v. 3, n. 4, 2023

International Journal of Biological and Natural Sciences

EFFECT OF NANOATRAZINE ON THE GROWTH OF AQUATIC BIOINDICATORS THROUGH TROPHIC TRANSFER

Rafaela Monique Fernandes Dionisio
Author, CNPq Scholarship (PIBIC): Graduation in Biological Sciences, UNIP, Campinas-SP

Rodrigo Fernandes Castanha
Employee: Technician, Embrapa - Environment, Jaguariúna-SP.

José Henrique Vallim
Employee: Analyst, Embrapa - Environment, Jaguariúna-SP.

Vera Lucia Scherholz Salgado de Castro
Employee: Researcher, Embrapa - Environment, Jaguariúna-SP.

Claudio Martín Jonsson
Advisor: Embrapa Researcher - Environment, Jaguariúna-SP.

Abstract – *Atrazine is a herbicide that has stood out in recent years for being one of the most commercialized pesticides in the country, being used to control weeds, mainly in corn, sugar cane and sorghum plantations. The nanoencapsulation of this compound has been a strategy to combat environmental impacts in agriculture. The objective of this work is to evaluate the effects of nanoatrazine on the growth of bioindicators through trophic transfer, focusing on harmful effects for species present in aquatic environments. The organisms were exposed to the compounds ATZ-NPZ (atrazine encapsulated in zein nanoparticles) and ATZ-PCL (atrazine encapsulated in poly-epsilon-caprolactone nanoparticles), in the presence and absence of organic matter. No significant difference (p>0.05) was observed between treatments and controls, with the exception of ATZ-PCL 0.002 mg/L in the absence of organic matter for Daphnia magna. This microcrustacean showed a significant increase of 31% in the growth rate compared to the control group. Our study shows that there are no adverse effects on the development of bioindicators, as the trophic transfer of these compounds would not pose risks to fish and microcrustaceans. The results indicate positive aspects in the use of nanoatrazine as a viable alternative for agriculture, aiming at a decrease in environmental impacts.*
Keywords: Ecotoxicology, Nanoatrazine, Toxicity, Bioindicators, Trophic transfer.

INTRODUCTION

It is notable that the use of pesticides has been increasing agricultural productivity in Brazil, however, the existence of numerous undesirable toxic effects of these substances on ecosystems is evident, which are being found in worrying amounts in water and underground bodies.

Atrazine is a herbicide used on a large scale in the country. According to the Brazilian Institute of the Environment - IBAMA, the compound was on the list of the ten best-selling active ingredients between 2009 and 2016. During this period, approximately 160,000 tons of active ingredients were sold, with atrazine being the fourth best seller in 2016. The annual pesticide bulletin prepared by IBAMA in 2020 reveals that in that year the total sale of chemical and biochemical products was 685,745.68 tons of active ingredients, presenting atrazine, again, as one of the 10 most commercialized (DIAS et al., 2018).

This triazine-type herbicide is used to control weeds in maize, sugar cane and sorghum plantations. Its mechanism of action is based on the inhibition of photosynthesis in susceptible plants, causing them to die. However, it is not restricted only to these target organisms, being found beyond the area of its application due to its high potential for leaching such as: rainwater and irrigation, in addition to the contamination of groundwater (UNESP, 2022).

Nanotechnology is a branch of science that studies and builds materials on a very small scale, the nanometer, and has been a possibility to combat environmental impacts in agriculture through the encapsulation of chemical pesticides in nanoparticles (nanopesticides). Its purpose is the slow and steady release of the active ingredient, such as atrazine. This way, the product is completely used in the control of pests and diseases, allowing the use of a smaller amount of the active ingredient in the plant and in the soil. (CROPLIFE, 2022)

Zein is a protein extracted from plants, used as a base for microencapsulation of compounds, increasing their solubility and prolonging their release. Poly-epsilon-caprolactone is a polymer used in pesticide nanoencapsulation with the aim of increasing its effectiveness due to its prolonged release.

(FREITAS, 2016)

Nanoparticles can be bioaccumulated and transferred from one trophic level to another through the food chain. Trophic transfer studies allow differentiating the importance of different exposure routes, which is useful in risk analysis (HOLBROOK et al., 2008). Algae are primary energy producers as a food source and any impacts at this level could affect the health of organisms at higher trophic levels (LEKAMGE et al., 2019). MCTEER et al. (2014) reported a significant reduction in feeding when daphnids were fed algae contaminated with silver nanoparticles compared to untreated algae, suggesting deleterious effects on growth and reproduction.

Bioindicator organisms have been used as experimental models in toxicological tests due to their high sensitivity, such as Daphnia magna. This is a microcrustacean found in fresh waters, commonly known as water flea. It is a filtering organism that feeds on algae, rotifers and infusoria, and plays an important role in the food chain. In addition, its easy cultivation and rapid reproduction make this organism viable for acute and subchronic toxicity experiments (TKACZYK et al. 2021; CROA CLUB DE ACUARISMO, 2022)

Danio rerio, better known as zebrafish or paulistinha, it is a freshwater fish with approximately five centimeters when adult, with transparent embryos, used in research mainly because it presents an easily observable and testable development (TESOLIN et al., 2014).

The objective of this work was to evaluate the effects of nanoatrazine on the growth of bioindicators through trophic transfer, focusing on harmful effects for species present in aquatic environments.

MATERIAL AND METHODS

The organisms were tested at different concentrations of atrazine encapsulated in zein nanoparticles (ATZ-NPZ), as well as in poly-epsilon-caprolactone nanoparticles (ATZ-PCL) in the absence and presence of NOM (Natural Organic Matter, 10 mg/ L), a complex organic matrix, built mainly by humic and fulvic acids present in the aquatic environment. The animal handling procedures were approved by the Ethics Committee for Animal Use of Embrapa Environment (protocol No. 006/2019).

EXPOSURE OF ALGAE TO NANOFORMULATIONS:

Cultures of the microalgae chlorophycea Raphidocelis subcapitata (Figure 1) with an initial inoculum algal concentration of 7 x 10^5 cells / mL were exposed to sublethal concentrations of ATZ-NPZ at concentrations equivalent to 0.058; 0.0116; and 0.0058 mg/L. Similarly, cultures were also exposed to concentrations of 0.02; 0.004; and 0.002 mg/L ATZ-PCL. The test concentration values represented, respectively, IC10-96h (inhibitory concentration at 10% of algal growth for 96 h); 1/5 IC10-96h; and 1/10 of IC10-96h, in order to maintain viability and allow the growth of microalgae with concomitant incorporation of the herbicide in these organisms. Exposures were performed in the absence and presence of NOM (10 mg/L) for 96h.

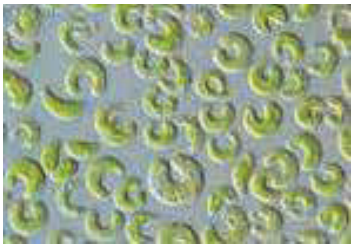

Figure 1. Microscopic detail of culture of *Raphidocelis subcapitata.*

Source: SAG 61.81 Raphidocelis subcapitatahttps://sagdb.unigoettingen.de/detailedList.php?str_number=61.81

After 8 days of growth, 50 ml Falcon tubes were autoclaved to concentrate the algae in each flask and re-suspend them in reconstituted water and embryo medium (composition for 1L: NaHCO3 – 35mg; MgSO4 – 60mg; KCl – 4mg; and CaSO42H2O – 60mg) (UNITED STATES ENVIRONMENTAL PROTECTION AGENGY, 2002), to be used in bioconcentration assays with D. *magna* (Figure 2) and embryos of *D. rerio* (Figure 3).

Figure 2: Daphnia magna neonate.

Source: https://commons.wikimedia.org/wiki/ File:Daphnia_magna_hatchling_from_restingegg.JPG

Figure 3: Initial stage of Danio rerio embryo.

Source: Danio rerio (zebrafish) embryonic development. http://bioimages.vanderbilt.edu/pages/zfish-devel.htm

EXPOSURE OF TEST ORGANISMS THROUGH FOOD.

Specimens of D. *magna* were kept in aquariums (40 × 20 × 15 cm), containing water reconstituted with nutrients, prepared according to the methodology of JONSSON and MAIA (1999). Temperature and light intensity were maintained at 20°C and 1,000 lux, respectively. The microalgae R. subcapitata was used to feed the cultures once a day. Neonates younger than 24 hours were separated from the cultures and used as test organisms.

Adults of *D. rerio* of the wild type were housed in a "rack" system. After mating, the eggs (Figure 3) were collected, washed and selected for their viability through microscopic observation. D. *rerio* larvae (Figure 4) (n=24), hatched from eggs, were used for toxicity assessment.

Figure 4: Larval stage of Danio rerio.

Source: collection of the Laboratory of Ecotoxicology and Biosafety.

The test organisms were placed in 14 polystyrene plates containing 24 wells each, two for control and 12 for concentrations of NPZ 0.058; 0.0116; 0.0058 mg/L and PCL 0.02 ;0.004; 0.002 mg/L, and were fed (30 uL/day for Daphnia and only 1x for D. rerio) with algae previously exposed to the two nanoformulations in medium absent or in the presence of NOM.

Body length measurements of D. rerio were taken after 96 h of egg hatching and up to 192 h of larval development. In the case of D. magna, measurements were taken from collection of neonates until 96 hours after feeding. Measurements were performed by obtaining microphotographs using a stereomicroscope with a camera (Optika Camera 4083B3, Optika^{R}) coupled to a measurement system (Optika View Version 7.1.1.5 software) at a 2xx magnification.

DATA ANALYSIS

Linear regression analysis of percentage growth as a function of time was applied, where the angular coefficient was equivalent to the growth rate (BASU; PAL, 2011). The "One Way ANOVA" module of the Statgraphics Centurion XVII program was used to compare growth rates between treatments according to Tukey's test.

Data were analyzed using the non-parametric Kruskal-Wallis test (followed by post-hoc Bonferroni analysis) when growth rates did not satisfy the assumption of homogeneity of variance. Data were considered statistically significant at a 95% certainty level ($p<0.05$).

RESULTS AND DISCUSSION

The highest concentrations evaluated for ATZ-NPZ and ATZ-PCL in which the algae were exposed to the nanoformulations were 30 and 10 times higher, respectively, than the maximum permissible concentration of the herbicide for the protection of aquatic communities according to Brazilian legislation (0.002 mg/ L; CONAMA, 2005).

The growth curves of the test organisms fed with algae that were exposed to the two nanomaterial formulations, both in the absence and in the presence of NOM, are shown in Figures 5 to 8. The curves allowed calculating the specific growth rates that were compared by the test of Tukey, where no significant difference ($p>0.05$) was found in relation to the controls for all treatments, with the exception of ATZ-PCL 0.002 mg/L in the absence of NOM for D. *magna* (Table 1).

In analogy to the results of the present work, ANDRADE et al. (2019) reported that atrazine nanoencapsulation protects aquatic biota from the adverse effects of this herbicide.

In this situation, there was a significant increase of 31% in the growth rate of the microcrustacean in relation to the control. There is no explanation for this effect in the present study. However, according to Marcus and Fiumera (2016), acceleration of arthropod development time at low concentrations of atrazine was observed. The authors relate this effect to a possible endocrine disruption that would involve cell signaling mediated by insulin.

CONCLUSION

There were no adverse effects associated with the development of bioindicators *D. magna and D. rerio* both in the absence or presence of organic matter, suggesting that the trophic transfer "via algae" of ATZ in the evaluated nanoformulations would not present a risk for fish and microcrustaceans and, therefore, would not represent a risk in its use and commercialization in formulations containing nanoparticles of NPZ and PCL. Therefore, in this scenario, the results obtained will provide important information for the development of new nanomaterials and their interactions with organic material present in the environment. In addition, they will provide information on the effects and ecological risks of environmental contamination by these agents, generating data that may help in their management to reduce impacts on aquatic biota. The data may be useful to support environmental legislation, at national and international levels.

ACKNOWLEDGMENTS

The authors would like to thank CNPq for granting the scientific initiation scholarship to the first author, Embrapa Environment for providing resources and opportunities for research development, and the Unesp Institute of Science and Technology (Campus Sorocaba) for providing the nanomaterial used in the toxicological tests.

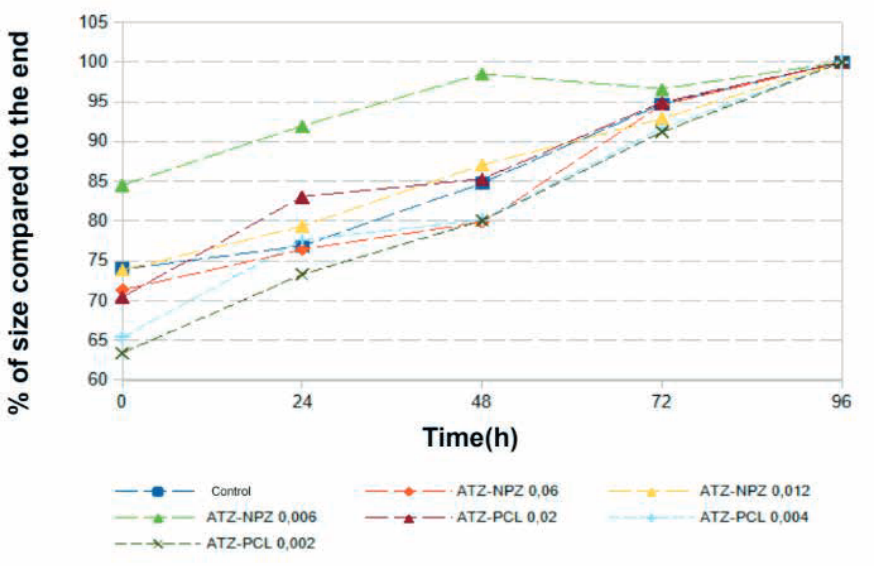

Figure 5: Body growth curves of Daphnia magna fed on algae (*Raphidocelis subcapitata*) previously exposed to ATZ-NPZ (atrazine encapsulated in zein nanoparticles) and ATZ-PCL (atrazine encapsulated in poly-epsilon-caprolactone nanoparticles) in the absence of NOM (humic acid), under different herbicide concentrations (mg/L).

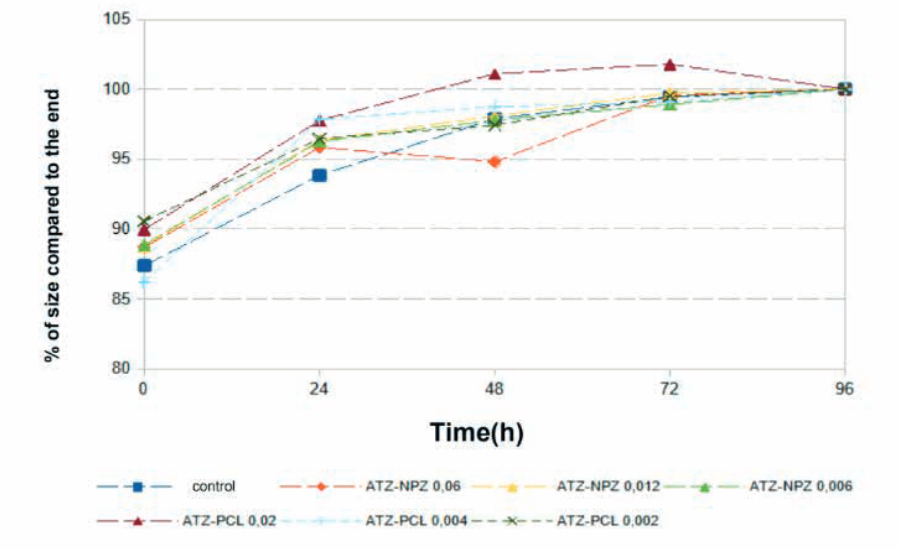

Figure 6: Body growth curves of Daphnia magna fed on algae (*Raphidocelis subcapitata*) previously exposed to ATZ-NPZ (atrazine encapsulated in zein nanoparticles) and ATZ-PCL (atrazine encapsulated in poly-epsilon-caprolactone nanoparticles) in the presence of NOM (Natural Organic Matter, 10 mg/L), under different herbicide concentrations (mg/L).

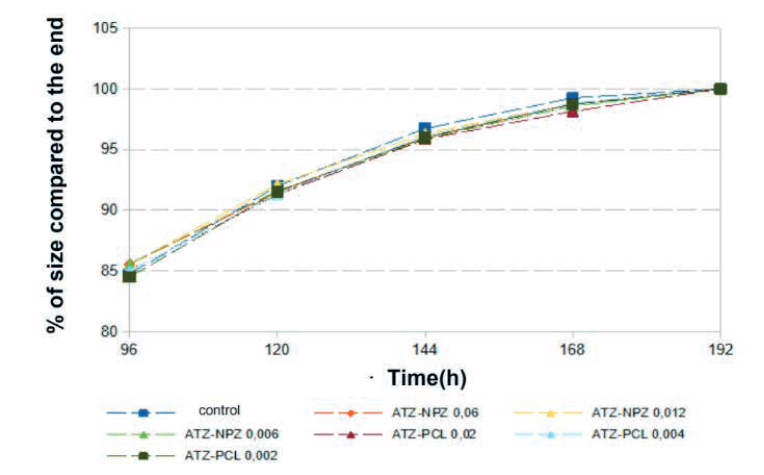

Figure 7: Body growth curves of *Danio rerio* larvae from 96 to 192 hours after egg hatching, fed with microalgae (*Raphidocelis subcapitata*) previously exposed to different concentrations of ATZ-NPZ (atrazine encapsulated in zein nanoparticles) and ATZ- PCL (atrazine encapsulated in poly-epsilon-caprolactone nanoparticles) in the absence of NOM (Natural Organic Matter, 10 mg/L).

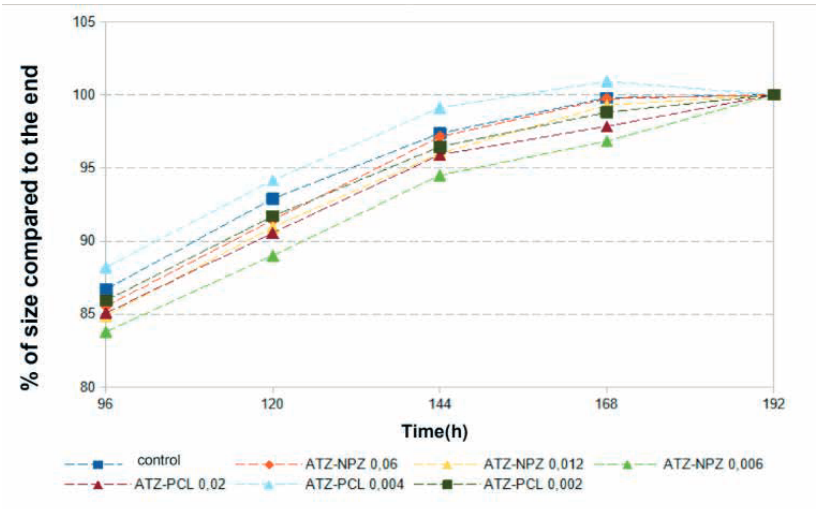

Figure 8: Body growth curves of larvae of *Danio rerio* in the period of 96 to 192 hours, after hatching of the eggs, under microalgae feeding (*Raphidocelis subcapitata*) previously exposed to different concentrations of ATZ-NPZ (atrazine encapsulated in zein nanoparticles) and ATZ-PCL (atrazine encapsulated in poly-epsilon-caprolactone nanoparticles) in the presence of NOM (Natural Organic Matter, 10 mg/L).

	Control	Algae in ATZ-NPZ (mg/L)			Algae in ATZ-PCL (mg/L)		
		0.006 (1/10 IC10-96h)	**0.016 (1/5 ICI10-96h)**	**0.06 (IC10-96h)**	**0.002 (1/10 IC10-96h)**	**0.004 (1/5 IC10-96h)**	**0.02 (IC10-96h)**
				Without NOM			
D. magna	0.2915 (0.0941)	0.2143 (0.0975)	0.2742 (0.0793)	0.3145 (0.1088)	0.3795* (0.0335)	0.3471 (0.0327)	0.2958 (0.0344)
				With NOM			
	0.1413 (0.0560)	0.1081 (0.0562)	0.1154 (0.0409)	0.1098 (0.0521)	0.0969 (0.0433)	0.1146 (0.0483)	0.1023 (0.0148)
				Without NOM			
D. rerio	0.1572 (0.0384)	0.14911 (0.0397)	0.1484 (0.0386)	0.1508 (0.0384)	0.1592 (0.0325)	0.1558 (0.0330)	0.1531 (0.0323)
				With NOM			
	0.1413 (0.0558)	0.1081 (0.0562)	0.1154 (0.0409)	0.1098 (0.0521)	0.0969 (0.0433)	0.1146 (0.0483)	0.1023 (0.0420)

* Significant difference compared to the control ($p<0.05$).

TABLE 1: Mean values (standard deviation) of the growth rate (% length/h) of the test organisms fed with microalgae (R. subcapitata) previously exposed to different concentrations of ATZ-NPZ (atrazine encapsulated in zein nanoparticles) and ATZ- PCL (atrazine encapsulated in poly-epsilon-caprolactone nanoparticles) in the absence and presence of NOM (Natural Organic Matter, 10 mg/L).

REFERENCES

ANDRADE, L. L. *et al.* Can atrazine loaded nanocapsules reduce the toxic effects of this herbicide on the fish Prochilodus lineatus? A multibiomarker approach**. Science of The Total Environment.** v. 663, p. 548-559, 2019.

BASU, P.; PAL, A. Spatio-temporal analysis of development of basal roots of common bean *(Phaseolus vulgaris L*)**. Plant Signaling & Behavior**, v. 6, n. 7, p. 982–985. 2011.

CONAMA. Resolução nº 357, de 17 de março de 2005. Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para o seu enquadramento, bem como estabelece as condições e padrões de lançamento de efluentes, e dá outras providências. **Diário Oficial [da] República Federativa do Brasil**, Brasília, DF, 18 mar. 2005. Seção 1, p. 58-63.

CROA CLUB DE ACUARISMO. **La Daphnia**: pulga de água. Disponível em: <https://www.croa.com.ar/la- daphmia-pulga-agua/>. Acesso em: 01 ago. 2022.

CROPLIFE**. Nanotecnologia potencializa a ação dos defensivos agrícolas**. Disponível em: <https://croplifebrasil.org/noticias/nanotecnologia-potencializa-a-acao-dos-defensivos- agricolas/#:~:text=O%20Brasil%20avan>. Acesso em: 29 jun. 2022.

DIAS, A. C. L. *et al.* Ocorrência de atrazina em águas no Brasil e remoção no tratamento da água: revisão bibliográfica. **Revista Internacional de Ciências**, v. 8, n. 2, p. 149–168, 2018.

FREITAS, D. Z. **Nanopartículas de zeína e quitosana contendo óleo essencial de *Ocimum gratissimum* l. quimiotipos eugenol e geraniol**. 2016. 157 f. Dissertação (Mestrado) - Centro de Ciências Agrárias, Universidade Federal de Santa Catarina, Florianópolis.

HOLBROOK, R. D. *et al.* Trophic transfer of nanoparticles in a simplified invertebrate food web. **Nature Nanotechnology**, v. 3, n. 6, p. 352-355, 2008.

JONSSON, C. M.; MAIA, A. de H. N. **Protocolo**: avaliação de agentes microbianos de controle de pragas para registro como biopesticidas: uma proposta para os órgãos federais registrantes: testes toxicopatológicos em organismos não-alvo do ambiente aquático: organismos zooplanctônicos, fitoplanctônicos e vertebrados. Jaguariúna: Embrapa Meio Ambiente, 1999. 33 p. (Embrapa Meio Ambiente. Documentos, 11).

LEKAMGE, S. *et al.* The toxicity of coated silver nanoparticles to *Daphnia carinata* and trophic transfer from alga *Raphidocelis subcapitata*. **PLoS One**, v. 14, n.4, 2019.

MARCUS, S. R.; FIUMERA, A. C. Atrazine exposure affects longevity, development time and body size in *Drosophila melanogaster*. **Journal of Insect Physiology**, v. 91-92, p.18-25, 2016.

MCTEER, J. *et al.* Bioaccumulation of silver nanoparticles into *Daphnia magna* from a freshwater algal diet and the impact of phosphate availability. **Nanotoxicology**, v. 8, n. 3, 305-316, 2014.

TESOLIN, G. A. S. *et al.* Avaliação da toxicidade de herbicidas usados em cana-de-açúcar para o Paulistinha (*Danio rerio*). **O Mundo da Saúde (Online)**, v. 38, p. 86-97, 2014.

TKACZYK, A. *et al. Daphnia magna* model in the toxicity assessment of pharmaceuticals: a review. **Science of the Total Environment,** v. 763, p. 143038, 2021.

UNESP. Instituto de Ciência e Tecnologia - Campus de Sorocaba. Unesp. **A atrazina e seu impacto em ambientes aquáticos**: pesquisa expõe impacto da atrazina e indica nanotecnologia como ferramenta para reduzi-lo. Disponível em: <https://www.sorocaba.unesp.br/#!/noticia/1135/a-atrazina-e-seu-impacto-em- ambientes-aquaticos/>. Acesso em: 26 jun. 2022.

UNITED STATES ENVIRONMENTAL PROTECTION AGENGY. **Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms.** 5 ed. Washington: EPA, Oct. 2002. (EPA-821-R-02-012).