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Original Article

Effects of Sodium Diclofenac on *Daphnia Magna*: Growth, Locomotion, Heartbeat and Risk Assessment

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Abstract

As the population of large urban centers grew following major world revolutions, the consumption of pharmaceuticals increased. Consequently, these substances are frequently detected in water treatment plants, surface waters, and even sediments. Sodium diclofenac (DCF), a non-steroidal anti-inflammatory drug (NSAID), can cause adverse effects on non-target organisms when released into the environment, particularly in aquatic ecosystems. This study aimed to assess the acute and chronic effects of DCF exposure on the freshwater crustacean *Daphnia magna*. The organisms were exposed to five nominal DCF concentrations for 48 hours (acute exposure) and 21 days (chronic exposure). The evaluated endpoints for acute exposure included EC_{50} (48h), NOEC, LOEC, and risk quotient (RQ). For chronic exposure, survival, body length, reproduction, and behavioral parameters (locomotion and heart rate) were analyzed. The EC_{50} (48h) value was determined as 42.47 mg L^{-1}, indicating a high environmental risk (RQ = 1.03-5.41). The LOEC value obtained in acute exposure resulted in 100% mortality within the first 10 days of prolonged exposure. The length (mm) of exposed organisms decreased with increasing concentrations. Reproduction was affected only in terms of the number of neonates at exposures above 3.75 mg/L. Locomotion and trajectory were impaired at 0.0375 mg L^{-1} onwards, while heart rate increased with rising DCF concentrations, with effects observed from the lowest concentration tested (0.00375 mg L^{-1}). This study highlights the toxicological effects of DCF on the freshwater organism *D. magna.* Given the widespread use of DCF, further studies across different trophic levels are necessary to support discussions on its regulation and potential inclusion in Brazilian and international regulatory frameworks.

Keywords: Aquatic toxicology; Freshwater; Pharmaceuticals; Diclofenac; *Daphnia magna*; Risk quotient

INTRODUCTION

With the increasing human population in large urban centers after the great world revolutions, the consumption of personal care products and pharmaceuticals increased accordingly. The presence of these substances is often detected in water treatment station effluents and wastewaters, superficial waters, and even in sediments in the range of µg L^{-1} and ng L^{-1} (Hernández-Tenorio *et al.*, 2022; Wojcieszyńska *et al.*, 2023). When ingested, pharmaceuticals can exert therapeutic or physiological effects in humans even at low doses. Once released into the environment, these compounds may pose risks to the aquatic biota, including non-target organisms, due to their persistent bioactivity (Bio & Nunes, 2020; Mikula *et al.*, 2024; Zhang *et al.*, 2021, 2022). The potential risks depends the exhibition, from the exposition time, absorption pathways, absorption levels, metabolism, and organisms' sensibility (Di Cicco *et al.*, 2021; Lee *et al.*, 2011a; Zhang *et al.*, 2022).

Sodium diclofenac (DCF) is a pharmaceutical that belongs to the class of nonsteroidal anti-inflammatory drugs (NSAIDs), with the molecular formula $C_{14}H_{11}Cl_2NO_2$. It is usually used in the form of oral tablets

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or topical gels for the treatment of pain and inflammation in humans or other animals (Fent *et al*., 2006). Since 2008, the global consumption of DCF has been growing (Van Nguyen *et al*., 2023; Zhang *et al*., 2008). The increase in demand leads to more concerns about the availability of DCF and its metabolites in the environment, attracting attention to environmental toxicology, especially in the aquatic environment (Kovacic *et al*., 2016; Nosek & Zhao, 2024).

The main action mechanism of DCF is the inhibition of the cyclooxygenase (COX) enzyme, leading to damage to critical processes in the organisms' development, such as cell proliferation and tissue differentiation (Praskova *et al*., 2014). For instance, heart malformations, reduction in body size, and spinal deformities are some of the effects caused by DCF in zebrafish (*Danio rerio*) (Chabchoubi *et al.*, 2023). Given these potential risks, understanding the metabolism and effects of DCF is important for developing mitigation strategies and improving its removal from wastewater. Its removal in treatment plants is still not effective, once DCF has been detected in various environmental matrices (water samples, urban effluent, groundwater, coastal water, hospital effluents) in the range of 0.001 - 230 μgL^{-1} (Leite *et al*., 2025).

Freshwater microcrustacean *Daphnia magna* is one of the most commonly used organisms in the investigation of the adverse effects of contaminants in the aquatic environment (Nogueira *et al*., 2022; Ogliari Bandeira *et al.*, 2025a; Puerari *et al*., 2021a, 2021b; Rossetto *et al.*, 2014; Silva *et al*., 2021; Vaz *et al.*, 2021). Therefore, *D. magna* was selected as an indicator organism of the freshwater ecosystem. As a microcrustacean, it is an essential organism for the aquatic environment and an important key to the trophic chain (ABNT, 2022). It also has several desirable advantages, including high sensitivity to environmental contaminants, ease of cultivation in the laboratory, parthenogenesis, short generation time, large hatching scale, and a wide range of endpoints for toxicological studies (Pikuda *et al*., 2019). Thus, this study aims to identify the acute and chronic effects of DCF exposure on *D. magna* and to determine if the drug causes changes in the organism's growth, heart rate, and locomotion. It also assessed the environmental ecological risk of the drug.

MATERIAL AND METHODS

Reagent preparation

Chemical standard DCF ($C_{14}H_{10}Cl_2NNaO_2$; CAS # 15307-79-6) was purchased from Sigma-Aldrich (Sigma-Aldrich Brasil Ltda, São Paulo, Brazil) with a purity of 100% (ACS grade). The preparation process involved dissolving the acquired reagents in ultrapure water (Milli-Q) to create stock solutions. A stock solution of 1 g/L DCF was prepared and subsequently diluted in ISO medium to the nominal concentrations specified by the experimental design.

Daphnia magna culture

Daphnia magna Straus 1820 (Cladocera, Crustacea) was cultivated and maintained under controlled conditions as established by the ISO 6341:2012 standard (ISO, 2012). The M4 culture media was renewed 3 times a week, and the daphnids were fed with a Desmodesmus subspicatus algal suspension at a concentration of 10^7 cells mL^{-1} as a nutrient source. The pH and dissolved oxygen values were evaluated to evaluate if it was within the range stipulated by the standard used (pH: 7.75 and DO: >3 mg L^{-1}). To ensure the quality of the culture and guarantee compliance with established standards according to current norms, sensitivity tests were conducted biweekly with the standard reference substance potassium dichromate (CAS # 7778-50-9). The sensitivity range adopted for the study was between 0.6 and 0.9 mg $L^{-1.}$ Table S1 shows the $CE50^{24h}$ sensitivity values during the testing period.

Toxicological evaluation

Acute toxicity

Acute toxicity tests were performed according to the ABNT NBR 12713:2016 (ABNT, 2016) and ISO 6341:2012 (ISO, 2012) standards, exposing neonates (< 24 h) to the test substance for a short time interval (48 h). The tests were performed in duplicate, with 10 organisms in each beaker, exposing 20 organisms by dilution at a temperature of 20 ± 2 °C, without feeding, in the dark, and without medium change. Exposure concentrations were established by examining the occurrence frequencies of DCF in different research studies, considered a thousand-fold risk of exposure scenario, according to Facin *et al.,* (2021). Preliminary tests were carried out to determine the best concentration range to be used, with tests carried out in the concentration range of 0.0023 - 230 mg L^{-1}. In this way, the nominal exposure concentrations used in this study for DCF were 12.5, 25, 37.5, 50, and 62.5 mg L^{-1}. For dilution, the ISO medium was used (ISO, 2012). Control tests were performed only with ISO medium. After 48 h, the EC_{50}, NOEC, and LOEC were estimated.

Chronic toxicity

In chronic toxicity tests, *D. magna* neonates (< 24 h) were exposed to DCF individually, based upon the recommendations of ISO 10706:2000 (ISO, 2000) and OECD 211:2012 (OECD, 2012). In a chronic test, ten

neonates were exposed to DCF for 21 days, a period that represents a significant part of the life cycle of an organism, where each exposure consisted of 10 replicates, containing 1 organism per beaker. DCF was tested at concentrations of 0.00375, 0.0375, 0.375, 3.75, and 37.5 mg L^{-1} concentrations. For dilution, the M4 medium was used in chronic tests. Control tests were performed with only M4 medium. *D. magna* was maintained under lighting with a photoperiod of 16h/8h, light/dark. Test maintenance occurred three times a week, including changing the exposition and control medium, and feeding with the microalgae Desmodesmus subspicatus (10^6 cells mL^{-1}). The exposed organisms were checked daily for the number of survivors and reproductive parameters. At the end of the exposure period (21 days), the effects on size (body length in mm), locomotion, and heart rate were analyzed.

Survival

Each exposed organism was assessed daily to determine its survival. The organism considered dead was the organism that did not move after 15 seconds. For the survival of each treatment, the longevity per replicate was presented, and then the mean and standard deviation of the treatment were calculated.

Reproductive parameters

The organisms were checked daily to determine reproductive parameters. The age at first brood was recorded as the day the exposed organism had its first offspring. The number of neonates generated was counted each time the organism produced offspring. The number of postures in each laying was assessed by counting the offspring bred in each posture. For each reproductive parameter, the parameter per replicate was presented, and then the mean and standard deviation of the treatment were calculated.

Body length

Each organism was transferred individually to a millimeter ruler to read its body length (mm). To calculate the length per treatment, the average growth per replicate was calculated to obtain the average length (mm) per concentration.

Determination of swimming behavior

The analysis of swimming behavior used was adapted from Ogliari Bandeira *et al*. (2025b) and Pikuda *et al*. (2019). After 21 days of exposure, the living organisms were individually transferred to a 10 cm diameter petri dish, filled with approximately 10-15 mL M4 medium. After an acclimation period of 2 min, each organism was recorded for 1 minute with a camera (4K, 1080p, gyro-EIS). Recorded files were processed with the Kinovea software (https://www.kinovea.org/). The distance and route traveled were evaluated. The parameter per replicate was presented, and then the mean and standard deviation of the treatment were calculated.

Determination of heart rate

The analysis of heart rate used was adapted from Bownik (2019). After 21 days, the surviving organisms were transferred individually to a microscope slide, with about 50 µL of M4 medium, where each organism was recorded for 1 minute with a camera attached to an electron microscope. The videos were analyzed using Kinovea software, where the speed of the videos was slowed down by 0.25 to facilitate counting (Fig. 1). The number of heartbeats per minute (BPM) was then evaluated. The parameter per replicate was presented, and then the mean and standard deviation of the treatment were calculated.

Statistical analysis

All data were tested for normality of distribution (Shapiro-Wilk test) and homogeneity (Bartlett test). Data were then subjected to univariate analysis of variance (ANOVA) followed by post hoc tests (Dunnett test) to compare control and treatment groups using Statistica software (version 7.0, StatSoft, Inc.). The significance criterion for all analyses was set at $p < 0.05$.

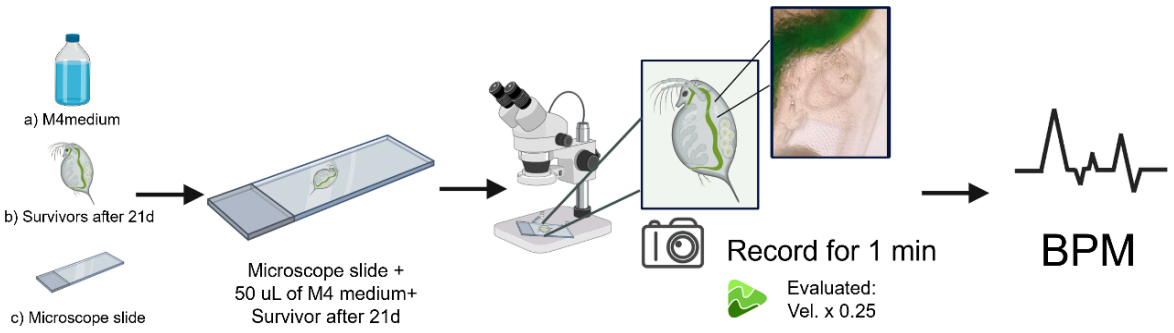

Figure 1. Step-by-step assessment of heart rate determination of *D. magna* in chronic exposures.

$EC50_{48h}$ values were calculated from nonlinear regression curves using GraphPad Prism (version 8.0.2, GraphPad Software, Inc.) to determine concentration-response curves. To evaluate the acute ecological risk assessment, the risk quotient (RQ) was calculated based on the European "Guideline on the environmental Risk Assessment of Medicinal products for human use (EMEA/CHMP/SWP/4447/00) (Ema, 2024) (Equation 1).

$$RQ = \frac{PEC}{PNEC} \quad (1)$$

Where PEC is the predicted environmental concentration detected in previous studies and various environmental matrices. PNEC is the predicted no-effect concentration (Equation 2).

$$PNEC = \frac{EC50}{AF} \quad (2)$$

Where AF is the safety factor (AF=1,000 (Blasco *et al*., 2020)). The results are expressed according to a binary classification (high and low risk): RQ>1 indicates high risk and RQ≤1 indicates low risk (Blasco *et al.*, 2020; dos Santos *et al.*, 2024; Leite *et al*., 2025; Rueanghiran *et al*., 2022).

Dunnett's test was used to determine LOEC and NOEC in acute and chronic studies. Locomotion, trajectory, and heart rate were evaluated using the software Kinovea (Version 0.9.5, Joan Charmant and contributors). Graphs, except for concentration-response curves, were created using Sigma Plot software (version 12.5, Systat Software, Inc.) with tools provided by the Bar Group.

RESULTS

Exposure of the microcrustacean *D. magna* to the anti-inflammatory DCF demonstrated that the contaminant had negative effects on different physiological parameters of the organism, with very pronounced toxicity data from the 48-hour exposure time. After 48 h of exposure, the $EC50_{48h}$ value found was 42.47 mg L^{-1} (CI = 40.40 – 44.66 mg L^{-1}; $R^2 = 0.97$), from the dose-response curve with the best fit linear correlation (Fig 2). The values of NOEC and LOEC were 25 and 37.5 mg L^{-1}, respectively. There was a significant difference ($p < 0.05$) in *D. magna* immobility by increasing DCF concentration (37.5, 50, and 62.5 mg L^{-1} treatments) when compared to control exposure.

For the risk assessment, the PNEC value obtained in this study was 0.04247 mg L^{-1}, and the RQ values are shown in Table 1. The RQ values obtained according to various environmental matrices, as shown in Table 1, are >1, showing that the drug presents an ecological risk when present in the environment. Thus, DCF exposure presents a high ecological risk under the exposure conditions of this study, based on the concentrations used in acute exposure.

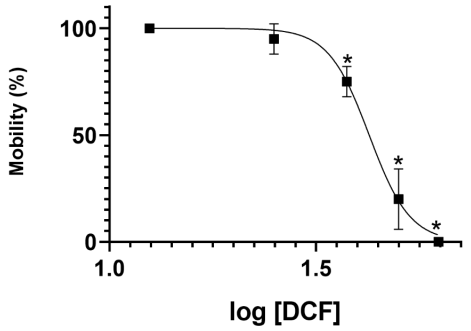

Figure 2. Dose-response curve for DCF considering *D. magna* mobility (48h). $EC50_{48h}$ values were inferred from the best fit of concentration-response curves. Data are mean ± SD. Asterisk (*) indicates significant differences between the control and the respective treatment (Dunnett's post hoc test, $p < .05$).

After chronic exposure (21 d) (Table 2), DCF reduced the number of *D. magna* survivors, with effects on 0.375 mg L^{-1} and 100% mortality at 37.5 mg L^{-1} (highest concentration tested), with mortality starting on day 1 and ending after day-10 exposure. The body length of the daphnids was affected at the lowest concentration tested (0.00375 mg L^{-1}), with effects in all the concentrations tested in this study. Reproductive parameters were only affected by the number of neonates produced at the highest concentration tested (3.75 mg L^{-1}). The number of postures and the day of the first posture did not differ when compared to the control organisms.

Table 1. Ecological risk assessment for acute exposure of *D. magna* (48 h) in DCF in various environmental matrices.

Environmental matrices	Variables			
	PEC (mg L^{-1})	Reference PEC	RQ	Inference
Influent wastewater treatment	0.16	(Ajibola *et al*., 2021)	3.91	High risk
Surface water (urban river)	0.23	(Peng *et al*., 2017)	5.41	High risk
River	0.04	(Scheurell *et al*., 2009)	1.03	High risk
Drainage sample	0.08		2.00	High risk

Note: The PNEC value obtained in this study was 0.04247 mg L^{-1}.

The number of heartbeats increased according to the increase in DCF concentration, with observed effects already at the lowest concentration tested (0.00375 mg L^{-1}) (Fig. 3a). When it comes to daphnids locomotion, from 0.0375 mg L^{-1} DCF there is a significant difference in distance traveled (in cm) (Fig 3 b). On the trajectory of locomotion (Fig. 3c), there is a decrease in the dispersion at which *D. magna* moves, with a difference from 0.0375 mg L^{-1}, as well as a decrease in the trajectory.

It is notable that at the highest concentration tested (3.75 mg L^{-1} DCF), there was a negative change in almost all parameters: survival, growth, heart rate, trajectory, and reproduction. As for the lowest concentration tested (0.00375 mg L^{-1} DCF), even though the survival rate was not affected, other parameters, such as heart rate and organism growth, were significantly different from the control test, demonstrating that even in low concentrations, the anti-inflammatory is harmful to *D. magna.*

Table 2. Results obtained after 21 d of DCF exposure in *D. magna*.

Concentration (mg L^{-1})	Survival (%)	Body length (mm)	Reproductive parameters (/female)		
			Number of neonates generated	Number of postures	Age at first brood (day)
0	100	3.85 ± 0.40	25.00 ± 4.43	3.71 ± 1.11	10.28 ± 0.95
0.00375	90	3.17 ± 0.18*	19.87 ± 2.94	3.75 ± 0.46	10.62 ± 0.74
0.0375	100	3.24 ± 0.23*	23.20 ±1.68	4.10 ± 0.31	10.70 ± 0.67
0.375	70*	3.16 ± 0.20*	22.83 ± 2.99	4.00 ± 0.89	10.33 ± 1.36
3.75	50*	3.15 ± 0.25*	10.33 ± 2.51*	3.00 ± 1.22	10.00 ± 0.00
NOEC	0.0375	< 0.00375	0.375	3.75	3.75
LOEC	0.375	0.00375	3.75	< 3.75	< 3.75

Note: Data are mean ± SD. Asterisk (*) indicates significant differences between the control and the respective treatment (Dunnett's post hoc test, $p < .05$). The organisms did not survive at the highest concentration tested (37.5mg L^{-1}).

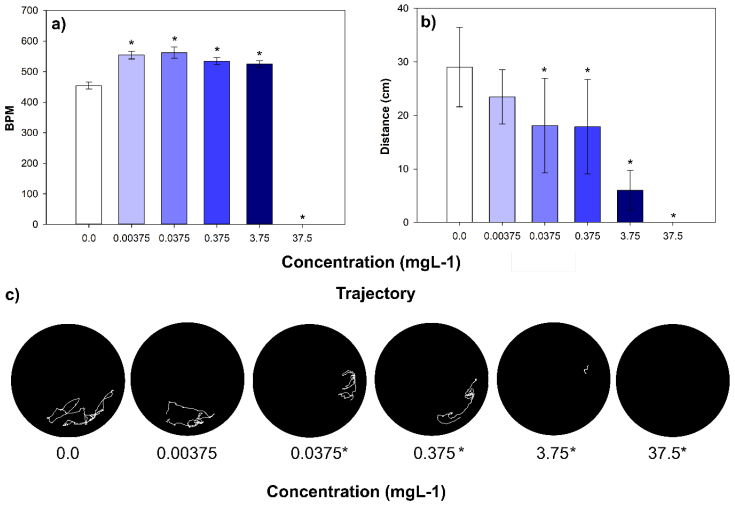

Figure 3. BPM (a), distance traveled (b), and trajectories (c) of daphnids after 21 d of exposure to DCF. Asterisk (*) indicates significant differences between the control and the respective treatment (Dunnett's post hoc test, $p < .05$). The organisms did not survive at the highest concentration tested (37.5mg L^{-1}).

DISCUSSION

DCF was selected for this study once it is largely used by the world population (Martins *et al.*, 2024; Munzhelele *et al.*, 2025; Parolini *et al.*, 2009), and due to inefficient wastewater treatment infrastructure, this pharmaceutical is already detected in several environmental matrices (Česen *et al.*, 2019; Pereira *et al.*,

2016; Rivera-Jaimes *et al*., 2018; Schmidt *et al*., 2018). The presence of DCF in aquatic environments is concerning once, due to its specific action mechanism that works even in low doses, it can cause negative effects on non-target organisms such as the aquatic biota (Hartmann *et al.*, 2021; Liu *et al.*, 2017).

In our study, significant acute and chronic toxicity of DCF to *D. magna* were demonstrated. In the mobility, DCF presented acute effects from the concentration of 37.5 mg L^{-1}, differing significantly from the control ($p < 0.05$). In line with these results, the value found for $EC50_{48h}$ corroborates the range of values already reported by Lee *et al.* (2011b) in two freshwater crustaceans (*D. magna* and Japanese medaka; 48.4 and 164.6 mg L^{-1}). The exposure of the rotifer *Brachionus calyciflorus* to DCF also resulted in a value of LC50 of 34.08 mg L^{-1} (Russo *et al.*, 2023). The EU Directive 93/67/EEC (https://eur-lex.europa.eu/eli/dir/1993/67/oj) describes EC50 values among 11 and 100 mg L^{-1} as "harmful to aquatic organisms". Hence, the EC50 values reported for DCF in this study and others demonstrate that this pharmaceutical poses a risk to aquatic organisms. The classification given by EU Directive 93/67/EEC corroborates with the risk analysis performed in this study, which concluded that the presence of DCF in environmental matrices, as said in the introduction, represents a high risk ($RQ > 1$), corroborating the findings of Leite *et al.* (2025). Even though the 93/67/EEC directive mentioned above considers the RQ value to determine risk, it is not considered a global standard. The lack of standardized regulation at a global level leads to fragmented approaches, different analysis criteria, and a lack of mandatory guidelines for these compounds (Puri *et al.*, 2023).

Regarding the results in this study, the tested concentrations in acute effects (in the range of mg L^{-1}) are hardly detected in the aquatic environment, where they are detected in the range of µg L^{-1} and ng L^{-1} (Patel *et al*., 2019). The acute exposition presented effects when exposed to concentrations in a range of mg L^{-1}, which are higher than environmentally relevant concentrations.

Pharmaceuticals that belong to the class of NSAIDs, such as DCF, can cause biochemical and molecular effects in aquatic invertebrate organisms (Joachim *et al*., 2021; Martins *et al*., 2024); still, few studies describe such effects. In the environment, exposure to DCF tends to occur over long periods; thus, this also needs to be considered.

In *D. magna*, long-term exposure to DCF in the range of mg L^{-1} (37.5 mg L^{-1}) caused total mortality, demonstrating that exposure to prolonged periods can also be harmful to the organism. Dealing with the effects of the exposition for 21 days, the reproductive parameters did not present a difference in the expositions when compared to the control. Nonetheless, considering the grow parameter, the lowest concentration tested (0.00375 mg L^{-1}), as well as the subsequent concentrations, presented differences in comparison to the control ($p < 0.05$). This effect is due to the effect of the pharmaceutical in the organism's system, with the inhibition of the COX and lipoxygenase enzymes, that act in anti-inflammatory processes, but that destabilizes important physiological processes if it experiences any changes in its activity, such as high doses of a pharmaceutical, that affect metabolism and energy allocation (Gan, 2010; Scholer *et al.*, 1986). Once the organisms allocated energy to the maintenance of the species (reproduction) as a defense response to the detriment of anabolic processes, such as optimal growth (Beauvieux *et al*., 2022). This energy allocation is a phenomenon of adaptative resilience of the species. This helps in the explanation of the effects not observed in the reproductive parameters (except for the treatment of 3.75 mg L^{-1} DCF concerning the number of neonates generated for 21 days).

Still, it was possible to observe effects in the alteration of the distance the organisms moved, as well as the loss of phototactic properties in *D. magna* due to the difference in dispersion with the increase in DCF concentration (dispersion ≠ when > 0.0375 mg L^{-1}). The alteration of the number of heartbeats is caused by the alteration of prostaglandins enzyme production, which occurs by the inhibition of the COX, which provokes a vasodilation that alters functions in the organism's systems (Simmons *et al*., 2004). Considering the stress, the exposition to DCF provokes an alteration in the vasodilation, as can be seen in the results presented in Figure 3a, where from 0.00375 mg L^{-1} DCF it was observed an increase in the BPM concerning the control ($p < 0.05$). The heartbeat is a physiological indicator of the organism, considered a direct indicator of physiological stress (Kotov, 2017; Oscar *et al.*, 2025). At the environmentally relevant concentration tested in this study (0.00375 mg L^{-1} DCF), the organism undergoes a stress that modifies its physiological capacity, which creates an alert for the potential risk of the presence of this pharmaceutical in the environment and highlights the need for regulations.

When considering environmentally relevant concentrations for the assessment of acute ecological risk, the concentrations used (0.16 - 0.23 mg L^{-1}) are within the range of concentrations tested in this study for chronic exposures, which showed negative effects, highlighting the possible adverse effects of the presence of DCF in the environment. At environmentally relevant concentrations (0.00375 mg L^{-1}), DCF effects *D. magna,* increasing heart rate, and growth were observed after 21 days of exposure. Alteration of these parameters, observed in the laboratory, can have very negative physiological and ecological impacts. The increase in the heart rate of *D.*

magna may cause an increase in energy expenditure, where other metabolic activities can be reduced. The decrease in *D. magna* growth makes it more vulnerable to predator attacks. However, with their reduced size, the organisms will provide a smaller amount of biomass and therefore a lower energy transfer. This may cause predators to have to feed on more organisms, leading to a decrease in the population and a reduction in ecosystem biodiversity. Decreased body size can also cause *D. magna* to feed less, thereby increasing the phytoplankton concentration and enabling blooms. It is therefore clear that the presence of DCF in the freshwater ecosystem at the concentration studied is worrying, because in addition to damaging *D. magna*, it can also lead to the deregulation of the entire food chain (Heath *et al*., 2014; Liu *et al*., 2024; Ripple *et al.*, 2016).

It is also important to consider global trends in DCF consumption, which indicate an increase in the production and consumption of this pharmaceutical in the coming years. This data is worrying, since it also demonstrates a possible increase in the presence of this substance in environmental matrices and its contact with organisms (Acuña *et al.*, 2020). Besides, the entrance of this contaminant into the environment is constant, which makes it a pseudo-persistent pollutant (Ort *et al*., 2009; Sathishkumar *et al*., 2020). Yet, despite the most observed effects not being on environmentally relevant concentrations of DCF in reported environmental matrices, these effects are concerning and deserve attention, once, in the aquatic environment, this chemical can interact with other contaminants. When mixed, these compounds may intensify their adverse effects (Kandaswamy *et al*., 2024; Leite *et al*., 2025). Further studies should be performed, for example, enzymatic analyses to verify the occurrence of oxidative stress in the exposure of *D. magna* to DCF, as well as in other organisms, in concentrations environmentally relevant and in prolonged exposures (chronic condition).

CONCLUSION

This study exhibited the toxicological effects of the drug DCF on the freshwater microcrustacean *D. magna.* The two highest DCF concentrations differed from the control in acute exposure regarding the organisms' immobility. The RQ values obtained according to various environmental matrices show that the drug presents an ecological risk when present in the environment, considering acute values. In chronic exposure, the three highest treatments showed effects on survival, heartbeats, and neonate' generation, which had a significant difference in all treatments. The presence of DCF in the freshwater environment is worrying because it causes physiological changes in the organism.

Knowing the existing effects of the DCF, it is necessary to conduct more studies at different trophic levels regarding the drug, to foment discussion and evaluation for inclusion in Brazilian and international regulatory standards. Incorporating combined‑risk assessments alongside toxicological studies at environmentally relevant concentrations is essential for producing comprehensive and reliable environmental evaluation data.

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CREDIT AUTHOR STATEMENT

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