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Bisphenol A Acute Toxicity to the Macroinvertebrate *Chironomus sancticaroli* (Diptera, Chironomidae) and Sensitivity Analysis of the Species to BPA Analogs

Toxicidade aguda do bisfenol A (BPA) para o macroinvertebrado *Chironomus sancticaroli* (Diptera, Chironomidae) e análise de sensibilidade da espécie para análogos do BPA

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ABSTRACT

Due to concerns about the potential health risks associated with bisphenol A (BPA), several alternatives have been developed. The most common BPA analogs include bisphenol F (BPF) and bisphenol S (BPS). The objective of this study was to evaluate the sensitivity of the tropical macroinvertebrate *Chironomus sancticaroli* to BPA by performing acute toxicity tests. In addition, species sensitivity distribution (SSD) curves were constructed to compare the sensitivity of *C. sancticaroli* to different taxonomic groups when exposed to BPA, as well as to evaluate the sensitivity of varying freshwater organisms exposed to BPF and BPS, addressing the SSD analysis. The Predicted No-Effect-Concentrations (PNECs) values of bisphenols were calculated. The average value of the 48-h EC50 of BPA based on the measured concentration for the first instar larva of *C. sancticaroli* was 6.71 mg L^{-1}. The SSD curve of BPA demonstrated that *C. sancticaroli* presented an intermediate sensitivity to BPA when compared to two other chironomid species commonly used in toxicity tests. As demonstrated by PNECS values developed in the present study, the order of toxicity based on all species was BPA > BPF > BPS. This study highlights the need to expand data on the acute and chronic toxicity of BPA and its analogs for tropical freshwater biota to estimate potential effects of bisphenols.

Keywords: emerging contaminants; ecotoxicology; hazardous concentration; tropics.

RESUMO

Em razão de preocupações sobre os riscos potenciais do bisfenol A (BPA) para a saúde humana e ambiental, vários análogos foram desenvolvidos como alternativas. Os análogos mais comuns do BPA incluem o bisfenol F (BPF) e bisfenol S (BPS). O primeiro objetivo deste estudo foi avaliar a sensibilidade do macroinvertebrado tropical *Chironomus sancticaroli* ao bisfenol A realizando testes de toxicidade aguda. Além disso, curvas de distribuição de sensibilidade de espécies (SSD) foram construídas para comparar a sensibilidade de *C. sancticaroli* a diferentes grupos taxonômicos quando expostos ao BPA, bem como para avaliar a sensibilidade de diferentes organismos de água doce expostos ao BPF e BPS, abordando a análise SSD. Os valores de concentrações previstas sem efeito (PNEC) de bisfenóis foram calculados. O valor médio da EC50 48h do BPA com base na concentração medida de BPA para a larva de primeiro ínstar de *C. sancticaroli* foi de 6,71 mg L^{-1}. A curva SSD do BPA demonstrou que *C. sancticaroli* apresentou sensibilidade intermediária ao BPA quando comparado a duas outras espécies de quironomídeos comumente usadas em testes de toxicidade. Conforme demonstrado pelos valores de PNEC para bisfenóis calculados no presente estudo, a ordem de toxicidade com base em todas as espécies foi BPA > BPF > BPS. Este estudo destaca a necessidade de ampliar os dados sobre a toxicidade aguda e crônica do BPA e de seus análogos para biota aquática de ecossistemas tropicais, a fim de estimar os efeitos potenciais dos bisfenóis.

Palavras-chave: concentração de risco; contaminantes emergentes; ecotoxicologia; trópicos.

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Introduction

Bisphenol A (BPA) is an industrial chemical used primarily in the production of plastics and resins. BPA is a key component in polycarbonate plastics, which are found in products such as water bottles, food containers, and eyewear lenses. Additionally, BPA is used in epoxy resins that coat the inside of metal cans, helping to prevent corrosion and contamination of food and beverages. However, concerns have been raised about BPA's potential effects on human health. Some studies suggest that BPA may disrupt the endocrine system, leading to possible health risks. As a consequence, many manufacturers have started producing BPA-free alternatives, especially in food-related packaging and children's products (Ramakrishna et al., 2022).

Due to concerns about the potential health risks of BPA, several analogs have been developed as alternatives. The most common BPA analogs include bisphenol S (BPS) and bisphenol F (BPF), both of which share a similar chemical structure and are used in various industrial applications. However, due to their ubiquity in ecosystems, potential effects of endocrine disruptors and associated ecological risks, bisphenol analogs (BPs) have raised concerns in recent decades (Gao et al., 2023).

BPF is an industrial chemical belonging to the bisphenol family, structurally similar to BPA. BPF has been used as an alternative to BPA in various applications, including the production of epoxy resins, coatings, adhesives, and certain plastics. BPF has been detected in environmental samples, food, and human biological fluids, indicating widespread exposure (Han et al., 2021). However, recent scientific studies have raised concerns about the safety of BPF. The study by Rochester and Bolden (2015) emphasized the need for further research to understand the potential health risks associated with BPF exposure. Ullah et al. (2019), investigating the effects of BPF on male rats' reproductive health, revealed that BPF exposure antagonized male reproductive hormones and induced alterations in testicular morphology, suggesting potential risks to male fertility. Tiwari et al. (2024) investigated the impact of BPF on neural stem cells and cognitive functions in rats. Regarding environmental monitoring, some studies describe BPF as an environmental pollutant of significant concern for both human and environmental health, particularly for aquatic organisms (Yang et al., 2019).

BPS has been used as a substitute for BPA in various consumer products due to health concerns associated with BPA. However, research has demonstrated that BPS can act as an endocrine disruptor, interfering with hormonal functions (Thoene et al., 2020). Ferguson et al. (2019) revealed that BPS could rapidly depress heart function through estrogen receptor pathways. According to the authors, this finding raises concerns about the potential cardiovascular risks of BPS, especially considering its widespread use as a BPA alternative. The Office of Environmental Health Hazard Assessment (OEHHA, 2024) published a document presenting findings that suggest BPS may adversely affect male reproductive health and cause female reproductive toxicity (OEHHA, 2024).

As a potential environmental endocrine disruptor, BPA has been extensively studied for its potential toxicity to several representatives of the aquatic community. Studies describing the toxicity of BPF and BPS have only limited data for freshwater aquatic species (Liu et al., 2021; Razak et al., 2023). Additionally, studies on the toxicity of BPA and its analogs are relatively limited for tropical aquatic species. *Chironomus sancticaroli* Strixino & Strixino, 1981 (Insecta: Diptera) is an aquatic macroinvertebrate belonging to the Chironomidae family described from specimens collected in the municipality of São Carlos (São Paulo, Brazil) (Corbi, 2021). *C. sancticaroli* is a benthic species widely distributed in Brazilian aquatic systems, easy to grow and maintain in the laboratory (Brovini et al., 2023), and has been used as an organism in several ecotoxicological investigations (Dornfeld et al., 2019; Prado et al., 2023). To our knowledge, there are no studies comparing the sensitivity of *C. sancticaroli* to BPA compared to other freshwater species.

Species sensitivity distributions (SSDs) are a useful tool for assessing the variation in sensitivity among species, and SSDs are applied in risk assessments of chemical agents in aquatic ecosystems (Belanger et al., 2016). These curves are used to calculate the hazardous concentration (HC), that is, the concentration of the chemical agent that affects a given proportion (%) of species. The HCs derived from SSD serve as a reference for establishing safe limits for exposure to toxic substances. The Predicted No-Effect-Concentrations (PNECs) values of bisphenols were calculated using SSD methods and an appropriate assessment factor (European Commission Joint Research Centre, 2003). These values guide the development of regulatory standards and policies, ensuring the protection of the majority of species.

The first objective of this study was to evaluate the sensitivity of the tropical macroinvertebrate *C. sancticaroli* to BPA by performing acute toxicity tests. In addition, SSD curves were constructed to compare the sensitivity of *C. sancticaroli* to different taxonomic groups (bacteria, microalgae, cnidarians, arthropods, molluscs, and fish) when exposed to BPA, as well as to evaluate the sensitivity of different freshwater organisms exposed to BPS and BPF, addressing the SSD analysis. This is the first research to present HC and PNEC values based on SSDs for BPA, BPF, and BPS for aquatic organisms belonging to different trophic levels.

Methodology

BPA toxicity tests

The cultures of the *C. sancticaroli* species were kept in plastic trays containing 1 cm of white quartz sand (Sigma, 50 MESH) and reconstituted water, covered by nylon cages. Tetramin® flocculated fish feed was used as food for larvae weekly at a rate of 0.04 mg L^{-1}. Cultures of *C. sancticaroli* were maintained under the controlled conditions of hardness (14 mg L^{-1} for $CaCO_3$), pH (between 6 and 8), temperature of 25±2°C, 12-h photoperiod, and constant aeration (Dornfeld et al., 2019; Corbi, 2021). The sensitivity of the chironomid cultures was evaluated by reference toxicant testing with potassium chloride. The response of the larvae to the reference KCl at concentrations of 2, 3, 4, 6, and 8 g L^{-1} was tested. After 96 h of exposure of larvae to the reference substance, the number of alive organisms was analyzed. The sensitivity test conditions were similar to those maintained during the cultivation of the organisms (Dornfeld et al., 2019). The cultures of the organisms have been kept in stock cultures at the Biology Laboratory of the Department of Environmental Engineer, UNESP (Brazil).

In the acute toxicity tests, the first instar larvae of *C. sancticaroli* were exposed to nominal BPA concentrations of 0, 1, 2, 4, 8, and 16 mg L^{-1} to assess the effect of the compound on larval survival. These concentrations were chosen based on preliminary studies of Spadoto (2013). A commercial BPA of high purity (purity ≥99%, Sigma-Aldrich) was used to prepare the test solutions. The acute toxicity tests were carried out in four replicates, with five larvae, during 48 h of exposure. The test system was static, and the larvae were not fed (OECD, 2011). The test conditions were maintained at 25°C with a 12-h photoperiod. Ten acute toxicity tests with BPA were carried out with *C. sancticaroli* larvae for the average EC50 value of the compound (Lima et al., 2019). Acute toxicity tests were carried out between May 2023 and June 2024. Based on these results, the median effective concentration of BPA that causes immobility in 50% of the test organisms (EC50) was determined. The results of the toxicity test with KCL and BPA were analyzed using the Trimmed Spearman-Karber method and expressed as 96-h LC50 and 48-h EC50 values, respectively (Hamilton et al., 1977). The value of BPA was checked using a GENESYSTM 50 UV-Vis spectrophotometer at a wavelength of 276 nm.

Ecotoxicity data collection

Ecotoxicity data of three bisphenols, namely BPA, BPF, and BPS, were collected from various reputable databases and publications, including the ECOTOX database (http://cfpub.epa.gov/ecotox/), Scopus (https://www.scopus.com/), Web of Science (https://www.webofscience.com) and PubMed (https://pubmed.ncbi.nlm.nih.gov/). The search terms used in the retrieval process included "bisphenol A," "bisphenol F," "bisphenol S," "toxicity," "freshwater," "invertebrate," "plants," "insect," "crustaceans," "molluscs," "fish," "cnidarian," "bacteria," and "algae."

The data retrieved from the literature were screened and curated based on the following selection criteria:

- Toxicity Metrics: Only studies reporting acute toxicity indicators such as LC50 (Lethal Concentration 50%), EC50 (Effective Concentration 50%), and IC50 (Inhibitory Concentration 50%) of different taxonomic groups were included. Acute toxicity data were more readily available to construct SSD curves for bisphenols, so chronic toxicity data were not included.
- Life Stages: When toxicity data were available for multiple life stages of a species, results from the most sensitive life stage were selected.
- Outlier Exclusion: If toxicity data for a single species exhibited a significant variation (greater than 10-fold difference), outliers were excluded from the dataset to ensure consistency.
- Experimental Conditions and Scientific Standards: Studies were included only in freshwater and were prioritized if they provided information about controlled physicochemical parameters, ensuring minimal interference from external factors. Studies meeting rigorous scientific testing principles, such as appropriate control group designs and quality control measures, were prioritized.

This systematic methodology ensured the reliability and relevance of the selected toxicity data for BPA and its analogs (Table 1) in environmental impact assessments.

Methodology for data analysis using species sensitivity distributions goodness-of-fit and model selection

The normality of residuals for each SSD dataset was assessed using the Anderson-Darling (AD) test, the Kolmogorov-Smirnov (KS) test, and the Cramer-von Mises test. The Akaike Information Criterion with corrections (AICc) was calculated to compare model fit quality, with the best-fit model being the one with the lowest AICc value that also satisfied normality and goodness-of-fit criteria.

For each selected model, the HC5 (hazardous concentration 5% of the species) and HC50 (hazardous concentration 50% of the species) and their 95% confidence intervals (CIs) were determined. These metrics represent the concentration thresholds below which 95 and 50% of species, respectively, are expected to be protected (Lima et al., 2019). The PNECS values of BPA, BPF, and BPS were calculated using SSD methods and an appropriate assessment factor (European Commission Joint Research Centre, 2003; Razak et al., 2023). All computations were performed using ETX 2.3 software, which facilitated the SSD curve fitting, calculation of HC values, and associated statistical tests. To construct reliable SSDs, datasets required at least seven data points (N≥10) and representation from diverse taxonomic groups. By combining these rigorous procedures, the methodology ensures robust derivation of SSDs, facilitating accurate assessment of ecological risks and the establishment of safe environmental concentration thresholds.

Table 1 – Identification and nomenclature of bisphenols.

Chemical name	IUPAC name	CAS number
Bisphenol A (BPA)	4-[2-(4-hydroxyphenyl)propan-2-yl]phenol	80-05-7
Bisphenol F (BPF)	4-[(4-hydroxyphenyl)methyl]phenol	620-92-8
Bisphenol S (BPS)	4-(4-hydroxyphenyl)sulfonylphenol	80-09-1

Results and Discussion

The average 96-h LC50 value of KCl for larvae of *C. sancticaroli* was 5.73 g L^{-1}, with a sensitivity range between 4.12 and 7.34 g L^{-1}. The 96-h LC50 values obtained for the species in this research are within the sensitivity range established for the species by Dornfeld et al. (2019), demonstrating adequate physiological conditions of the cultures before subjecting them to acute toxicity tests with BPA.

The average values of the measured concentrations of BPA used in the acute toxicity tests were 0 (control treatment); 1.0, 2.0, 4.0, 8.0, and 15.45 mg L^{-1}. At the end of the acute toxicity tests, the survival rate of the larvae in the control treatment was over 90%, meeting the performance required by the OECD (2011). After carrying out ten acute toxicity tests, an increase in larval immobility was observed with the increase in BPA concentration (Figure 1). The average value of the 48-h EC50 of BPA based on the measured concentration of BPA for the first instar larva of *C. sancticaroli* was 6.71 mg L^{-1}, and the 95% confidence interval was between 4.53 and 9.98 mg L^{-1} of BPA.

The SSD curves for BPA, BPF, and BPS (Figures 2, 3, and 4) were prepared based on data obtained in the acute ecotoxicological tests of this research and in the literature, as detailed in Supplementary Material 1. Based on the literature review, 43 scientific articles on the acute toxicity of BPA and 10 articles on the acute toxicity of BPS and BPF for freshwater species were reviewed. In this sense, we emphasize the need to expand studies on the toxicity of BPS and BPF to a greater number of freshwater species. The SSD curve of BPA demonstrated that *C. sancticaroli* presented an intermediate sensitivity to BPA when compared to two other chironomid species commonly used in toxicity tests, with the sensitivity of *C. sancticaroli* being lower than that observed for *Chironomus tentans* and higher than that of *Chironomus riparius*. *C. sancticaroli* was more sensitive to BPA than standardized microalgae (*Scenedesmus quadricauda*, *Desmodesmus subspicatus*, *Schoenoplectus acutus*, and *Chlorella vulgaris*), cladocerans (*Daphnia magna* and *Daphnia similis*), and fish (*Danio rerio* and *Oncorhynchus mykiss*) commonly used in toxicity tests (Campbell et al., 2022; Turan and Arslan, 2023), confirming the feasibility of using this macroinvertebrate as a test species in evaluations of the BPA effects in tropical regions.

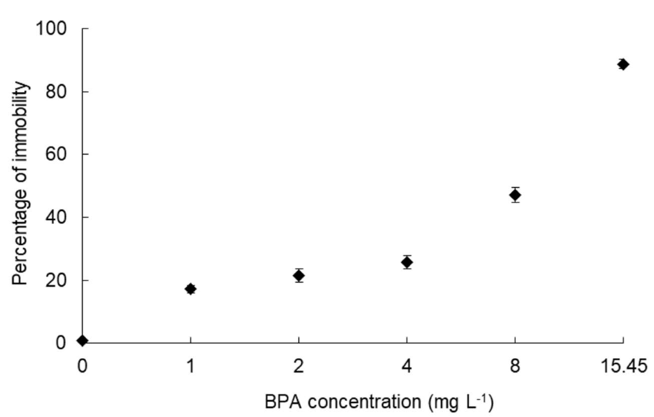

Figure 1 – Average percentage of immobility of *C. sancticaroli* in acute toxicity tests (n=10). Error bars corresponding to the standard deviation.

The fish *Carassius auratus* was the most sensitive to BPA, while the cladoceran *Moina micrura* and the diatom *Navicula* sp. were more sensitive to BPF and BPS, respectively. BPA has immunotoxic effects in *C. auratus* with functional disorder of macrophages and lymphocytes (Yin et al., 2007). Among the arthropod species tested for the toxicity of BPA and its analogs, the tropical cladoceran *M. micrura* was the most sensitive. According to Razak et al. (2023), BPA and its analogs negatively affect the heart rate and survival rate of *M. micrura* and stimulate the expression of stress-related genes in the invertebrate. The high sensitivity of *M. micrura* compared to other cladoceran species to physical, chemical, and biological factors has already been demonstrated (Miracle et al., 2011; Mau et al., 2019). The greater toxicity of BPS to the diatom *Navicula* sp. compared to *C. vulgaris* was associated with a significant decrease in the chlorophyll a content in the diatom compared to that observed for the chlorophyte (Li et al., 2021). Similar to the present study, the SSD analysis performed by Jung et al. (2020) and Chae et al. (2023) demonstrated that *C. auratus* and *Navicula* sp. were the most sensitive species to BPA and BPS, respectively.

The algae *Chlorella pyrenoidosa*, *Chlamydomonas mexicana*, *C. vulgaris*, and *Scenedesmus obliquus* demonstrated low sensitivity to BPA. The chlorophytes *D. armatus*, *C. vulgaris*, and *S. obliquus* demonstrated lower sensitivity to BPF, while the green algae *C. pyrenoidosa* was the least sensitive species to BPS. Studies have shown that the chlorophytes *C. vulgaris*, *Chlorella sorokiniana*, *S. obliquus*, and *Desmodemus* sp. accumulated, biodegraded, and biotransformed BPA and its analogs (Eio et al., 2015; Ding et al., 2020; Azizullah et al., 2022; Atengueño-Reyes et al., 2024). Additionally, variations in the results of acute toxicity of biphenols for freshwater organisms may be related to the intra- and interspecific characteristics of each species tested, such as cell wall composition in microalgae, body size in invertebrates and vertebrates, and defense system (Czarny-Krzymińska et al., 2023).

The effects of BPA on reducing chlorophyll content, reducing cell growth, and oxidative stress for the alga *C. pyrenoidosa* were observed by Li et al. (2021). Additionally, Czarny-Krzymińska et al. (2023) and Le et al. (2024) reported effects of BPA associated with inhibition of cell division, disintegration of chloroplasts, and damage to the cell wall and cell organelles in green algae. In crustaceans, bisphenol exposure can lead to neurotoxic effects, induce oxidative stress, and disturb carbohydrate metabolism (Fabrello et al., 2024; Kim et al., 2024; Wang et al., 2024). BPA has also been shown to affect the expression of genes involved in molting, growth, and reproduction in cladoceran (In et al., 2019; Chen et al., 2021). Bisphenols can affect mollusks by disrupting gametogenesis and spawning. BPA has been shown to reduce the size of the sexual organs in males of the gastropod *Marisa cornuarietis*

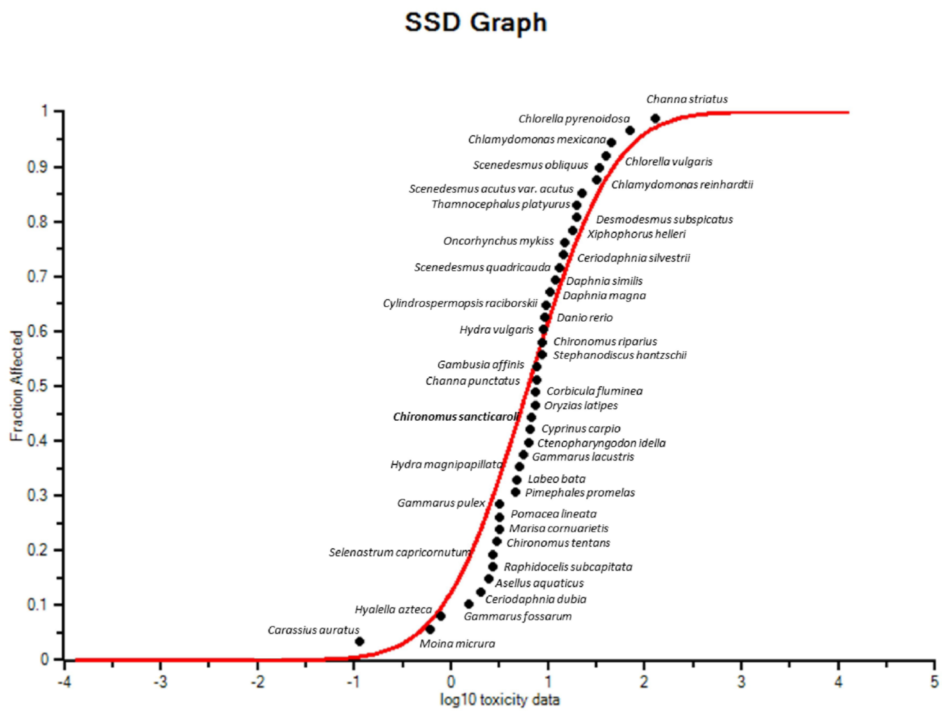

Figure 2 – Species sensitivity distribution curve for bisphenol A based on LC50, EC50, and IC50 values of aquatic species.

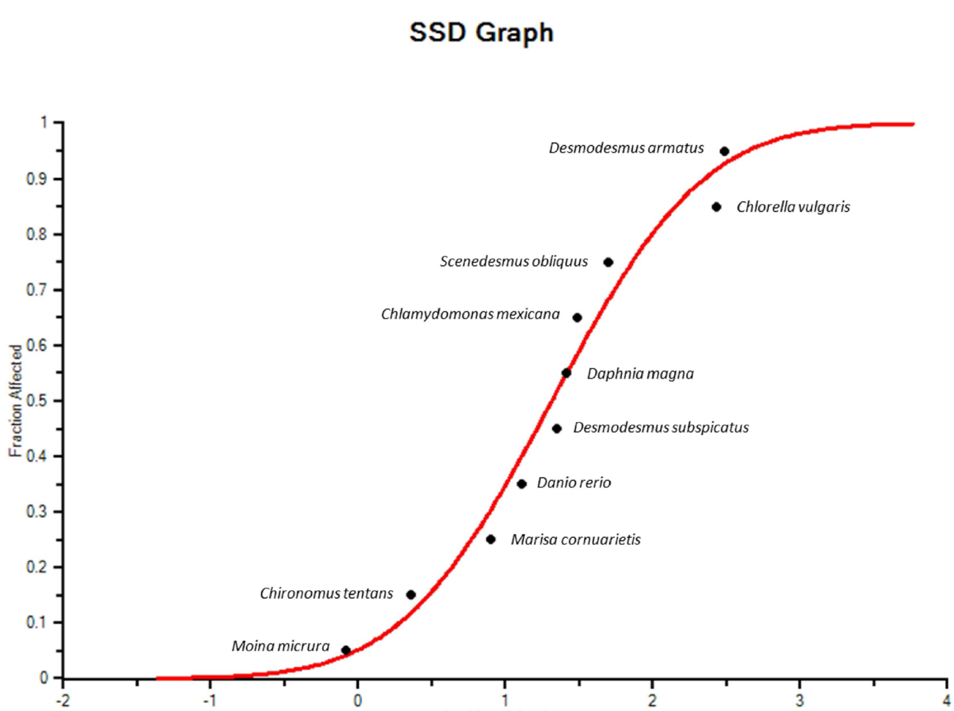

Figure 3 – Species sensitivity distribution curve for bisphenol F based on LC50, EC50, and IC50 values of aquatic species.

(Oehlmann et al., 2000) and alter several genes related to the endocrine system and stress response in the gastropod *Physa acuta* (Morales et al., 2018).

Herrero et al. (2018) and Morales et al. (2020) verified that exposure to BPs altered the transcriptional profile of genes related to the ecdysone pathway in *C. riparius*, increasing cellular vulnerability to apoptosis with detrimental effects on the hormonal system and various metabolic pathways of the insect.

BPA has been linked to developmental abnormalities, such as delayed hatching, reduced larval survival, and malformations in fish. In relation to reproductive effects on fish, BPF and BPS have similar effects, though their potency may differ (Han et al., 2021; OEHHA, 2024). Ahmad et al. (2024) described genotoxic effects and histological and biochemical changes in organs of the fish species *Labeo rohita* exposed to BPF. In the presence of BPS, cholesterol and total protein content in gonad, muscle, and liver tissues of *C. striatus* decreased significantly, disrupting metabolic pathways and antioxidant defense mechanisms in fish (Mohan et al., 2024).

The curve-derived HC5 for BPA was 0.86 (0.48–1.35) mg L^{-1}, and the HC50 was 7.24 (5.20–10.07) mg L^{-1} (Table 2). The PNEC of BPA was 0.17 mg L^{-1}. BPA concentrations of 2 and 6.4 µg L^{-1} have been recorded in Canada (Gewurtz et al., 2021), 2.7 µg L^{-1} in the United States (Elliott et al., 2018), 4 µg L^{-1} in Portugal (Azevedo et al., 2001), 4.7 µg L^{-1} in China (Huang et al., 2012), 14 µg L^{-1} in India (Lalwani et al., 2020), 76 µg L^{-1} in Colombia (Bedoya-Rios et al., 2018), 410 µg L^{-1} in Germany (Fromme et al., 2002), and 4.3 µg L^{-1} in Taiwan (Chen et al., 2017), with high values being associated with environments under the influence of industrial areas and sewage treatment systems. In Brazilian surface waters, BPA was found in concentrations ranging from 0.0012 to 64.2 µg L^{-1} (Peteffi et al., 2019; Ferreira et al., 2023). BPA demonstrates potential toxicity to aquatic biota. Based on the HC5 and PNEC values of BPA calculated in the present study and relating to the

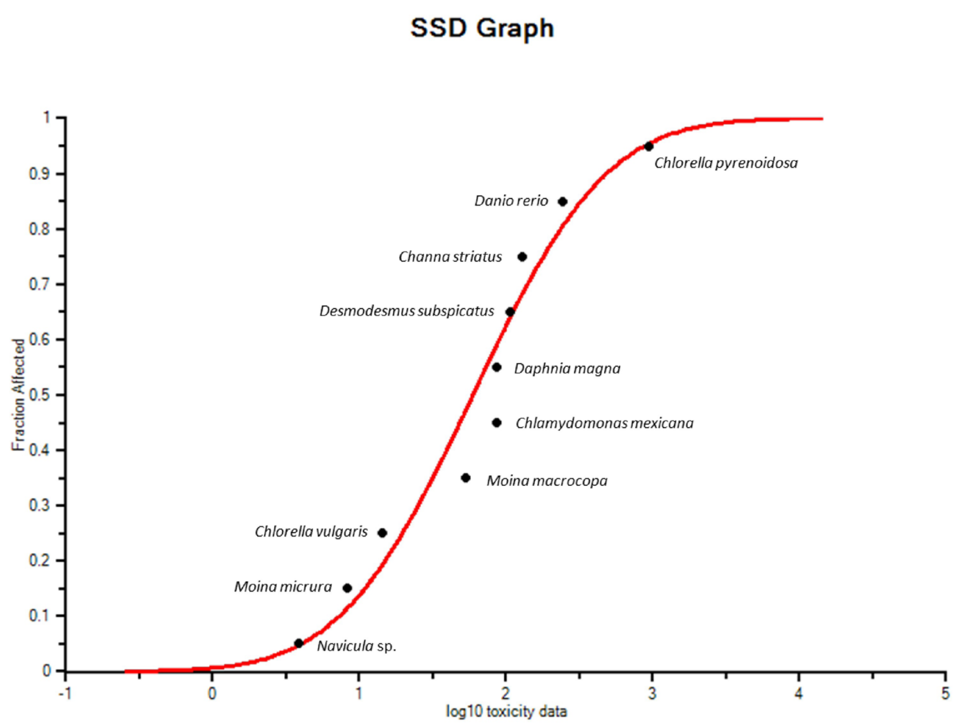

Figure 4 – Species sensitivity distribution curve for bisphenol S based on LC50, EC50, and IC50 values of aquatic species.

Table 2 – Hazardous concentrations for 5 and 50% of species (HC5 and HC50 respectively; (mg L^{-1}) and their lower and upper confidence limits, derived from the species sensitivity distribution curve for bisphenol A, bisphenol S, and bisphenol F.

	N (number of common species for datasets)	HC5 (mg L^{-1})	HC50 (mg L^{-1})	AD	KS	CM
BPA	43	0.86 (0.48–1.35)	7.24 (5.20–10.07)	0.44	0.60	0.07
BPF	10	0.90 (0.09–3.10)	20.64 (7.01–60.72)	0.25	0.40	0.03
BPS	10	3.65 (0.51–11.22)	59.42 (22.98–153.63)	0.32	0.64	0.05

AD: statistic of the Anderson-Darling test; KS: statistic of the Kolmogorov-Smirnov test; CM: statistic of the Cramer von Mises test.

environmental concentrations of the compound, it is likely that a small percentage of aquatic biota representatives are at risk or have a low risk of impact of BPA on aquatic communities. Research on the environmental risk assessment of BPA has described that this compound poses a low risk to the freshwater community (Spadoto et al., 2017).

Regarding BPF, the HC5 derived from the curve was 0.90 (0.09–3.10) mg L^{-1}, and the HC50 was 20.63 (7.01–60.72) mg L^{-1}. PNEC of BPF was 0.20 mg L^{-1}. A variation in the environmental concentrations of BPA analogs is observed. BPF has been detected at concentrations of 1 µg L^{-1} in rivers of China, Korea, and Japan, reaching concentrations of 3 µg L^{-1} in Lake Taihu (China) and in the Tamagawa River (Tokyo Bay, Japan) (Yan et al., 2017) and 6 µg L^{-1} Laoyu Dianchi River (China).

The HC5 calculated from the curve for BPS was 3.65 (0.50–11.22) mg L^{-1}, and the HC50 was 59.42 (22.98–153.62) mg L^{-1} (Table 2). PNEC of BPS was 0.73 mg L^{-1}. Regarding BPS values, values of 0.02 µg L^{-1} were recorded in the Rhône River (France) (Schmidt et al., 2020), 3.6 µg L^{-1} in the Cooum River (India) (Yamazaki et al., 2015), 7 µg L^{-1} in the Adyar River (India) (Yamazaki et al., 2015), and 31 µg L^{-1} in Lake Taihu (China) (Chen et al., 2017). Environmental concentrations of BPF and BPS pose a low risk of impact to aquatic communities because these concentrations are below the HC5 and PNEC values calculated for these compounds (a low percentage of the community is expected to be at risk). Wang et al. (2018) indicate that BPF concentrations occur in freshwater worldwide with negligible risks.

As demonstrated in the SSD curves developed in the present study and PNEC values, the order of toxicity based on all species was BPA > BPF > BPS, as previously established by the study by Razak et al. (2023) when assessing the risk of BPA and its analogs for the group of microalgae, cladocerans, and freshwater fish through the analysis of PNEC values. According to Chen et al. (2016), different chemical properties and physiological effects of BPA analogs may contribute to the compounds having different degrees of toxicity to organisms. In the present study, to prepare the SSD, data from 43 species were used for BPA and from 10 species for BPS and BPF. In the study by Razak et al. (2023), a smaller number of data was used, being from 23 species for BPA and from four species for BPF. The minority of species tested for bisphenol toxicity are native to tropical and subtropical regions (*Pomacea lineata*, *Ceriodaphnia silvestrii*, *C. sancticaroli*, *M. micrura*, *Labeo bata*, and *Channa punctata*, for example), revealing the need to expand research on the potential deleterious effects of these chemical compounds for tropical species since the contamination of these aquatic environments with BP and its analogs is a topic of environmental interest (Shehab et al., 2020; Roledo et al., 2024).

Canada, the United States, and Australia are countries that control the presence of BPA in water with legislation (Chen et al., 2016). In Brazil, there is still no legislation on the monitoring of bisphenol in surface waters, and according to the National Environmental Council CONAMA 357/2005 (Brasil, 2005), the compound can be analyzed within the group of total phenols (Spadoto et al., 2017). According to this resolution, the maximum value of total phenols in natural freshwater is between 3 and 10 µg L^{-1}, depending on the quality of the water body and its priority use. In view of the above, the concentration established by CONAMA Resolution 357/2005 may be adequate to avoid acute toxic effects of BPA and its analogs on freshwater biota.

The results of this study show that the values of bisphenols A, F, and S analyzed in environmental samples present a low risk of causing lethal effects to representatives of the freshwater community. However, the environmental concentrations of these contaminants in aquatic ecosystems are underestimated and are expected to increase continuously (Czarny-Krzymińska et al., 2023). The chronic exposure of organisms through aqueous exposure to bisphenols and through diet in aquatic environments contaminated with BFs cannot be ignored. Chae et al. (2023) indicated a high environmental risk of BPS in aquatic organisms based on chronic risk quotient values. Mukherjee et al. (2024) detected chronic toxic effects of BPA on the reproduction of the fish *L. bata* at environmentally relevant concentrations. Wu et al. (2025) verified that ambient concentrations of BPF promote early hatching in zebrafish embryos. The bioaccumulation and trophic amplification of several bisphenols have already been reported by Wang et al. (2017) in Lake Taihu (China). In laboratory experiments, Guo et al. (2017) detected chronic toxicity in the rotifer *Brachionus calyciflorus* fed with the microalgae *C. pyrenoidosa* containing BPA. Additionally, aquatic organisms may be exposed to complex mixtures of bisphenol analogs that can cause potentiated toxic effects compared to the effects of a single isolated compound (Han et al., 2021). We highlight the need to expand ecotoxicological data on the chronic toxicity of BPA and its analogs for tropical freshwater ecosystems to estimate potential effects of bisphenols and, thus, assist in the development of more protective environmental laws.

Conclusion

The insect *C. sancticaroli* was a suitable test organism to test the effects of BPA. Although environmental concentrations of BPA present a low risk of causing immediate lethal effects, the chronic risk, especially through prolonged exposure and through different routes, should not be neglected. The continuous increase in the concentration of these pollutants in aquatic environments and the possibility of toxic effects potentiated by exposure to bisphenol mixtures indicate the urgent need for further ecotoxicological research, especially in species native to tropical regions. In ecological and environmental science aspects, this study contributed information for the development of more effective environmental laws aimed at mitigating the impacts of bisphenols on aquatic communities, thus promoting ecosystem preservation and public health.

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Authors' Contributions

Rodgher, S.: conceptualization, data curation; formal analysis, funding, acquisition, investigation, methodology, project administration, resources, supervision, validation, visualization, writing – original draft, writing – review & editing. **Tavares**, D.: conceptualization, data curation; formal analysis, investigation, methodology, validation, visualization, writing – original draft. **Moraes**, M.V.: data curation; formal analysis, investigation, methodology, validation, visualization, writing – original draft.

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