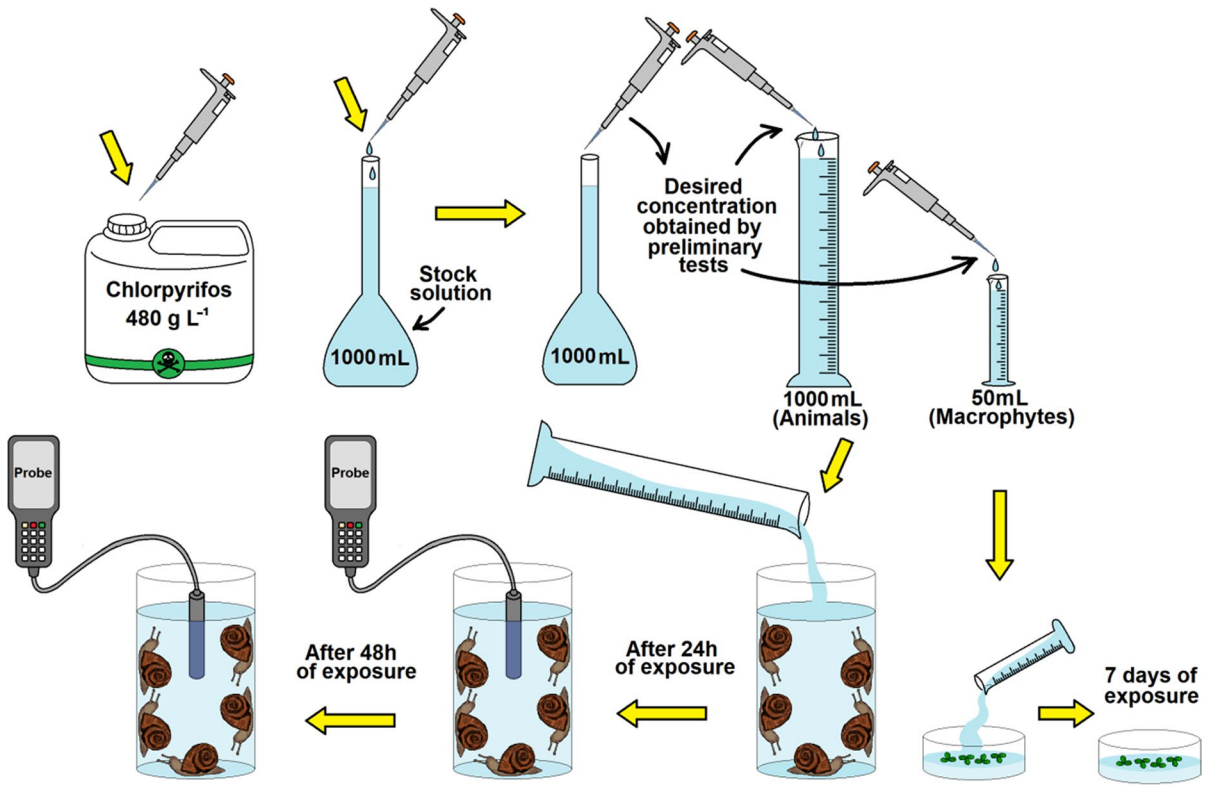


Fig. 1 Schematic of preparation of chlorpyrifos dilutions for toxicity assays with aquatic organisms

plants for each replicate. The *W. brasiliensis* assay used a homogeneous number of individuals, which were sampled by a syringe connected to 0.45 mm tubes, as described by Pereira et al. (2019).

Toxicity for *L. minor* was evaluated by inhibiting plant growth, occurrence of necrosis, and chlorosis. The toxicity for *A. caroliniana* and *W. brasiliensis* was evaluated by the percentage of necrosis and chlorosis.

Before carrying out the toxicity assays with the insecticide, the health and sensitivity of the macrophytes was evaluated by testing with the reference substance sodium chloride (NaCl). Sensitivity tests were performed under the same acclimatization conditions (OECD, 2002).

The growth inhibition concentration (IC50;7d) of NaCl for *L. minor* was 0.30 mg L^{-1}, with 0.20 mg L^{-1} of lower limit (LL) and 0.35 mg L^{-1} of upper limit (UL). The lethal concentration (LC50;7d) of the same substance for *A. caroliniana* was 0.40 mg L^{-1}, with 0.30 mg L^{-1} LL and 0.53 mg L^{-1} UL. For *W. brasiliensis*, the LC50;7d was 4.85 mg L^{-1}, with 3.42 mg L^{-1} of LL and 6.88 mg L^{-1} of UL. These values confirm that the macrophytes were in normal conditions of health and sensitivity, according to the laboratory control.

CPF toxicity assays for macrophytes were performed by exposing the plants to different concentrations of the insecticide diluted in the cultivation water (*Hoagland's* solution). CPF concentrations were determined in preliminary toxicity tests in an acclimatized room with the same acclimatization conditions.

The CPF toxicity assays were performed by the plants exposure to the following CPF concentrations: 100.0, 200.0, 400.0, 800.0, 1600.0, 3200.0 mg L^{-1} and a control (*Hoagland's* solution without CPF) for seven days.

2.2 Acute Toxicity Assay for Snail

Snails (*P. canaliculata*) were cultivated in 250.0 L containers with dechlorinated water and substrate (earth, sand, and organic matter, equal measures) until reproduction. They were fed the aquatic macrophyte *Hydrilla verticillata* and extruded fish feed, containing 28% protein as recommended by Venturini et al. (2008).

After reproduction, young offspring weighing 5.0 to 7.0 g were acclimated in aquariums containing dechlorinated water, *H. verticillata* at 25.0 ± 1.0 °C, continuous aeration, light intensity of 6500 Lux, and photoperiod (12 h light:12 h dark) for 7 days. Toxicity assay with the snail was conducted in accordance with standard NBR 15088 (ABNT, 2016) for fish, adapted for snails, which ensured the standardization of the assays. The snails were not fed and there was no aeration of the aquariums during the toxicity assays (48 h). The snail assay was carried out with three repetitions for each concentration tested. For each repetition, five snails were used.

Before carrying out the toxicity assays with the CPF, the health and sensitivity of the snails was evaluated by testing with the reference substance potassium chloride (KCl). Sensitivity assays were performed under the same acclimatization conditions (ABNT, 2016).

The immobility/mortality assessments were performed with the snail response to a single physical stimulation at 24 and 48 h after the exposure and visual observation of beathing signs. The results were expressed in effective concentration after 48 h of exposure (EC50;48h).

The KCl EC50;48h for the snail was 1.63 mg L^{-1} with LL = 1.34 mg L^{-1} and UL = 1.98 mg L^{-1}. Thus, the organisms were considered in good health and with sensitivity levels comparable to other sets of organisms previously born and grown in the same system.

For the CPF toxicity assay, the snails were exposed for 48 h to the following concentrations: 2.8, 4.8, 8.2, 13.9, 23.7, and 40.3 mg L^{-1} and a control (dechlorinated natural water without CPF). CPF concentrations were determined in preliminary toxicity tests in an acclimatized room with the same acclimatization conditions. At 24 and 48 h of CPF exposure, pH, dissolved oxygen, temperature, and electric conductivity were measured with YSI Professional Plus multiparameter probe (ABNT, 2016).

2.3 Acute Toxicity Assay for Freshwater Shrimp

Freshwater shrimp (*M. acanthurus*) were grown in 250.0 L containers with dechlorinated natural water and were fed with the aquatic macrophyte *H. verticillata* and extruded fish feed, containing 28% protein.

Young individuals with 1.0 to 2.0 g were acclimatized in bioassay room at 25 ± 1.0 °C, continuous aeration, 6500 Lux light intensity, and photoperiod (12 h light:12 h dark) for 7 days, according to the NBR 15088 regulation for fish (ABNT, 2016), adapted for shrimps, which ensures destandardization of the results. The shrimps were not fed and there was no aeration of the aquariums during the toxicity assays (48 h). The tests were conducted with three replicates for each concentration of insecticide. For each replica, a shrimp was used.

The shrimp sensitivity was assessed by an acute toxicity assay (LC50;48h) with KCl as reference substance. The KCl LC50;48h for the shrimp was 1.53 mg L^{-1} with LL = 1.00 mg L^{-1} and UL = 2.33 mg L^{-1}. Thus, the organisms were considered in good health and with sensitivity levels comparable to other sets of organisms previously born and grown in the same system.

For the CPF toxicity assay, the shrimps were exposed for 48 h to the following concentrations: 0.001, 0.004, 0.006, 0.008, 0.010 mg L^{-1} and a control (dechlorinated natural water without CPF). The tests were carried out with three repetitions for each concentration of CPF with 5 individuals each. The mortality assessments were performed at 24 and 48 h of exposure. At 24 and 48 h of CPF exposure, pH, dissolved oxygen, temperature, and electric conductivity were measured with YSI Professional Plus multiparameter probe (ABNT, 2016).

2.4 Acute Toxicity Assay for the Fish

Specimens of platyfish (*X. maculatus*) and serpae tetra (*H. eques)* were purchased from a specialized commercial aquaculture producer. After a quarantine period, fish were acclimatized for 7 days in 250.0-L cultivation tanks in open circulation system with renewal of the total volume of water every 24 h in bioassay room at 25.0 ± 2.0 °C, photoperiod (12 h light:12 h dark), with pH 7.0–7.6 and hardness 40–48 mg $CaCO_3$/L. Water was constantly aerated (dissolved oxygen > 6.0 mg L^{-1}). The fish were fed with 2% of body weight, every other day with the same commercial diet (28% protein). The fish selected for the acute toxicity assays were healthy and had body weight between 0.8 and 1.0 g.

The toxicity assays for fish were performed according to the fish standard NBR 15088 (ABNT, 2016). The tests were conducted in an acclimatized room (same condition as the acclimatization) for 48 h without feeding the animals during this period. Assays for each fish species were performed with three replicates for CPF concentration. For replica, five specimens were used. Fish sensitivity was evaluated by acute toxicity assay with the reference substance KCl. The lethal concentration of KCl after 48 h of exposure (LC50; 48 h) was 1.33 mg L^{-1}, with LL = 1.14 mg L^{-1} and UL = 1.75 mg L^{-1} for *X. maculatus*; and the LC50; 48h was 1.40 mg L^{-1}, with LL = 1.24 mg L^{-1} and UL = 1.68 mg L^{-1} for *H eques*. Thus, the organisms were considered in good health and with sensitivity levels comparable to other sets of organisms previously born and grown in the same system.

CPF toxicity assays were performed by exposing the fish to different concentrations of the insecticide diluted in the cultivation water (natural and dechlorinated). CPF concentrations were determined in preliminary toxicity tests in an acclimatized room with the same acclimatization conditions.

In the acute toxicity assay with *X maculatus*, fish were exposed for 48 h at concentrations of 0.05, 0.10, 0.50, 1.00, 1.50, 2.00 mg L^{-1} and a control (culture water without CPF). *H. eques* were exposed to 0.50, 1.00, 2.00, 2.50, 3.00, 4.00 mg L^{-1} and control (culture water without CPF). CPF concentrations were determined in preliminary toxicity tests in an acclimatized room with the same acclimatization conditions. Fish mortality was assessed daily with the removal of dead animals from the aquariums, considering in this state the animals that did not present opercular beat, spasm, or any movement.

At 24 and 48 h of CPF exposure, pH, dissolved oxygen, temperature, and electric conductivity were measured with YSI *Professional Plus* multiparameter probe (ABNT, 2016).

2.5 Data Analysis

IC50;7d, LC50;7d, EC50;48h, and LC50;48h values were calculated using the Trimmed Spearman Karber software with 95% confidence limits (Hamilton et al., 1977). The toxicity data were used to classify the CPF according to the EPA (2021) ecotoxicity categories for aquatic organisms (Table 1).

2.6 Environmental Risk Assessment

The risk of CPF environmental poisoning was determined by the quotient (Q) method proposed by Goktep et al. (2004). In this method, the risk is calculated by the ratio between the estimated environmental concentration (EEC), which is the dose of the insecticide applied in the field, and the values of IC50;7d, LC50;7d, EC50;48h, and LC50;48h obtained in the toxicity assays. The Q value, which is also called risk quotient (RQ), is a pure number, which is used to classify the environmental risk in categories according to a RQ interval.

If $RQ > 0.5$, the environmental risk of the insecticide is high. If $0.05 < RQ < 0.5$, the environmental risk is medium and when $RQ < 0.05$, the pesticide presents low environmental risk (Goktepe et al., 2004).

To estimate the EEC, contamination scenarios adapted from the procedure proposed by Kokta and Rothert (1992) were considered. In these scenarios, it was considered that

I) The substance must be evenly distributed in two theoretical water bodies, both presenting 1-hectare (10,000 m^2) surface area, one with 0.3 m and the other with 2.0 m deep.
II) The average water density is 1.0 g cm^{-3}.
III) The insecticide must be applied on the water body in dilutions corresponding to 100, 50, 25, 12.5, 6.25, and 3.12% of its highest recommended dose.
IV) The volume of the reservoir and all the concentrations of the applied insecticides are known.

The chlorpyrifos risk evaluation used the highest recommended dose for coffee, tomato, and wheat (720 g ha^{-1}) according to the manufacturer guidelines for the commercial formula Capataz BR®.

Table 1 Ecotoxicity categories for aquatic organisms.

Toxicity category	Aquatic organisms: acute concentration (mg L^{-1})
Very highly toxic	< 0.1
Highly toxic	0.1–1
Moderately toxic	> 1–10
Slightly toxic	> 10–100
Practically nontoxic	> 100

Source: Adapted from EPA (2021)

3 Results and Discussion

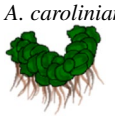
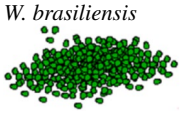
3.1 Toxicity Assay

The CPF toxicity results (IC50;7d, LC50;7d, EC50;48h, LC50;48h), its 95% confidence limits, and duration of the assays are shown in Table 2. There was no mortality of neotropical aquatic organisms in the containers controls. The toxicity values (IC50;7d, LC50;7d) of the CPF for aquatic macrophytes (*L. minor*, *A. caroliniana*, *W. brasiliensis*) were greater than 100.0 mg L^{-1} (Table 2). Thus, according to EPA (2021), the insecticide is classified as practically nontoxic for these species. Our results corroborate those found by Carraschi et al. (2015) in which *L. minor* was tolerant to concentrations of the insecticide thiamethoxan above 100.0 mg L^{-1}. The lower sensitivity of *L. minor* to diflubenzuron compared to other aquatic organisms was also recorded in the study by Souza et al. (2011) in which the LC50;48h of the organophosphorus for macrophyte was 459.50 mg L^{-1}.

The acute toxicity of CPF for the neotropical aquatic plants studied was lower than that observed for the algae *Microcystis wesenbergii* (Sun et al., 2015), *Oscillatoria sp*., *Nitzschia sp*., and *Chlorella sp.* (Tien & Chen, 2012).

From the third day of exposure to CPF, *L. minor* fronds showed chlorosis at all concentrations tested, except for the control. The first signs of necrosis occur on the third day of exposure at a concentration of 3200.0 mg L^{-1}. On the third day of exposure of *A. caroliniana* to the insecticide, the occurrence of chlorosis was observed from a concentration of 100.0 mg L^{-1}. At the concentration of 800.0 mg L^{-1}, 48% of chlorotic and necrotic plants were recorded. After seven days of exposure, lethality of 62% of the plants was recorded at the concentration of 1600.0 mg L^{-1} and 100% at the concentration of 3200.0 mg L^{-1}. On the third day of exposure of *W. brasiliensis* to CPF, chlorosis was recorded in all treatments, except for the control. The highest lethality at the end of the assay (after 7 days) was 93% of the plants at the concentration of 3200.0 mg L^{-1} of CPF.

Table 2 Results of chlorpyrifos toxicity assays for neotropical aquatic organisms of different aquatic trophic levels

Species	Assay duration	IC50;7d, LC50;7d EC50;48h, LC50;48h (mg L^{-1})	Linear equation	R^2
L. minor 	7 days	1299.60 (1170.20–1443.31)*	$y=5.7343x–18.80$	0.7481
A. caroliniana 	7 days	849.72 (742.47–972.47)*	$y=6.2349x–11.21$	0.9447
W. brasiliensis 	7 days	1271.63 (1117.05–1447.59)*	$y=5.4857x–13.62$	0.8330
P. canaliculata 	48 h	30.66 (24.71–30.90)*	$y=29.32x–46.65$	0.7115
M. acanthurus 	48 h	0.002 (0.0017–0.0023)*	$y=30x–45.55$	0.8500
X. maculatus 	48 h	0.07 (0.03–0.16)*	$y=25.337x–16.91$	0.9477
H. eques 	48 h	1.65 (1.28–2.18)*	$Y=21.429x–39.68$	0.8209

*CL: Confidence limits between parentheses (95%)

The depigmentation (chlorosis) of fronds and leaves of the tested macrophytes during CPF exposure highlights the oxidative stress by antioxidant enzymatic activation and significative decrease on chlorophyll pigment content as observed on the green macroalgae *Ulva pertusa* (Schweikert & Burritt, 2012).

The OPs insecticides induce the oxidative stress, which increases the antioxidants and detox defenses. It causes the initial increase in carbonila protein levels and lipid hyperoxides, followed by a fast decrease on both markers of the oxidative stress (Schweikert & Burritt, 2012). According to Bertrand et al. (2017), the macrophyte *Potamogeton pusillus* exposed to an environmental concentration of 0.0000035 mg L^{-1} CPF for 96 h, presented oxidative stress and chlorosis on the leaves. The same authors observed that the OPs compounds are responsible for effects in the photosynthetic systems, which indicate that the CPF presence in the aquatic environment may represent low toxicity and lethality to the macrophyte but may harm their metabolism.

EC50;48h of CPF for snail was 30.66 mg L^{-1} (Table 2). This acute toxicity value indicates that the insecticide is slightly toxic ($10 < EC50;48h < 100$) for the bioindicator according to EPA classification (2021). CPF was more toxic to snails than to aquatic macrophytes. This greater toxicity may be associated with the neurotoxic mechanism of OPs in invertebrates and vertebrates.

Generally, insecticides are slightly toxic to freshwater snails. Some studies have shown a sensitivity of these organisms to insecticide concentrations between 44.50 and 87.14 mg L^{-1} in short-term toxicity assays (Carraschi et al., 2015; San Juan et al., 2020). Subchronic effects of CPF (0.004 and 0.005 mg L^{-1}) were observed in freshwater snail *Planorbarius corneus* after 14 days of exposure to the organophosphate. The insecticide caused an inhibition of the cholinesterase enzyme and reduced egg hatching and offspring survival (Rivadeneira et al., 2013).

Mollusks are the second largest group in the Animalia Kingdom and are not considered in the environmental risk assessment, mainly due to the lack of standardized protocols. Gastropods with abundant and widely distributed species in aquatic environments should be included in environmental risk and xenobiotic toxicity assessments (Tallarico, 2015). Wide distribution, bioaccumulative capacity, and ease of sampling are essential features of an ideal bioindicator (Hall et al., 2009). Snails, such as the species *P. canaliculara*, have these characteristics and are an interesting group of bioindicators of toxins in the environment (Martín et al., 2019).

The species used in the present study (*P. canaliculata*) is a freshwater gastropod native to South America (Schneiker et al., 2016). This snail is not considered in standardization norms for ecotoxicological tests. However, these organisms are in direct contact with the sediment and are exposed to contaminants that adsorb on the sediment and can be turned over and again bioavailable (Duft et al., 2003). Therefore, we recommend the use of this aquatic bioindicator in programs for monitoring and evaluating the toxicities of contaminants. According to Tallarico (2015), the tests developed with mollusks may be more susceptible to extrapolation in risk assessment programs than those based on less diverse and less numerically significant phyla.

The water quality variables observed during the snail's exposure to CPF were maintained according to the protocol established by the ABNT (2016) for the assays validation. The water conditions are as follows: 23 ± 1 °C, 110 ± 2.0 µS cm $^{-1}$, 4.5 ± 0.2 mg O_2 L^{-1}, and pH 8.0 ± 0.5.

Shrimp (*M. acanthurus*) was the most sensitive organism to CPF (LC50;48h = 0.0020 mg L^{-1}) and can be considered an excellent aquatic neotropical bioindicator of CPF contamination. The insecticide is classified as very highly toxic (LC50;48h < 0.1 mg L^{-1}) for this crustacean according to EPA (2021). After 24 h of exposure to CPF, at the highest concentrations tested (0.008 and 0.010 mg L^{-1}), there was 100% mortality of organisms. After 48 h of exposure, 100% mortality of individuals exposed to 0.004 and 0.006 mg L^{-1} of CPF was recorded. At the end of the exposure period, 33% mortality of shrimp was obtained in 0.001 mg L^{-1} of CPF.

The crustaceans most used in ecotoxicological assessments and internationally standardized are daphnids, popularly known as water fleas. CPF is more toxic to microcrustaceans *Ceriodaphnia silvestrii* (EC50; 48h = 0.000039 mg L^{-1}), *Diaphanosoma birgei* (EC50;48h = 0.000211 mg L^{-1}), *Daphnia laevis* (EC50;48h = 0.000216 mg L^{-1}), *Moina micrura* (EC50;48h = 0.000463 mg L^{-1}), and *Macrothrix flabelligera* (EC50;48h = 0.000619 mg L^{-1}) (Raymundo et al., 2019) than for the shrimp in this study (*M. acanthurus*).

CPF can cause neurotoxic effects in microcrustaceans by inhibiting the activity of acetylcholinesterase enzymes, which prolongs nerve transmission and results in neurotoxic symptoms and death at high concentrations (Maggio et al., 2021). The acute toxicity of CPF may vary depending on the species of crustacean. The crayfish *Astacus leptodactylus* is less sensitive (LC50;96h = 0.04955 mg L^{-1}) to CPF (Banaee et al., 2019) than *M. acanthurus*. Thus, our results indicate that the freshwater shrimp *M. acanthurus* is a promising bioindicator in toxicity studies with CPF and environmental monitoring due to its sensitivity to pesticide.

The water quality variables observed during the shrimp's exposure to CPF were maintained according to the protocol established by the ABNT NBR (2016) for the assays validation. The water conditions are as follows: 26 ± 1 °C, 150 ± 2.0 µS cm $^{-1}$, 4.5 ± 0.2 mg O_2 L^{-1}, and pH 8.0 ± 0.5.

The fish species evaluated in our research were also sensitive and excellent bioindicators for CPF with LC50;48h of 0.07 mg L^{-1} for *X. maculatus* and 1.65 mg L^{-1} for *H. eques* (Table 2). Thus, the insecticide was considered very highly toxic for *X. maculatus* (LC50;48h < 0.10 mg L^{-1}) and moderately toxic for *H. eques* (1.0 < LC50;48h ≤ 10.0 mg L^{-1}), according to EPA (2021).

After 48 h of exposure of *X. maculatus* to pesticide, the mortality at 0.05, 0.10, and 0.50 mg L^{-1} of CPF were 44, 55, and 89%, respectively. In fish that survived at 0.10 and 0.50 mg L^{-1}, signs of intoxication such as lethargy, erratic swimming, and loss of panting ability were observed. These signs may have occurred due to the action of organophosphates, such as CPF, in the inhibition of the acetylcholinesterase enzyme, which is responsible for the transmission of the nervous impulse. In the assay with *H. eques*, the lethality at 2.00, 2.50, 3.00, and 4.00 mg L^{-1} CPF after 48 h was 56, 78, 89, and 100%, respectively. There was no mortality at 0.50 and 1.00 mg L^{-1}. Fish surviving the test showed no signs of intoxication as seen in *X. maculatus.*

Comparing our acute toxicity results for fish with data from the literature, we found that the toxicity ranges of OPs vary according to the species studied and active ingredient (Abe et al., 2019; Barbieri & Ferreira, 2011; Jeon et al., 2016; Souza et al., 2011). Tilapia juveniles (*Oreochromis niloticus*) exposed to different concentrations of parathion methyl showed LC50;48h of 8.91 mg L^{-1} and inhibition of acetylcholinesterase activity in plasma (Barbieri & Ferreira, 2011). In the study with the insecticide diflubenzuron, the authors recorded LC50;96h of 151.98 mg L^{-1} for the fish *Poecilia reticulata* (Souza et al., 2011). The fish *O. niloticus* and *H. eques* exposed to temephos had LC50;48h of 10.91 and 7.30 mg L^{-1}, respectively (Abe et al., 2019).

*Danio rerio (*zebrafish) adults had a LC50;96h of 0.70 mg L^{-1} for CPF (Jeon et al., 2016), which is the same toxicity value found in our study for *X. maculatus*. Zebrafish has been widely used as a model organism in ecotoxicological assays to test the biological effects of different contaminants (Padilla & Glaberman, 2020). Therefore, our results suggest that *X. maculatos* fish has similar sensitivity to *D. rerio* for CPF and may be a promising fish species and alternative for toxicity tests with other environmental contaminants.

The sensitivity to CPF, adequate size, ease of cultivation, and economic importance of *H. eques* allow us to indicate this neotropical organism as an advantageous species for ecotoxicological tests. According to Aguinaga et al., 2014 and Cruz et al., 2016, *H. eques* has a great economic importance as it is a small, ornamental, and freshwater fish, becoming the main source of income for the families that use fish marketing as a means of livelihood. Being sensitive to changes in its natural environment, *H. eques* is a potential bioindicator of environmental changes caused by contaminants (AGUINAGA et al., 2014; CRUZ et al., 2016).

The water quality variables observed during the fish's exposure to pesticide were maintained according to the protocol established by the ABNT 2016 for the assays validation. The water conditions are as follows: 24 ± 1 °C, 105 ± 2.0 µS cm $^{-1}$, 6 ± 0.2 mg O_2 L^{-1}, and pH 8.5 ± 0.4.

The difference in CPF ecotoxicity for distinct aquatic trophic levels (producers, primary, and secondary consumers) found in our research shows the relevance of evaluating the effects of contaminants considering food chains and highlights the importance of studying these levels in environmental monitoring programs.

3.2 Environmental Risk Assessment

According to the calculations of risk ratios obtained in the present study and the classification standards proposed by Goktepe et al. (2004), the risk of environmental poisoning in the CPF varied according to the trophic level evaluated and the scenario considered.

For water bodies with 1 hectare, 0.3 m deep and considering the highest application rate of the CPF recommended for coffee, tomatoes, and wheat (situation 1), the insecticide presents a low risk of environmental poisoning (RQ < 0.05) for the three species of macrophytes and for the snail regardless of the percentage of the EEC that reaches the aquatic environment (even if 100% of the ECC reaches the watercourse). The low risk for these organisms is associated with lower CPF toxicity at these aquatic trophic levels.

For situation 1, the risk of CPF environmental poisoning for shrimp will always be high (RQ > 0.5) even if 3.12% of the EEC is present in surface water. However, in this situation, the risk of environmental poisoning varies from medium (0.05 < RQ < 0.5) to

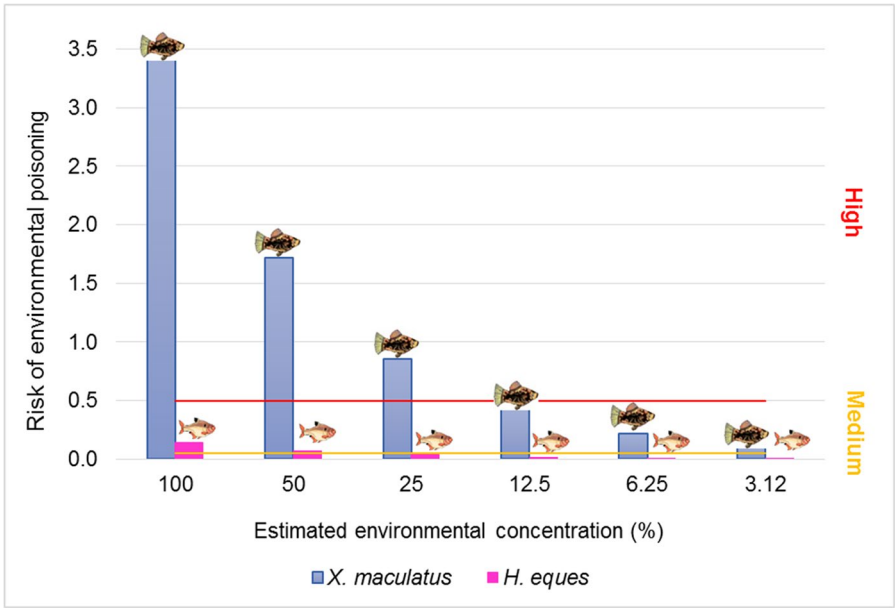


Fig. 2 Risk quotient (RQ) and the respective risk classifications of the organophosphorus chlorpyrifos, represented by the ratio between dilutions of the EEC and LC50;48h presented by the fish, considering the highest recommended dose for coffee, tomatoes, and wheat (720 g i.a. ha^{-1}) in a 1 hectare water body with 0.3 m deep

high (RQ < 0.05) for the two fish species depending on the percentage of the EEC (Fig. 2).

In situation 2 (water body with 1 hectare, 2.0 m deep and considering the highest application rate of the recommended CPF for coffee, tomatoes, and wheat), the pesticide presents a low risk of environmental poisoning (RQ < 0.05) for the three species of macrophytes and for the snail regardless of the percentage of the EEC. However, the risk of environmental poisoning *for M. acanthurus* remains high even if the EEC is at a dilution of 3.12%. For *X. maculatus,* the risk of CPF environmental poisoning is low when there is up to 6.25% of the EEC dilution. For *H. eques*, the risk will always be low regardless if we consider the 100% contribution from the EEC (Fig. 3).

One of the main effects of the persistence of pesticides in the environment is the risk of poisoning non-target organisms. Those substances present high toxicity and bioaccumulation, which may harm the entire food chain (Mello et al., 2019). Sumon et al. (2018) observed the toxic effects of CPF, diazines, and quinalphos to some organisms from distinct trophic

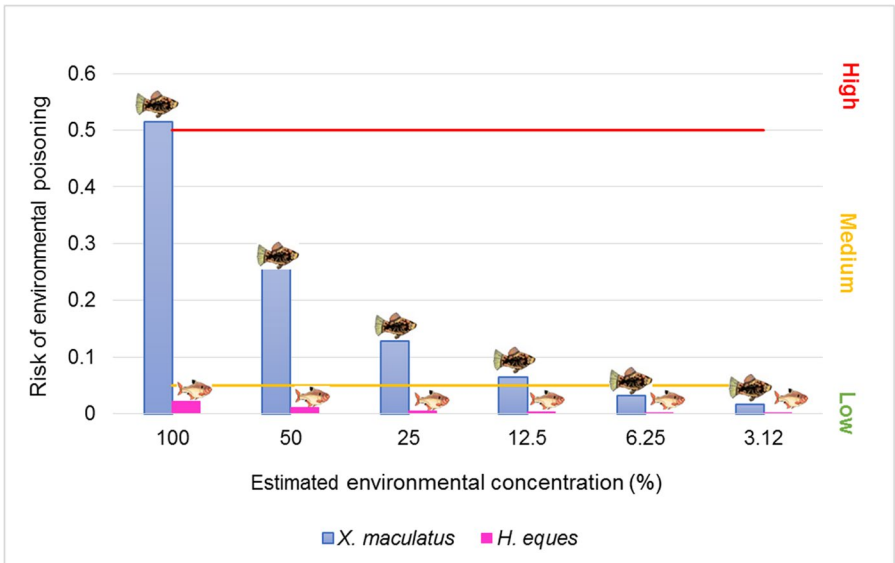


Fig. 3 Risk quotient (RQ) and the respective risk classifications of the organophosphorus chlorpyrifos, represented by the ratio between dilutions of the EEC and LC50;48h presented by the fish, considering the highest recommended dose for coffee, tomatoes, and wheat (720 g i.a. ha^{-1}) in a 1 hectare water body with 2.0 m deep

levels. The shrimps presented higher intoxication risks for OPs than the fish, as in our research.

A research conducted in Thailand found that OPs present a high environmental risk for crustaceans (Satapornvanit et al., 2004) and this indicates that these organisms are sensitive bioindicators to OPs. On surface waters in Malaysia, the environmental risk of CPF and diazinon was compared. CPF presented higher risk than diazinon, which suggests that the aquatic organisms could be exposed to dangerous levels of OPs in this environment (Wee & Aris, 2017).

Chen et al. (2020) investigated an aquatic system in Shanghai, China, in order to determine the ecological risks for organisms co-exposed to various pesticides and to determine the primary pesticide that carries the risk to aquatic organisms. They detected several pesticides and found that CPF and butachlor were the most toxic, being responsible for the increase in the overall toxicity of the pesticide mixtures. Among fish, green algae and daphnids, the latter was exposed to a greater risk in the aquatic system.

Tropical regions generally have an agriculture-based economy and rely on intensive use of pesticides to improve productivity. The presence of pesticides in the environment is a matter of concern due to the risk of pollution, especially for more vulnerable ecosystems such as inland waters. The potential risk of damage to the environment in these regions is greater because safety measures to reduce the negative impact of these contaminants are often not applied (Castillo et al., 1997). According to Cornejo et al. (2021), tropical streams usually receive higher concentrations of pesticides than watercourses from temperate regions, with greater damage to populations and ecosystems.

The presence of these contaminants in the environment does not only affect aquatic organisms. These xenobiotics can also affect human health. Thus, the removal of pesticides from water is a challenging issue and new technologies (including the use of adsorbents and catalysts) with alternative materials must be researched and used to decrease the negative impact caused by these contaminants on the environment (Bagheri et al., 2021; Salomão et al., 2019).

4 Conclusion

The sensitivity order of neotropical aquatic organisms to chlorpyrifos was *Macrobrachium acanthurus* (0.002 mg L^{-1}) > *Xiphophorus maculatus* (0.07 mg L^{-1}) > *Hyphessobrycon eques* (1.65 mg L^{-1}) > *Pomacea canaliculata* (30.66 mg L^{-1}) > *Azolla caroliniana* (849.72 mg L^{-1}) > *Wolffia brasiliensis* (1271.63 mg L^{-1}) = *Lemna minor* (1299.60 mg L^{-1}).

The shrimp was the best and more sensible bioindicator to chlorpyriphos in comparison to the other organisms and may be employed in organophosphorus insecticides monitoring programs in the aquatic environment. The fish species also presented suitable response to CPF exposure and may be included in organophosphorus toxicity complementary assessments.

We suggest the use of the crustacean *Macrobrachium acanthurus* and the fish *Xiphophorus maculatus* in ecotoxicological evaluations with organophosphate, mainly in neotropical regions, because these bioindicators have sensitivity, adequate size for laboratory tests, economic and ecological importance in aquatic ecosystems.

The risk of poisoning by chlorpyriphos may vary according to the environmental concentration of the insecticide and the exposed trophic level. Therefore, we suggest that any environmental risk assessment of contaminants should consider different trophic levels (producers, primary and secondary consumers) because organisms have different toxicity ranges and exposure levels. Furthermore, the negative impacts on survival at any level can consequently impair the survival of other organisms belonging to the food chain.

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Data Availability Data generated from the experiment.

Code Availability Not applicable.

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