Environmental Risk Assessment of Drugs in Tropical Freshwaters Using *Ceriodaphnia silvestrii* as Test Organism

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Abstract
In this study we evaluated the acute (immobility/mortality) and chronic (survival and reproduction) effects of the drugs caffeine, diclofenac sodium salt, ketoprofen, paracetamol and salicylic acid on the cladoceran *Ceriodaphnia silvestrii*. The environmental risks of these substances for tropical freshwaters were estimated from the risk quotient MEC/PNEC. Sensitivity in acute exposures varied up on the drug as follows: salicylic acid (EC₅₀ = 69.15 mg L⁻¹) < caffeine (EC₅₀ = 45.94 mg L⁻¹) < paracetamol (EC₅₀ = 34.49 mg L⁻¹) < ketoprofen (EC₅₀ = 24.84 mg L⁻¹) < diclofenac sodium salt (EC₅₀ = 14.59 mg L⁻¹). Chronic toxicity data showed negative effects of the drugs on reproduction. Paracetamol and salicylic acid caused reduction in fecundity in concentrations starting from 10 mg L⁻¹ and 35 mg L⁻¹, respectively. Ketoprofen caused total inhibition at 5 mg L⁻¹. MEC/PNEC values were relatively low for all drugs. The risk was estimated as low or insignificant, except for caffeine, whose MEC/PNEC value was greater than 1 (moderate risk).

Keywords Caffeine · Diclofenac sodium salt · Ketoprofen · Paracetamol · Salicylic acid

Introduction
The high consumption of pharmaceuticals and their occurrence in freshwater environments have raised concerns over potential adverse effects on aquatic organisms. As these organisms are constantly exposed to sublethal concentrations, toxic effects may appear in the long term (Dionísio et al. 2020). Although, the risk associated with the presence of these substances in the aquatic environment may be assessed with both short- or long-term ecotoxicological studies (Ågerstrand and Rudén 2010), the use of short-term data is important but results from chronic toxicity tests based on life cycle or partial life cycle exposures can represent in a complementary way the environmental effects of drugs and provide more comprehensive risk assessments for aquatic ecosystems (Li 2013).

The drugs selected for this study (caffeine, diclofenac sodium salt, ketoprofen, paracetamol and salicylic acid) are vastly used in human medicine. With exception of caffeine, they belong to the class of non-steroidal anti-inflammatory drugs (NSAIDs). These compounds were chosen because they are high consumed worldwide and often reported in studies to monitor the aquatic environment (Lin et al. 2008; Lin and Tsai 2009; Santos et al. 2010; Li 2013; Sotelo et al. 2014; Du et al. 2016).

Caffeine is one of the most consumed psychoactive compounds in the world (Rah et al. 2017), and has often been described as a potential marker of anthropic contamination in surface waters (Sotelo et al. 2014). This drug was among the most frequently detected compounds (95%) and in the highest concentration levels, in ng L⁻¹, in water samples from European rivers (Loos et al. 2009). Caffeine was found in almost all samples (ng L⁻¹) of surface water and municipal wastewater treatment plants in Canada (Verenitch et al. 2006). The presence of caffeine was also reported in surface waters in Brazil in concentrations of 127 µg L⁻¹ (Montagner et al. 2019) and 24.96 µg L⁻¹ (Sousa et al. 2018).

Diclofenac sodium salt was detected in 83% of water samples collected in more than 100 European rivers (Loos et al. 2009) and it is among the 10 compounds most commonly found in aquatic environments (Sotelo et al. 2014).
Santos et al. (2010) showed that among NSAIDs measured in different aquatic environments, diclofenac (ng L\(^{-1}\)) was the second most frequently detected compound found in rivers, groundwater, drinking water, and in effluents from pharmaceutical production facilities, sewage treatment plants (STP), household and hospital. In a classification created by Voogt et al. (2009) for pharmaceuticals relevant to the water cycle, diclofenac was classified in the group of high priority - Class 1.

Ketoprofen (ng L\(^{-1}\)) was the second most abundant drug in secondary effluent from Tokyo municipal sewage treatment plants (Nakada et al. 2006). This drug was detected in effluent from sewage treatment plants in Canada and Sweden in concentrations of 0.09 µg L\(^{-1}\) (Lee et al. 2005) and 0.33 µg L\(^{-1}\) (Bendz et al. 2005), respectively. Concentrations up to 239 ng L\(^{-1}\) were detected in rivers throughout Europe (Loos et al. 2009), and a maximum of 0.62 µg L\(^{-1}\) was detected in the Iguaçu River in Brazil (Ide et al. 2017).

Paracetamol has been the most widely used drug in the world and is often detected in the aquatic environment (Oliveira et al. 2018; An et al. 2009). This drug was classified as Class 2 – priority pharmaceuticals (Voogt et al. 2009), and was considered as a compound with high priority in studies for ecotoxicological assessment (Murray et al. 2010). Significant levels of paracetamol were reported in rivers in Taiwan (up to 15.7 µg L\(^{-1}\)) and in wastewater from hospitals and pharmaceutical production facilities (up to 417.5 µg L\(^{-1}\)) (Lin and Tsai 2009).

Salicylic acid (ng L\(^{-1}\)) was found in samples of surface water in Canada (Verenitch et al. 2006), and was among the acid drugs constantly detected in sewage influent and effluent samples, with a reported maximum concentration of 12.7 µg L\(^{-1}\) (Lee et al. 2005). Concentrations of up to 5.17 µg L\(^{-1}\) were reported in surface waters in southern Brazil (Ide et al. 2017). Salicylic acid was the most frequently detected drug in drinking water and in 80% of surface water samples in France (Vulliet et al. 2011).

Considering these scenarios, the presence of drugs in Brazilian water bodies may represent a potential threat to tropical aquatic life. Therefore, in this study we evaluated the acute and chronic effects of the drugs caffeine, diclofenac sodium salt, ketoprofen, paracetamol and salicylic acid on the neotropical cladoceran Ceriodaphnia silvestrii to estimate the environmental risks they may cause to tropical freshwater ecosystems. In Brazil, C. silvestrii has been recommended as the test organism to evaluate potential adverse effects of drugs in tropical freshwaters (Oliveira et al. 2018). We also evaluated the sensitivity of C. silvestrii relative to other species through species sensitivity distributions (SSDs) curves and the risk characterization was performed from hazard concentrations.

### Materials and Methods

#### Test Organism and Culture Conditions

Neonates of C. silvestrii were obtained from an in-house culture at Nucleus of Ecotoxicology and Applied Ecology (NEEA) from the Centre of Water Resource and Environmental Studies (CRHEA), University of São Paulo. The cultures were kept in ASTM reconstituted water with total hardness of 40–48 mg CaCO\(_3\) L\(^{-1}\), pH between 7.0 and 7.6, controlled temperature of 25 ± 2 °C and photoperiod 12L:12D. The organisms were fed three times a week with the chlorophyceae alga Raphidocelis subcapitata \((2.0 \times 10^5\) cells/mL per organism) and a suspension based on fermented fish feed and yeast was added as a feed supplement (0.02 mL per organism). Tests were carried out with organisms originating from a healthy matrix. They were less than 24 h old and born between the 3rd and 5th offspring.

The sensitivity of the cultures was evaluated monthly by acute tests with the reference substance sodium chloride (NaCl). All procedures were conducted as recommended by the Brazilian Association of Technical Standards (ABNT NBR 13373, 2017).

#### Drugs and Test Solutions

Caffeine (CAS: 58-08-2), diclofenac sodium salt (CAS: 15307-79-6), ketoprofen (CAS: 22071-15-4), paracetamol (CAS: 103-90-2) and salicylic acid (CAS: 69-72-7) were used in the experiments (Table S1). All drugs consisted of grade of purity 99%. The test solutions were prepared by serial dilutions from the stock solutions of the drugs in ASTM reconstituted water. Although a chemical analysis of the test solutions to confirm actual nominal concentrations of the drugs was not performed as the required equipment was not available in the laboratory, precautions were taken that justify the confidence in the drugs solutions concentrations such as using an analytical precision balance (± 10 µg Mettler AE 240 S) and the fact that volumes were measured with volumetric flasks and precision pipettes. Furthermore, according on the report of the EPI Suite™ - Estimation Program Interface v4.11 of the US EPA (2022) the half-life for drugs caffeine, diclofenac sodium salt, ketoprofen, paracetamol and salicylic acid on water are 360, 900, 360, 360 and 360 h, respectively. These data are relevant for the experimental design adopted in the present study, because acute toxicity test (48 h) and chronic (8 days, with renewed means every 48 h) were performed. Which leads us to believe, in the face of the presented, that the compounds would be present in the solutions in the nominal concentrations established.
Toxicity Test

Acute toxicity tests with C. silvestrii were performed for each drug following the procedures recommended by ABNT (NBR 12713, 2016). They were performed with five organisms (<24 h of life) in non-toxic polypropylene plastic cups containing 10 ml of the test solution and in 4 replicates per concentration. Solutions containing the organisms were maintained under static conditions at a temperature of 25 ± 2 °C, photoperiod of 12L:12D and without feeding for 48 h. The number and range of concentrations (5 to 7) was established by preliminary tests for each drug. The minimum and maximum nominal concentrations were obtained as follows: 30–80 mg L\(^{-1}\) for caffeine, 3.12–100 mg L\(^{-1}\) for diclofenac sodium salt, 3.12-50 mg L\(^{-1}\) for ketoprofen, 6.25–66.25 mg L\(^{-1}\) for paracetamol and 55–80 mg L\(^{-1}\) for salicylic acid. Acute toxicity tests were repeated three times for each drug, the immobility/mortality of C. silvestrii were checked, and the mean values of the EC\(_{50}\) (48 h), EC\(_{20}\) (48 h) and EC\(_{10}\) (48 h) were calculated.

Chronic tests with C. silvestrii followed the procedures described by ABNT (NBR 13373, 2017). They were performed in non-toxic polypropylene plastic cups containing 15 ml of the test solution added of a neonate aged between 6 and 24 h per container in 10 replicates. Every 48 h the test solutions were renewed, and the organisms were fed with the algae R. subcapitata (2.0 × 10\(^{6}\) cells/mL per organism) and a plus a suspension based on fermented fish feed and yeast (0.02 mL per organism). The solutions were maintained at a temperature of 25 ± 2 °C, photoperiod 12L:12D and light intensity of 1000 lux. Sublethal concentrations for each drug were previously established in the acute toxicity tests, i.e., concentrations that did not cause immobility/mortality. A range of five concentrations was defined for each drug in addition to the control test. The minimum and maximum nominal concentrations were obtained as follows: 2.5–32.5 mg L\(^{-1}\) for caffeine, 2–6 mg L\(^{-1}\) for diclofenac sodium salt, 0.31-5 mg L\(^{-1}\) for ketoprofen, 2.5–20 mg L\(^{-1}\) for paracetamol and 15–55 mg L\(^{-1}\) for salicylic acid. For 8 days, the organisms were checked daily for survival and reproductive state and, if neonates had been produced, they were counted and immediately removed. Results were expressed in NOEC, LOEC and EC\(_{50}\) reproductive (8 days). Parameters such as p\(H\) (Micronal B374), conductivity (ORION 145 plus) and dissolved oxygen (YSI 55-25FT) were recorded at the beginning and end of all toxicity tests, as well as at each renewal of test solutions for the chronic tests.

Statistical Analysis

EC\(_{50}\) (48 h), EC\(_{20}\) (48 h), EC\(_{10}\) (48 h), EC\(_{50}\) (8 days) and their corresponding 95% confidence intervals were determined by non-linear regression analysis, using a logistic equation to the data by the least squares method. The NOEC (no observed effect concentration) and LOEC (lowest observed effect concentration) values for the reproduction were obtained from the results of ANOVA analysis. Analyses were performed using the Statistica software version 7. The normality (Shapiro-Wilk) and homogeneity of the data (Levene) were verified and differences between treatments were assessed by analysis of variance (ANOVA). This was followed by the post-hoc Dunnett’s test in case of data that met the normality and homoscedasticity criteria and was applied to each endpoint of the chronic test to verify significant statistical differences between the groups of different drug concentrations and the control group (\(p < 0.05\)).

Environmental Risk Assessment

The environmental risk assessment for the different drugs was carried out using the risk quotient MEC/PNEC (Measured Environmental Concentration/Predicted No Effect Concentration), according to the European Medicines Agency (EMEA 2006). MEC values were obtained in the scientific literature and summarized in Table S2. PNEC values were calculated based on NOEC values applying a standard assessment factor of 100, as recommended in the Technical Guidance Document on Risk Assessment (TGD) of the European Commission (2003), using the following equation: \(\text{PNEC} = \frac{\text{NOEC}}{100}\). The worst-case scenario was considered for the risk assessment, i.e., using the maximum environmental concentrations. The risk was classified as negligible (MEC/PNEC < 0.1), low (0.1 < MEC/PNEC ≤ 1), moderate (1 < MEC/PNEC ≤ 10), and high (MEC/PNEC > 10), according to the classification described by Ågerstrand and Rudén (2010) and Komori et al. (2013).

Species Sensitivity Distribution (SSD)

The SSD for the drugs caffeine, diclofenac sodium salt and paracetamol were built using the software ETX 2.0 from the chronic toxicity values obtained in this study for the species C. silvestrii when exposed to chemicals, and also from the chronic toxicity values taken from the literature reported in US-EPA database (US-EPA 2020). The chronic toxicity data (endpoint NOEC) were used for parameters mortality or immobilization, growth, feeding and reproduction, with test duration (days) > 4. The values of hazard concentrations (HCs) for 5% and 50% of the species, i.e., HC\(_5\) and HC\(_{50}\), and their 95% confidence intervals were calculated with the
same software based on the method described by Aldenberg and Jaworska (2000).

**Results**

**Toxicity Tests**

The sensitivity of *C. silvestrii* to sodium chloride during the testing period resulted in an EC$_{50}$ (48 h) of 1.28 g L$^{-1}$ which fell within the range of the laboratory control chart (from 1.05 to 1.43 g L$^{-1}$) (Casali-Pereira et al. 2015). The mean EC$_{50}$, EC$_{20}$ and EC$_{10}$ (48 h) for *C. silvestrii* exposed to the drugs are listed in Table 1. These results showed the difference in the sensitivity of *C. silvestrii* to the drugs as follows: salicylic acid (EC$_{50}$ = 45.94 mg L$^{-1}$) < caffeine (EC$_{50}$ = 34.49 mg L$^{-1}$) < paracetamol (EC$_{50}$ = 34.49 mg L$^{-1}$) < ketoprofen (EC$_{50}$ = 24.84 mg L$^{-1}$) < diclofenac sodium salt (EC$_{50}$ = 14.59 mg L$^{-1}$). The organisms were more sensitive to diclofenac sodium salt and more tolerant to salicylic acid.

Mobility of organisms was normal in the control treatment after 48 h of exposure. All measured parameters were within the acceptable levels, except the pH value for the highest test concentration of the salicylic acid drug (pH < 5), as follows: dissolved oxygen above 6.9 mg L$^{-1}$; pH range from 7.05 to 7.58; conductivity from 131.5 to 161.4 µS cm$^{-1}$; and temperature of 25 ± 2 °C. Chronic toxicity data showed adverse effects in reproduction for ketoprofen, paracetamol and salicylic acid (p < 0.001) (Fig. 1). The LOEC, NOEC and EC$_{50}$ (8 days) values for ketoprofen were 2.5 mg L$^{-1}$, 1.25 mg L$^{-1}$ and 1.94 mg L$^{-1}$, respectively. The latter was around 12 times below the concentration recorded in the 48-hour acute toxicity tests (EC$_{50}$ = 24.84 mg L$^{-1}$). For paracetamol, the eight-day EC$_{50}$ (8.19 mg L$^{-1}$) was 4 times below the 48-hour EC$_{50}$ (34.49 mg L$^{-1}$), and the LOEC and NOEC values recorded were 10 mg L$^{-1}$ and 5 mg L$^{-1}$, respectively. The LOEC, NOEC and EC$_{50}$ values (8 days) obtained for the salicylic acid drug were 35 mg L$^{-1}$, 25 mg L$^{-1}$ and 42.9 mg L$^{-1}$, respectively. No reproductive effect was observed in the concentrations tested for the drugs caffeine (p = 0.448) and diclofenac sodium salt (p = 0.930). Thus, it was not possible to determine the LOEC and EC$_{50}$ values (8 days) for these drugs. The NOEC values for caffeine and diclofenac sodium salt were 32.5 mg L$^{-1}$ and 6 mg L$^{-1}$, respectively.

All measured parameters in the chronic toxicity tests fell within the acceptable levels as follows: dissolved oxygen above 6.7 mg L$^{-1}$; pH range of 6.3 to 7.6; conductivity of 137.4 to 169.2 µS cm$^{-1}$; and temperature of 25 ± 2 °C. No lethality of adult organisms was observed in the control treatment and in the different test concentrations for the studied drugs. The mean number of neonates produced by female in the control was greater than 15 (NBR 13373, 2017).

**Table 1** Mean values of EC$_{50}$, EC$_{20}$, EC$_{10}$ and their corresponding 95% confidence intervals (in brackets) for *Ceriodaphnia silvestrii* exposed of the drugs caffeine, diclofenac sodium salt, ketoprofen, paracetamol and salicylic acid.

| Drugs               | EC$_{50}$ – 48 h (mg L$^{-1}$) | EC$_{50}$ – 48 h (mg L$^{-1}$) | EC$_{50}$ – 48 h (mg L$^{-1}$) |
|---------------------|-------------------------------|-------------------------------|-------------------------------|
| Caffeine            | 45.94 (42.80–48.73)           | 40.65 (36.38–44.60)           | 37.84 (32.76–42.57)           |
| Diclofenac sodium salt | 14.59 (9.19–20.71)           | 8.56 (4.36–12.55)            | 6.29 (2.59–10.56)             |
| Ketoprofen          | 24.84 (16.14–31.72)           | 19.03 (10.23–25.36)          | 14.59 (9.19–20.71)            |
| Paracetamol         | 34.49 (29.73–39.98)           | 26.16 (19.06–31.01)          | 22.33 (14.28–29.21)           |
| Salicylic acid      | 69.15 (63.76–73.75)           | 61.20 (54.97–67.11)          | 57.63 (49.93–65.13)           |

Fig. 1 Effects on reproduction of *Ceriodaphnia silvestrii* exposed for 8 days to diclofenac sodium salt (A), paracetamol (B), caffeine (C), salicylic acid (D) and ketoprofen (E). The error bars correspond to the standard deviation and (*) indicates statistically significant differences between groups in different drug concentration and control (Dunnett test, p < 0.05).
Table 2 Values of hazard concentrations at 5% (HC₅) and 50% (HC₅₀) for organisms evaluated in the species sensitivity distribution curves (SSD) in exposures to caffeine, diclofenac sodium salt and paracetamol. * Lower Limit. ** Upper Limit

|                  | LL HC₅          | UL HC₅          | LL HC₅₀ | UL HC₅₀ |
|------------------|-----------------|-----------------|---------|---------|
| Caffeine         | 8.29 × 10⁻¹⁰    | 0.005           | 0.006   | 56.83   |
| DIC               | 3.98 × 10⁻⁵     | 0.80            | 2.78    | 6.08    |
| Paracetamol      | 0.007           | 0.09            | 0.95    | 10.87   |
| LL* HC₅          | 0.06            | 0.80            | 1.27    | 6.08    |
| **UL** HC₅       | 0.32            | 0.80            | 2.78    | 6.08    |

DIC, diclofenac sodium salt; LL*, lower limit; **UL**, upper limit

Environmental Risk Assessment

The environmental risk for the drugs evaluated in this study was classified as negligible (MEC/PNEC ≤ 0.1) for most data of drug concentrations in tropical fresh surface waters reported in the literature. Only for a few higher environmental concentrations reported for caffeine and paracetamol (Table S2), their environmental risk was classified as low or moderate. The MEC/PNEC ratio determined for paracetamol concentrations (Campanha et al. 2015; Montagner et al. 2019) were between 0.1 and 1 (low risk). The MEC/PNEC maximum ratio obtained for caffeine was greater than 1 (moderate risk), based on data obtained by Ferreira (2005), who reported the maximum caffeine concentration (357.0 µg L⁻¹) in an urban river in Rio de Janeiro, Brazil. For diclofenac sodium salt, ketoprofen and salicylic acid, the MEC/PNEC ratios determined for the reported concentrations resulted lower than 0.1 (negligible risk).

SSD

When analyzing the SSD curve constructed for diclofenac sodium salt (Fig. 2A), it can be observed that neotropical cladoceran *C. silvestrii* presented low sensitivity when compared to the evaluated species, being more sensitive than the cladocerans *Daphnia magna* and *Moina macrocopa*. Regarding paracetamol, *C. silvestrii* was the most sensitive invertebrate species after mussel, *Mytilus galloprovincialis*. The fish *D. rerio* and amphibians *Bufo americanus* and *Lithobates pipiens* are species with greater sensitivity (Fig. 2B). For caffeine, *C. silvestrii* presented moderate sensitivity to other species (Fig. 2C). However, few data were found available in the literature of chronic effects of caffeine on different test organisms.

SSD curves were not constructed for salicylic acid due to the limited data available in the literature. They were found only for *Daphnia longispina* and *D. magna* species (> 10 mg L⁻¹) (Marques et al. 2004). For ketoprofen, no chronic toxicity (NOEC) values were found, which highlights the importance of this study, as it was presented for the first time, the evaluation of its toxicity.

HC₅ and HC₅₀ values with their respective 95% confidence intervals (95% CI) obtained from the SSD curves constructed with the toxicity data of caffeine, diclofenac sodium salt and paracetamol are presented in Table 2. The value of HC₅ (95% CI) obtained from the exposures to diclofenac sodium salt indicated that the reproduction of the cladoceran *C. silvestrii* and the parameters mortality or immobilization, growth, feeding or reproduction of the other species selected to assess the chronic toxicity (endpoint NOEC) would not be affected in concentrations ranging from 0.75 to 24.06 mg L⁻¹ of diclofenac sodium salt (Fig. 2A; Table 2). For exposure to paracetamol, also considering the confidence interval (lower limit of 0.007 mg L⁻¹) all species would also be protected, including the most sensitive, *D. rerio*, with NOEC of 0.07 mg L⁻¹ (Fig. 2B; Table 2). The same protection factor pattern in relation to the value of HC₅ (95% CI) for exposure to caffeine was observed, indicating that the species would not be affected in concentrations ranging from 0.0006 to 100 mg L⁻¹ of caffeine (Fig. 2C; Table 2).

![Fig. 2](Image) Species sensitivity distribution (SSD) constructed based on NOEC values for diclofenac sodium salt (A), paracetamol (B) and caffeine (C) obtained in this study for *Ceriodaphnia silvestrii* (in bold) and from literature for other species.
Discussion

The mean EC$_{50}$ (48 h) value for *C. silvestrii* exposed to caffeine was 45.94 mg L$^{-1}$. Studies by Di Lorenzo et al. (2019) and Bang et al. (2015) recorded EC$_{50}$ values (48 h) of 395 mg L$^{-1}$ and 445.3 mg L$^{-1}$ for *D. magna*, respectively, while Li (2013) recorded a LC$_{50}$ (96 h) of 377.6 mg L$^{-1}$ for *Dugesia japonica*. Comparing these data with the EC$_{50}$ (48 h) obtained in the present study, *C. silvestrii* is among these species the most sensitive to caffeine, with regard to acute toxicity.

The results of the chronic toxicity tests showed no impairment on the survival and reproduction of *C. silvestrii* under tested conditions. However, previous studies have shown adverse effects to other aquatic biota organisms, especially to fish, such as, negative effects on zebra fish (*D. rerio*) (Rah et al. 2017) and for fish embryos of the species *Oryzias latipes* (Lee and Wang 2015). Furthermore, as demonstrated by Di Lorenzo et al. (2019), caffeine represented an environmental risk in all water bodies investigated in Spain. In our study, caffeine was the only drug that reached a moderate level of environmental risk, due to the greater MEC (357 µg L$^{-1}$). Although for most available data the risk was classified as low and insignificant.

For diclofenac sodium salt, in the acute test, *C. silvestrii* was more sensitive (EC$_{50}$ 48 h = 14.59 mg L$^{-1}$) in comparison to the other drugs tested. Cleuvers (2003) and Cleuvers (2004) also showed that the cladoceran *D. magna*, the algae *Desmodesmus subspicatus* and the macrophyte *Lemma minor* were more sensitive to diclofenac than to other anti-inflammatories, such as acetysalicylic acid, ibuprofen and naproxene, in the evaluation of acute toxicity. Therefore, our results support findings by others studies, showing that diclofenac is probably the most acutely toxic compound within the NSAID class (Fent et al. 2006).

Chronic toxicity results showed no implications on the survival and reproduction of *C. silvestrii* exposed to sublethal diclofenac sodium salt concentrations considering an exposure period of eight days. This was also observed by Oliveira et al. (2018) for the same species at concentrations of 0.0625 to 2 mg L$^{-1}$ and same exposure time. The same response was also observed for *D. magna* at 21 days of exposure at concentrations of 29.5 to 72 mg L$^{-1}$ (Oliveira et al. 2015a). However, the exposure of *D. magna* to lower concentrations of diclofenac (0.0005 to 7.2 mg L$^{-1}$) over a period of 48 h caused adverse effects on neuron regulation biomarkers such as total cholinesterases (ChEs) by a significant decrease of ChE activity in concentrations of 0.0005 mg L$^{-1}$ and 7.2 mg L$^{-1}$, and on the defense of the enzymatic oxidative stress, e.g., by a decrease in the activity of glutathione peroxidase selenium-dependent (Se-GPx) in concentrations of 0.0005 mg L$^{-1}$- 0.5 mg L$^{-1}$ (Oliveira et al. 2015b).

The mean EC$_{50}$ (48 h) value for ketoprofen was 24.84 mg L$^{-1}$. As this concentration is over thousandfold bigger than the levels of ketoprofen registered, for instance, in effluent from sewage treatment plants in Canada (0.09 µg L$^{-1}$) (Lee et al. 2005), and in rivers in Europe (239 ng L$^{-1}$) (Loos et al. 2009) and Brazil (0.62 µg L$^{-1}$) (Ide et al. 2017), it is very unlikely to observe acute effects of this drug on the aquatic biota.

The results of chronic toxicity tests showed a high sensitivity of *C. silvestrii* to ketoprofen. The reproductive EC$_{50}$ (8 days) of 1.94 mg L$^{-1}$ for ketoprofen was the lowest among the drugs evaluated. A significant reduction in fecundity by 2.5 mg L$^{-1}$ (LOEC) and total inhibition in neonate production by 5 mg L$^{-1}$ was also observed. EC$_{50}$ values (8 days) for ketoprofen were on average 12 times lower than EC$_{50}$ values (48 h) for the acute toxicity tests. Thus, as stated by Du et al. (2016), it is likely that the degree of toxic effects of pharmaceuticals to this organism may depend not only on the drug concentrations in the aquatic environment, but also on other factors such as the time of exposure.

The mean EC$_{50}$ (48 h) for *C. silvestrii* exposed to paracetamol in the present study was 34.49 mg L$^{-1}$ and the sensitivity to the drug was very close to that observed in the studies by Oliveira et al. (2018) for the same species (EC$_{50}$, 48 h = 40.3 mg L$^{-1}$). For *D. magna*, EC$_{50}$ (48 h) values of 30.1 mg L$^{-1}$ (Kim et al. 2007) and 50 mg L$^{-1}$ (Henschel et al. 1997) have been described. Acute effects have also been reported on the *Oryzias latipes* (LC$_{50}$ = 160 mg L$^{-1}$) (Kim et al. 2007) and the *Dugesia japonica* (LC$_{50}$ = 150.8 mg L$^{-1}$) (Li 2013).

The results of chronic toxicity tests with *C. silvestrii* exposed to paracetamol showed toxicity in the reproduction of the organisms with the LOEC value of 10 mg L$^{-1}$ and reproductive EC$_{50}$ (8 days) of 8.19 mg L$^{-1}$. Studies in the literature reported the occurrence of toxic effects on fecundity and rate of population growth in *C. silvestrii* exposed to 2 mg L$^{-1}$ of paracetamol for 8 days (Oliveira et al. 2018), and in *D. magna* exposed to concentrations of 0.01 mg L$^{-1}$ and 1 mg L$^{-1}$ (48 h) significant decrease in ChE and Se-GPx activities, respectively (Oliveira et al. 2015b). Comparing these results with the levels at which paracetamol has been detected in natural waters in the USA (10 µg L$^{-1}$) (Kolpin et al. 2002), Brazil (above 13 µg L$^{-1}$) (Campanha et al. 2015), Taiwan (above 100 µg L$^{-1}$) (Lin et al. 2008) and UK (above 69 µg L$^{-1}$) (Roberts and Thomas 2006), we suggest that there is a risk for aquatic organisms. Kim et al. (2007) reported possible environmental effects of paracetamol on the aquatic environment and advised further investigations. Thus, although the environmental risk for paracetamol in this study was classified from low to insignificant, we
agree that investigations to evaluate the long-term adverse effects of paracetamol on the aquatic ecosystems must be conducted. Salicylic acid was the least acutely toxic drug for *C. silvestrii*, with an EC$_{50}$ (48 h) of 69.15 mg L$^{-1}$, about 2 to 5 times less than the observed toxicity for the drugs diclofenac sodium salt, ketoprofen and paracetamol. Henschel et al. (1997) reported for salicylic acid EC$_{50}$ (72 h) > 100 mg L$^{-1}$ for *Scenedesmus subspicatus* algae, EC$_{50}$ (24 h) of 118 mg L$^{-1}$ for *D. magna* and LC$_{50}$ (48 h) of 37 mg L$^{-1}$ for zebra fish embryos (*D. rerio*). Considering the concentrations necessary to cause acute effects, we do not expect that the registered environmental levels of salicylic acid have a direct impact on the survival of aquatic organisms. Although reproductive toxicity was observed in the chronic exposures, *C. silvestrii* had a lower sensitivity to salicylic acid. Chronic toxicity tests performed by Marques et al. (2004) with *D. magna* showed no reproductive impairment after 21 days of exposure to concentrations of 1–10 mg L$^{-1}$ of salicylic acid.

In conclusion, although the drug concentrations that caused immobility/mortality and adverse effects to *C. silvestrii* in the present study were higher than their environmental levels, the assays consisted of exposures to a single drug with specific exposure times and endpoints. Therefore, the potential risk to tropical aquatic biota in the long-term and in exposure scenarios using multiple stressors remains to be investigated, as toxicity tests with the complete life cycle of the species, multigenerational tests and tests with mixtures. In addition, SSD curves allowed the recognition of the absence of data in the literature for non-target invertebrate organisms in aquatic biota when exposed to drugs, especially, ketoprofen and salicylic acid.

**Supplementary Information** The online version contains supplementary material available at [https://doi.org/10.1007/s00128-023-03733-z](https://doi.org/10.1007/s00128-023-03733-z).

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**Declarations**

**Conflict of Interest** The authors declare that they have no conflict of interest.

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