UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de Sorocaba

PROGRAMA DE PÓS-GRADUAÇÃO em
Ciências Ambientais

FELÍCIA PEREIRA DE ALBUQUERQUE

EFEITOS ECOTOXICOLÓGICOS EM LARVAS DE *CHIRONOMUS SANCTICAROLI* STRIXINO & STRIXINO, 1981 (DIPTERA: CHIRONOMIDAE) EXPOSTAS A NANOPARTÍCULAS LIPÍDICAS SÓLIDAS CARREGADAS COM ATRAZINA

Sorocaba
2020

FELÍCIA PEREIRA DE ALBUQUERQUE

EFEITOS ECOTOXICOLÓGICOS EM LARVAS DE *CHIRONOMUS SANCTICAROLI* STRIXINO & STRIXINO, 1981 (DIPTERA: CHIRONOMIDAE) EXPOSTAS A NANOPARTÍCULAS LIPÍDICAS SÓLIDAS CARREGADAS COM ATRAZINA

Tese apresentada como requisito para obtenção do título de Doutor em Ciências Ambientais da Universidade Estadual Paulista "Júlio de Mesquita Filho" na Área de Concentração Diagnóstico, Tratamento e Recuperação Ambiental.

Orientador: Profª. Drª. Viviane Moschini Carlos
Coorientador: Prof. Dr. Leonardo Fernandes Fraceto

Sorocaba
2020

A345e

Albuquerque, Felícia Pereira de

Efeitos ecotoxicológicos em larvas de Chironomus sancticaroli Strixino & Strixino, 1981 (Diptera: Chironomidae) expostas a nanopartículas lipídicas sólidas carregadas com atrazina / Felícia Pereira de Albuquerque. -- Sorocaba, 2020

101 f. : il., tabs., fotos

Tese (doutorado) - Universidade Estadual Paulista (Unesp), Instituto de Ciência e Tecnologia, Sorocaba

Orientadora: Viviane Moschini Carlos

Coorientador: Leonardo Fernandes Fraceto

1. Ecotoxicologia. 2. Nanopesticida. 3. Nanoatrazina. 4. Organismo aquático. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca do Instituto de Ciência e Tecnologia, Sorocaba.
Dados fornecidos pelo autor(a).

Essa ficha não pode ser modificada.

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Sorocaba

CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: Efeitos ecotoxicológicos em larvas de Chironomus sancticaroli Strixino & Strixino, 1981 (Diptera: Chironomidae) expostas a nanopartículas lipídicas sólidas carregadas com atrazina.

AUTORA: FELÍCIA PEREIRA DE ALBUQUERQUE
ORIENTADORA: VIVIANE MOSCHINI CARLOS
COORIENTADOR: LEONARDO FERNANDES FRACETO

Aprovada como parte das exigências para obtenção do Título de Doutora em CIÊNCIAS AMBIENTAIS, área: Diagnóstico, Tratamento e Recuperação Ambiental pela Comissão Examinadora:

Prof. Dr. JULIANO JOSÉ CORBI
Departamento de Hidrobiologia / Universidade de São Paulo (USP)

Profª. Drª. ODETE ROCHA
Departamento de Ecologia e Biologia Evolutiva / Universidade Federal de São Carlos

Prof. Dr. DANIEL CLEMENTE VIEIRA RÊGO DA SILVA
- / EEL/USP

Prof. Dr. LUCAS BRAGANÇA DE CARVALHO
Engenharia Ambiental / Unesp - ICT Sorocaba

Profª. Drª. VIVIANE MOSCHINI CARLOS
Engenharia Ambiental / Unesp - Instituto de Ciência e Tecnologia de Sorocaba

Sorocaba, 13 de fevereiro de 2020

“Não há saber mais ou saber menos: há saberes diferentes.”

Paulo Freire

Agradecimentos

A Deus que em todos os momentos está ao meu lado. Todas as coisas posso naquele que me fortalece (Filipenses 4:13).

A Prof.ª Dr.ª Viviane Moschini-Carlos pela orientação e ao Prof.º Dr. Leonardo Fernandes Fraceto pela co-orientação, oportunidade única, aprendizagem, dedicação e disposição, serei sempre grata.

Ao Programa de Pós-Graduação em Ciências Ambientais e a Universidade Estadual Paulista "Júlio de Mesquita Filho", Sorocaba - SP, pela oportunidade e infraestrutura.

As agências de fomento: CAPES pela concessão da bolsa de doutorado e FAPESP pelo financiamento das pesquisas dos professores envolvidos neste trabalho.

Aos professores do Programa de Pós-Graduação em Ciências Ambientais que contribuíram para minha formação.

Ao Prof.º Dr. Marcelo Luiz Martins Pompêo pela colaboração e sugestões.

Ao Prof.º Dr. Mário Antônio Navarro da Silva da Universidade Federal do Paraná (UFPR) pela parceria e disponibilidade do Laboratório de Entomologia Médica e Veterinária.

A Prof.ª Dr.ª Renata de Lima da Universidade de Sorocaba (UNISO) por disponibilizar o freezer do Laboratório de Avaliação de Bioatividade e Toxicologia de Nanomateriais.

Aos pesquisadores Dr. Jhones Luiz de Oliveira, Dr. Vinicius Sobrinho Richardi e MSc. Leila dos Santos Machado pela parceria e diversas contribuições.

Ao Prof.º Dr. Julio César López Doval pela receptividade e conhecimento compartilhado.

As técnicas da UNESP de Sorocaba, Léticia B. F. Gonçalves, Suzan da Silva Lessa e Sandra Mara V. C. Gavetti por serem sempre prestativas.

Aos integrantes do laboratório de Limnologia e ao Grupo de Nanotecnologia Ambiental, da UNESP de Sorocaba - SP, agradeço a colaboração, os almoços e momentos de descontração. Desejo sucesso e muitas conquistas a todos.

Sou imensamente grata a minha querida família, em especial meu esposo Rafael Nunes Machado, pelo amor dedicado, incentivos diário, auxílio com o RStudio e principalmente pela compreensão ao longo destes anos de estudo. Vocês são essenciais em minha vida.

Aos amigos que a vida me presenteou, agradeço por vibrarem com as minhas conquistas.

Resumo

Os pesticidas são compostos sintéticos ou biológicos, utilizados para atrair, matar, repelir ou interferir no ciclo de vida de diferentes tipos de seres vivos. A atrazina é um herbicida pré e pós emergente, usado para controlar plantas daninhas em muitas culturas, destacando-se o milho, o sorgo e a cana de açúcar. Apresenta alto potencial de contaminação ambiental e seu uso tem sido controverso em alguns países. Entretanto, com a implementação da nanotecnologia na agricultura tem sido desenvolvidos eficientes sistemas transportadores da atrazina. Nanopartícula lipídica sólida carregada com atrazina é um exemplo, esta nanoformulação demonstrou eficácia no controle do organismo alvo mesmo quando aplicada em doses reduzidas, mas seus efeitos tóxicos precisam ser verificados em organismos não alvo representativo de ambientes aquáticos e terrestres. A presença de nanosistemas em ecossistemas aquáticos pode afetar organismos bentônicos, pois estes se desenvolvem no sedimento, local que ocorre o acúmulo de contaminantes, inclusive com propriedades em nanoescala. Neste contexto, no presente estudo foram analisados os efeitos ecotoxicológicos de nanopartículas lipídicas sólidas (com ou sem atrazina) e atrazina (ingrediente ativo) sobre larvas de *Chironomus sancticaroli*, avaliando a mortalidade, deformidade do mento, desenvolvimento e biomarcadores bioquímicos (acetilcolinesterase, esterases alfa e beta e glutationa S-transferase). As concentrações de contaminantes utilizadas foram 0,002, 0,47, 0,95 e 1,9 $mg.L^{-1}$ nos bioensaios agudos (96 h) e 0,002 $mg.L^{-1}$ nos bioensaios subcrônicos (10 dias). As nanoformulações mantiveram a estabilidade do diâmetro hidrodinâmico durante os experimentos. O valor da atrazina livre na água permaneceu estável no decorrer dos bioensaios. Atrazina na concentração ambientalmente relevante (0,002 $mg.L^{-1}$) causou efeitos letais e subletais para as larvas, indicando a necessidade de revisão do valor permitido. As nanopartículas com atrazina apresentaram efeitos tóxicos (mortalidade e alteração nos biomarcadores bioquímicos) semelhantes à atrazina. A nanopartícula controle (sem atrazina) causou alterações nos biomarcadores bioquímicos e mortalidade, indicando um possível efeito tóxico da nanoformulação. A maioria das concentrações da nanoformulação carregada com atrazina não foram dose dependente para mortalidade. Somente atrazina na concentração de 0,47 $mg.L^{-1}$ apresentou efeito considerado estatisticamente significativo para a deformidade do mento. A atrazina e as nanopartículas não afetaram o desenvolvimento larval. *Chironomus sancticaroli* foi sensível para monitorar a nanoatrazina, indicando potencial no uso em avaliações ecotoxicológicas de nanopesticidas na região Neotropical.

Palavras-chave: Ecotoxicologia; Nanopesticida; Nanoatrazina; Organismo aquático.

Abstract

Pesticides are synthetic or biological compounds, used for attracting, killing, repelling or interfering in the life cycle of different types of living beings. Atrazine is a herbicide pre- and post-emergence used to control weeds in many crops, highlighting maize, sorghum and sugar cane. Due to its high potential for environmental contamination, its use has been controversial in some countries. Althougth, with the implementation of nanotechnology in agriculture, efficient atrazine carrier systems have been developed. Solid lipid nanoparticle loaded with atrazine is an example, this nanoformulation showed to be effective in controlling the target organism even when applied at low doses, but its toxic effects need to be verified in nontarget organisms representative of aquatic and terrestrial environments. The presence of nanosystems in aquatic ecosystems can affect benthic organisms, because these species develop in the sediment, local where contaminants accumulate, including with nanoscale properties. In this context, the present study analyzed the ecotoxicological effects of solid lipid nanoparticles (with or without atrazine) and atrazine (active ingredient) on *Chironomus sancticaroli* larvae, evaluating the endpoints mortality, mentum deformity, development rate and biochemical biomarkers (acetylcholinesterase, alpha esterase, beta esterase and glutathione S-transferase). The contaminant concentrations used were 0.002, 0.47, 0.95, and 1.9 $mg.L^{-1}$ in acute (96 h) and 0.002 $mg.L^{-1}$ in subchronic (10 days) bioassays. The nanoformulations maintained hydrodynamic diameter stability during the bioassays. The value of atrazine free in the water during experiments remained stable. Atrazine at the environmentally relevant concentration (0.002 $mg.L^{-1}$) caused lethal and sublethal effects on the larvae, indicating the need to review the allowed value. Nanoparticles loaded with atrazine showed toxic effects (mortality and alteration in biochemical biomarkers) similar to atrazine. Nanoparticle control (without atrazine) caused biochemical alterations and mortality, indicating a possible toxic effect of the nanoformulation. Most concentrations of nanoformulation loaded with atrazine were not dose dependent for the endpoint mortality. Only the atrazine at concentration 0.47 $mg.L^{-1}$ showed effect considered statistically significant for mentum deformities. Atrazine and nanoparticles did not affect larval development. *Chironomus sancticaroli* was sensitive to monitor nanoatrazine, indicating potential use in ecotoxicological evaluations of nanopesticides in the Neotropical region.

Keywords: Aquatic organism; Ecotoxicology; Nanopesticide; Nanoatrazine.

Sumário

Introdução Geral

Anualmente na agricultura são consumidos aproximadamente 4 milhões de toneladas de pesticidas (Zhang et al., 2018), estas substâncias são utilizadas para atrair, matar, repelir ou interferir no ciclo de vida de diferentes tipos de seres vivos (Lushchak et al., 2018). Os pesticidas não se restringem ao organismo alvo, circulam nos ecossistemas, podem ser acumulados por diversas espécies e até mesmo migrar através de cadeias alimentares, causando desequilibrio ecológico (Lushchak et al., 2018).

Para o desenvolvimento de uma agricultura menos impactante é essencial o investimento em pesquisas de inovação que rastreiem esses pesticidas em organismos (alvo e não alvo) e no ambiente. Assim como, é de grande relevância propor formulações e ou métodos de produção que reduzam o impacto ambiental e à saúde humana (Kah et al., 2018).

O uso de nanotecnologia na agricultura tem demonstrado grande potencial na criação de novas formulações, bem como para melhorar a eficiência e segurança de pesticidas já existentes (Zhao et al., 2018). Neste contexto, a nanoatrazina foi desenvolvida como uma alternativa para minimizar os efeitos adversos da atrazina convencional ao meio ambiente (Clemente et al., 2013; Grillo et al., 2010; Grillo et al., 2012; Kah et al., 2014; Oliveira et al., 2015a, b, c; Pereira et al., 2014; Souza et al., 2012; Taverna et al., 2018; Xiao-Ting and Wang, 2019). A nanopartícula lipidica sólida carrregada com atrazina e simazine é um exemplo de nanosistema que apresentou excelente atividade herbicida contra o organismo alvo ***Raphanus raphanistrum***, reduziu a fitotoxicidade para *Zea mays* (planta) e citotoxicidade para células 3T3 de fibroblastos de rato. No entanto, esta mesma formulação apresentou efeito tóxico sobre o organismo de solo *Caenorhabditis elegans* (Jacques et al., 2017), indicando a necessidade de novos estudos ecotoxicológicos.

Para compreender os potenciais efeitos tóxicos da nanoatrazina são necessários estudos com diferentes organismos representativos de ecossistemas aquáticos e terrestres

(Albuquerque et al., 2020). Nanopartículas em ambientes aquáticos podem se depositar no sedimento e afetar diretamente os macroinvertebrados bentônicos, destacando-se algumas espécies da família Chironomidae que possuem o hábito de ingerir partículas de sedimento, podendo acumular altas concentrações de contaminantes em seu corpo, incluindo compostos químicos com propriedades em nanoescala (Oberholster et al., 2011; Strixino & Strixino, 1981, 1982).

Na região Neotropical, o amplo uso de *Chironomus sancticaroli* em bioensaios ecotoxicológicos está associado as suas características, pois esta espécie possui um ciclo de vida que dura aproximadamente 13 dias, tem um tamanho relativamente pequeno, pode ser cultivada em laboratório, é facilmente coletada em ambientes naturais e sua distribuição ocorre no Brasil e Argentina (Araújo et al., 2006; Fonseca & Rocha, 2004; Strixino & Strixino, 1981, 1982). Sendo assim, *Chironomus sancticaroli* pode ser considerado um organismo representativo para avaliar os potenciais efeitos tóxico da nanoatrazina.

Diante do exposto, o principal objetivo da presente tese foi avaliar os efeitos ecotoxicológicos de nanopartícula lipídica sólida (vazia e carregada com atrazina) sobre larvas de *Chironomus sancticaroli* (organismo não alvo), em condições de laboratório através de bioensaios agudo (96 h) e subcrônico (10 dias), a fim de obter dados sobre o potencial toxicológico destes nanosistemas em comparação com atrazina (ingrediente ativo).

A tese foi dividida em dois capítulos, os quais são compostos por um artigo de revisão publicado na revista Science of the Total Environment e outro de práticas.

No primeiro capítulo é apresentado uma revisão de literatura da tese, enfatizando o potêncial de contaminação da atrazina além da área de aplicação, seus efeitos toxicológicos sobre organismos aquáticos e o uso de nanotecnologia para reduzir a toxicidade deste herbicida através da micro/nanoencapsulação.

O segundo capítulo consta os bioensaios agudos e subcrônicos com larvas de *Chironomus sancticaroli*, quais foram desenvolvidos para avaliar a toxicidade das nanopartículas lipídicas sólidas carregadas com atrazina, nanopartículas controle (sem atrazina) e atrazina (ingrediente ativo), através da análise do efeito letal, deformidade do mento, desenvolvimento larval e biomarcadores bioquímicos (acetilcolinesterase, esterase alfa, esterase beta e glutationa S-transferase).

Após, consta uma conclusão geral e os testes com substâncias de referência, realizados para verificar a sensibilidade da cultura de *Chironomus sancticaroli* e validar os bioensaios.

Referências

Albuquerque, F.P., Oliveira, J.L., Moschini-Carlos, V., Fraceto, L.F., 2020. An overview of the potential impacts of atrazine in aquatic environments: Perspectives for tailored solutions based on nanotechnology. Sci Total Environ 700, 1–9. https://doi.org/10.1016/j.scitotenv.2019.134868.

Araújo, R.P.A., Shimizu, G.Y., Almeida, C.A., Rocha, O., 2006. Testes com invertebrados para avaliar a toxicidade de contaminantes associados ao sedimento de ecossistemas de água doce. in: Mozeto, A.A., Umbuzeiro, G.A., Jardim, W.F. (Eds.), Métodos de coleta, análises físico-químicas e ensaios biológicos e ecotoxicológicos de sedimentos de água doce. São Carlos, pp. 111–133.

Clemente, Z., Grillo, R., Jonsson, M., Santos, N.Z.P., Feitosa, L.O.; Lima, R., Fraceto, L.F., 2013. Ecotoxicological evaluation of poly (epsilon-caprolactone) nanocapsules containing triazine herbicides. J. Nanosci. Nanotechnol. 13, 1–7. https://doi.org/10.1166/jnn.2013.8681.

Fonseca, A.L., Rocha, O., 2004. Laboratory cultures of the native species *Chironomus Xanthus* Rempel, 1939 (Diptera-Chironomidae). Acta Limnol. Bras. 16, 153–161.

Grillo, R., Melo, N.F.S., Lima, R., Lourenço, R.W., Rosa, A.H., Fraceto, L.F., 2010. Characterization of atrazine-loaded biodegradable poly (hydroxybutyrate-co-hydroxyvalerate) microspheres. J. Polym. Environ. 18, 26–32. https://doi.org/10.1007/s10924-009-0153-8.

Grillo, R., Santos, N.Z.P., Maruyama, C.R., Rosa, A.H., Lima, R., Fraceto, L.F., 2012. Poly (-caprolactone) nanocapsules as carrier systems for herbicides: Physico-chemical characterization and genotoxicity evaluation. J. Hazard. Mater. 231, 1–9. https://doi.org/10.1016/j.jhazmat.2012.06.019.

Jacques, M.T., Oliveira, J.L., Campos, E.V.R., Fraceto, L.F., Ávila, D.S., 2017. Safety assessment of nanopesticides using the roundworm *Caenorhabditis elegans*. Ecotoxicol. Environ. Saf. 139, 245–253. https://dx.doi.org/10.1016/j.ecoenv.2017.01.045.

Kah, M., Machinski, P., Koerner, P., Tiede, K., Grillo, R., Fraceto, L.F., Hofmann, T., 2014. Analysing the fate of nanopesticides in soil and the applicability of regulatory protocols using a polymer-based nanoformulation of atrazine. Environ. Sci. Pollut. Res. 21, 11699–11707. https://doi.org/10.1007/s11356-014-2523-6.

Kah, M., Kookana, R.S., Gogos, A., Bucheli, T.D.A, 2018. critical evaluation of nanopesticides and nanofertilizers against their conventional analogues. Nat. Nanotechnol. 13, 677–684. https://doi.org/10.1038/s41565-018-0131-1.

Lushchak, V.I., Matviishyna, T.M., Husaka, V.V., Storey, J.M., Storey, K.B., 2018. Pesticide toxicity: a mechanistic approach. Experimental and Clinical Sciences 17, 1101–1136. https://dx.doi.org/10.17179/excli2018-1710.

Oberholster, P.J., Musee, N., Botha, A.M., Chelule, P.K., Focke, W.W., Ashton, P.J., 2011. Assessment of the effect of nanomaterials on sediment-dwelling invertebrate *Chironomus tentans* larvae. Ecotoxicol. Environ. Saf. 74, 416–423. https://doi.org/10.1016/j.ecoenv.2010.12.012.

Oliveira, H.C., Stolf-Moreira, R., Martinez, C.B.R., Grillo, R., Jesus, M. B., Fraceto, L.F., 2015a. Nanoencapsulation enhances the post-emergence herbicidal activity of atrazine against mustard plants. Plos One 10, 1–12. https://doi.org/10.1371/journal.pone.0132971.

Oliveira, J.L., Campos, E.V.R., Silva, C.M.G., Pasquoto, T., Lima R., Fraceto, L. F., 2015b. Solid lipid nanoparticles co-loaded with simazine and atrazine: preparation, characterization, and evaluation of herbicidal activity. J. Agric. Food Chem. 63, 422–432. https://doi.org/10.1021/jf5059045.

Oliveira, H.C., Stolf-Moreira, R., Martinez, C.B.R., Sousa, G.F.M., Grillo, R., Jesus, M.B., Fraceto, L.F., 2015c. Evaluation of the side effects of poly(epsilon-caprolactone) nanocapsules containing atrazine toward maize plants. Front. Chem. 3, 1–9. https://doi.org/10.3389/fchem.2015.00061.

Pereira, A.E.S., Grillo, R., Mello, N.F.S., Rosa, A.H., Fraceto, L.F., 2014. Application of poly (epsilon-caprolactone) nanoparticles containing atrazine herbicide as an alternative technique to control weeds and reduce damage to the environment. J. Hazard. Mater. 268, 207–215. https://dx.doi.org/10.1016/j.jhazmat.2014.01.025.

Souza, P.M.S., Lobo, F.A., Rosa, A.H., Fraceto, L.F., 2012. Desenvolvimento de nanocápsulas de poli-e-caprolactona contendo o herbicida atrazina. Quim. Nova 35, 132–137. https://dx.doi.org/10.1590/S0100-40422012000100024.

Strixino, S.T., Strixino, G., 1981. Nova espécie do gênero *Chironomus* Meigen do sul do Brasil (Diptera: Chironomidae). Rev. Bras. Entomol. 25, 333–340.

Strixino, S.T., Strixino, G., 1982. Ciclo de vida de *Chironomus sancticaroli* Strixino & Strixino, (Diptera, Chironomidae). Rev. Bras. Entomol. 26, 183–189.

Taverna, M.E., Busatto, C.A., Lescano, M.R., Nicolau, V.V., Zalazar, C.S., Meira, G.R., Estenoz, D.A., 2018. Microparticles based on ionic and organosolv lignins for the controlled

release of atrazine. J. Hazard. Mater. 359, 139–147. https://doi.org/10.1016/j.jhazmat.2018.07.010.

Xiao-Ting, C., Wang, T., 2019. Preparation and characterization of atrazine-loaded biodegradable PLGA nanospheres. J. Integr. Agric. 18, 1035–1041. https://doi.org/10.1016/S2095-3119(19)62613-4.

Zhang, W., 2018. Global pesticide use: Profile, trend, cost / benefit and more. Proc. Int. Acad. Ecol. Environ. Sci. 8, 1–27.

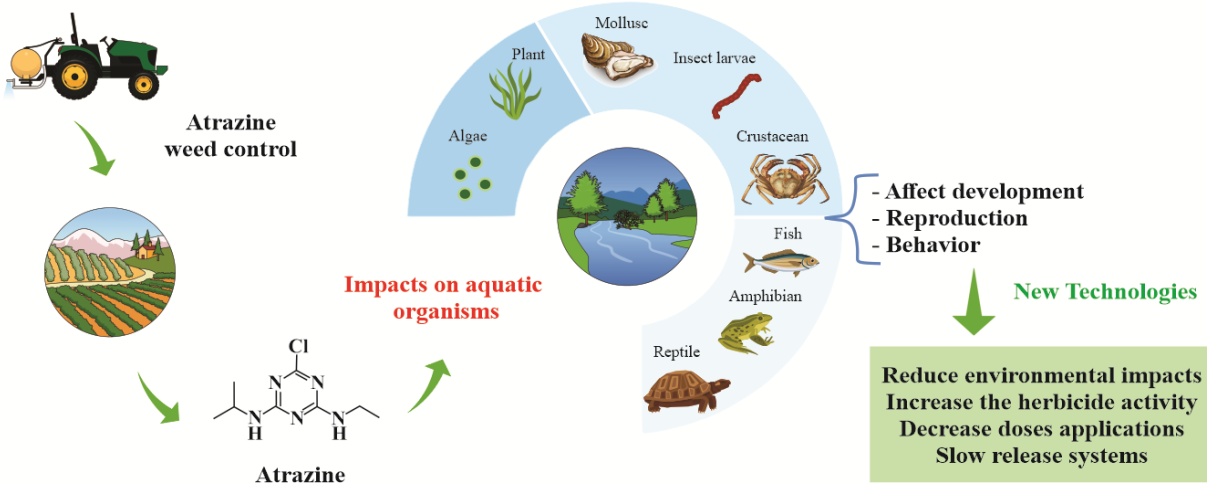
Zhao, F., Li, Y., Huang, L., Gu, Y., Zhang, H., Zeng, D., Tan, H., 2018. Individual and combined toxicity of atrazine, butachlor, halosulfuronmethyl and mesotrione on the microalga *Selenastrum capricornutum*. Ecotoxicol. Environ. Saf. 148, 969–975. https://doi.org/10.1016/j.ecoenv.2017.11.069.

CHAPTER I

An overview of the potential impacts of atrazine in aquatic environments: perspectives for tailored solutions based on nanotechnology

Science of the Total Environment, v. 700, 2020.

doi: https://doi.org/10.1016/j.scitotenv.2019.134868



An overview of the potential impacts of atrazine in aquatic environments: perspectives for tailored solutions based on nanotechnology

Felícia Pereira de Albuquerque*, Jhones Luiz de Oliveira, Viviane Moschini-Carlos, Leonardo Fernandes Fraceto*

São Paulo State University (UNESP), Institute of Science and Technology of Sorocaba, Av. Três de março, 511, Alto da Boa Vista, 18087-180, Sorocaba, Brazil

* **Corresponding author at:** São Paulo State University (UNESP), Institute of Science and Technology of Sorocaba, Av. Três de março, 511, Alto da Boa Vista, 18087-180, Sorocaba, Brazil

E-mail address: felicia.pa@hotmail.com (F.P. de Albuquerque); leonardo.fraceto@unesp.br (L.F. Fraceto).

- The effects of atrazine are not restricted to the target organism.
- At environmentally relevant concentrations, atrazine can cause sublethal effects in aquatic organisms.
- Atrazine affects the development, reproduction, and behavior of many aquatic species.
- Nanoencapsulation of atrazine could potentially be used to reduce its adverse effects.

Abstract

Atrazine is a pre- and post-emergence herbicide used to control weeds in many crops. It was introduced in the late 1950s, but its use has been controversial because of its high potential for environmental contamination. In agriculture, the implementation of sustainable practices can help in reducing the adverse effects atrazine. This review addresses aspects related to the impacts of atrazine in the environment, with focus on its effects on aquatic species, as well as the potential use of nanoencapsulation to decrease the impacts of atrazine. The application of atrazine leads to its dispersal beyond the immediate area, with possible contamination of soils, sediments, plantations, pastures, public supply reservoirs, groundwater, streams, lakes, rivers, seas, and even glaciers. In aquatic ecosystems, atrazine can alter the biota, consequently interfering in the food chains of many species, including benthic organisms. Nanoformulations loaded with atrazine have been developed as a way to reduce the adverse impacts of this herbicide in aquatic and terrestrial ecosystems. Ecotoxicological bioassays have shown that this nanoformulations can improve the targeted delivery of the active ingredient, resulting in decreased dosages to obtain the same effects as conventional formulations. However, more detailed analyses of the ecotoxicological potential of atrazine-based nanoherbicides need to be performed with representative species of different ecosystems.

Keywords: Agrochemical; aquatic organism; ecotoxicity; nanotechnology; triazinic herbicide.

1. Introduction

Herbicides are applied in various crops, in order to kill weeds following uptake of the chemicals into the leaves, stems, or roots (Kim et al., 2017; Lushchak et al., 2018). These chemical compounds have different mechanisms of action and can be classified as growth

regulators, cell membrane disruptors, and inhibitors (Lushchak et al., 2018). They can affect seedling growth, photosynthesis, and the biosynthesis of amino acid, lipids, and pigments (Lushchak et al., 2018).

Atrazine is a synthetic herbicide that has been available on the market for more than 50 years. It was patented in Switzerland in 1958 and was registered for commercial use in the United States in 1959, after which it became used worldwide (Solomon et al., 1996). However its use has been controversial due to its persistence and mobility, resulting in it being detected in the soil, plantations, pastures, reservoirs used for public water supply, groundwater, streams, lakes, rivers, seas, and even glaciers in remote areas (Carmo et al., 2013; Garcia et al., 2012; Hénault-Ethier, 2016; Jablonowski et al., 2011; Nödler et al., 2013; Solomon et al., 1996; Sun et al., 2017).

Due to the high potential of atrazine to contaminate groundwater, in 2004 it was removed from the list of approved products in the European Union (Ackerman, 2007). The use of this herbicide is decreasing in Canada, where many formulations are already restricted in the west of the country (Hénault-Ethier, 2016). However, atrazine still is widely used in agriculture in other countries, highlighting the United States, Brazil, China, and India (Balakrishnan and Athilakshmi, 2016; Rusiecki et al., 2004; Sass and Colangelo, 2006; Sun et al., 2017). It was reported in 2002 that the amount of atrazine used in China is up to 5.000 tons per year (Jin and Ke, 2002). In India, the annual consumption of atrazine reached 340 tons in 2008 (Kadian et al., 2008). In Brazil, 24.731 tons of the herbicide were sold in 2017 (Brasil, 2019), while in the United States, over 30.000 tons are applied annually (Lorber-Pascal and Laurente, 2011).

Surprisingly, even after being banned, atrazine continues to be detected in European coastal waters. An example is the Aegean Sea, which is influenced by the exchange of water with the Marmara Sea and the Black Sea, bordered by countries where atrazine is still used.

This shows that even the prohibition of chemical products at an international level is unable to prevent continuing contamination (Nödler et al., 2013). Atrazine residues were detected in the urine of pregnant women, when it had already been banned, in the Brittany region of France (Chevrier et al., 2011). In Croatia, atrazine residues were found in urine samples from rural workers, both before and after application of the chemical (Mendas et al., 2012).

The effects of atrazine are not restricted to the target organism, with the presence of this herbicide in terrestrial and aquatic environments having the potential to affect a great diversity of species, consequently threatening environmental sustainability (Singh et al., 2018). The present review discusses the adverse impacts of atrazine, focusing on its harmful effects on species present in aquatic environments. Particular attention is given to new technologies that could assist in reducing the toxicity of this herbicide, such as micro/nanoencapsulation techniques.

2. Principle of action of atrazine and its potential for contamination

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) is a selective pre- and post-emergence herbicide used to control weeds in cultivations including maize, sorghum, sugar cane, and pineapple, as well as in landscaping (Nwani et al., 2010; Solomon et al., 1996). This pesticide acts on the target organism by inhibiting photosynthesis, with blocking of electron transport in photosystem II (Forney and Davis, 1981). The blocking occurs because atrazine attaches at the plastoquinone Q_B (electron acceptor) binding site, consequently interrupting the flow of electrons between the photosystems (Fig. 1) (Marchi et al., 2008). With this inhibition, the electrons are not converted into chemical energy (adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH)) and impose a high energy load on chlorophyll molecules, leading to lipid peroxidation in the membranes, destruction of leaf chlorophyll, inhibition of carbohydrate synthesis, reduction of

the carbon stock, and accumulation of carbon dioxide within plant cells (Marchi et al., 2008; Shabana, 1987).

In the case of pre-emergence application, atrazine is absorbed by the plants through the roots, subsequently being transported in the xylem to the leaves, where its action causes chlorosis, necrosis, and death. In post-emergence application, the chemical is absorbed by the leaves (Fig. 1) (Souza et al., 2012).

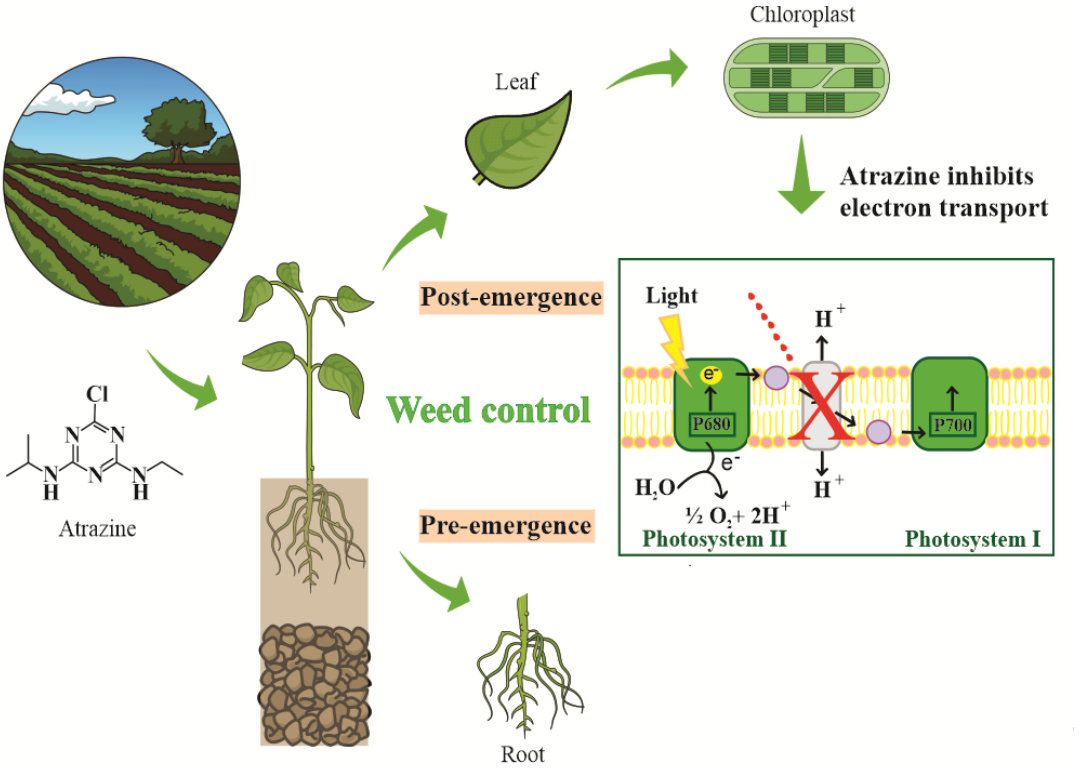


Fig. 1. Absorption of atrazine in weeds (target organisms), during the pre-emergence, it occurs mainly through the roots and in post-emergence through the leafs. The atrazine inhibits photosynthesis on target organism, blocking the electron transport in photosystem II.

Atrazine is considered to be a selective herbicide, enabling its use in several crops. Due to the different metabolic pathways and rates of metabolization of the chemical in weeds and crops, the species that are resistant rapidly convert the herbicide into nontoxic metabolites (Ebert and Dumford, 1976; Forney and Davis, 1981; Roman et al., 2007).

Atrazine has low vapor pressures, is moderately soluble in water, with solubility of 33.0 $mg.L^{-1}$ at 22 ºC, has low soil adsorption coefficient (k_{oc}) 100 cm^3 g^{-1} and an octanol partition coefficient water (log K_{ow}) of 2.75 (Balci et al., 2009; Baranowska et al., 2008; Carmo et al., 2013). However, it has high potential to contaminate groundwater, since it is poorly adsorbed in the organic fraction of the soil, consequently presenting high leaching potential, especially in a well-structured soil profile with macropores (Dias et al., 2018; Graymore et al., 2001).

When released into the environment, atrazine undergoes chemical, photochemical, and biological reactions that are responsible for its degradation to other compounds (Chevrier et al., 2011), each transformation product varying in its persistence and toxicity (Graymore et al., 2001). Examples are its transformation products deethylatrazine and deisopropylatrazine, which are frequently detected in surface and groundwater, in many regions worldwide, and can persist in water and soil for decades (Dores and De-Lamonica-Freire, 2001; Jablonowski et al., 2011).

Rainfall contributes to the dispersal of atrazine in ecosystems due to surface runoff, allowing it to be transported from the field to aquatic systems around the application area (Jablonowski et al., 2011). The transport of this contaminant in the environment can be attributed to its characteristics including resistance to decomposition by microorganisms, stability in soil and water, and half-life times varying from 14 days to 4 years in soil and from 6 months to several years in water (Guan et al., 2013; Stara et al., 2018).

Industries may also be a source of local contamination. In China (Changxing County, Zhejiang Province), high concentrations of atrazine were detected in a region close to a pesticide factory where atrazine was one of the main products (Sun et al., 2017).

Also, the application of pesticides in residential and agricultural areas can lead the simultaneous mixing of chemical compounds in aquatic environments with different

mechanisms of action, causing an increase in toxicity, greater disturbance of aquatic ecosystems than expected considering the individual toxicities of the substances in isolation (Pérez et al., 2013).

3. Presence of atrazine in aquatic environments

Atrazine is frequently detected in aquatic ecosystems, where its presence affects the reproduction of aquatic fauna and flora, interfering with the structure of every community (Graymore et al., 2001), because its adverse effects on aquatic plants may alter the survival, growth, and/or reproduction of herbivores and predators (Solomon et al., 1996).

Atrazine can significantly inhibit algal growth and photosynthesis (Zhu et al., 2016). In evaluation of the ecological risk of atrazine in North American surface waters, phytoplankton were considered to be the organisms most sensitive to this contaminant, followed by macrophytes, benthic invertebrates, zooplankton, and fish (Solomon et al., 1996).

Studies have demonstrated that atrazine has the potential to reduce cellular metabolism and to influence the formation of reactive oxygen species (ROS), altering the antioxidant activity in fish (Nwani et al., 2010; Owolabi and Omotosho, 2017; Santos and Martinez, 2012), crustaceans (Schmidt et al., 2017; Stara et al., 2018), and chironomid larvae (Londoño et al., 2004).

Some European countries had already banned the use of atrazine before the decision of the European Union (Ackerman, 2007). At the same time when this herbicide was banned by the European Union, the United States renewed authorization of its usage, rejecting the allegations that atrazine may cause serious risks to the environment and human health (Ackerman, 2007; Sass and Colangelo, 2006).

Before atrazine was banned by the European Union, a uniform limit of 0.1 µg.L^{-1} of the pesticide residue was established for drinking water and groundwater (Ackerman, 2007). The World Health Organization allows a maximum concentration of 100 µg.L^{-1} for atrazine and its chloro-s-triazine metabolites in drinking water (WHO, 2017). In Australia is allowed 20 µg.L^{-1} of atrazine (NHMRC/NRMMC, 2011), in the United States 3 µg.L^{-1} (USEPA, 2009) and in the Brazil 2 µg.L^{-1} for freshwater, considering the use of the water for human consumption and the protection of aquatic life (CONAMA).

A review of the literature, considering articles published in the last five years, provides ample evidence that atrazine at different concentrations, including those that are environmentally relevant, can affect the development, behavior, and reproduction of many species present in the aquatic environment (Table 1; Fig. 2).

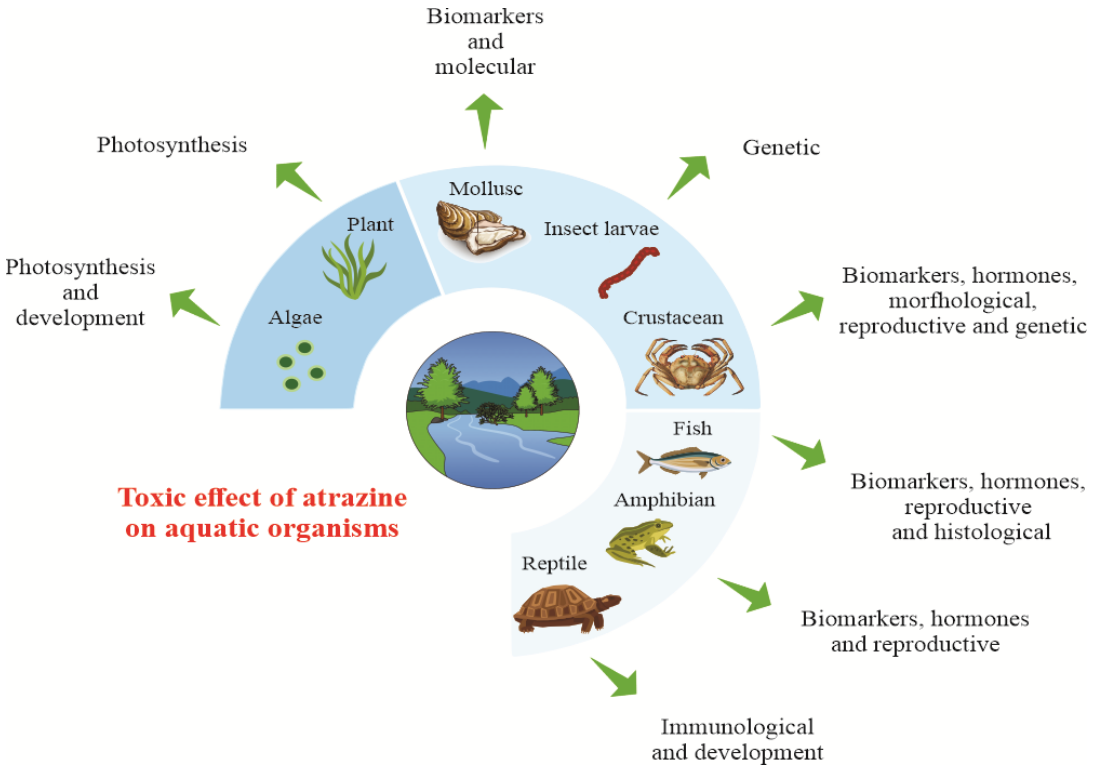


Fig. 2. The presence of atrazine in aquatic environments causes toxic effects on organisms representative this ecosystem. Each group represents different species that were exposed to atrazine, together with the corresponding toxic effects. Illustration adapted from Table 1.

Despite having the potential to cause toxic effects presenting toxic effects towards aquatic species, atrazine continues to be used in several countries, indicating the need to search for solutions and technologies that can reduce its impact (Singh et al., 2018), especially because agricultural activity is an important source of contamination of the environment with this herbicide (Sun et al., 2017).

4. Use of nanotechnology in the search for less harmful formulations

In 2020, there is estimated to be an increase of half a million tons in the global production of nanomaterials developed with specific characteristics for different applications (Rocha et al., 2015). The chemical structures of nanomaterials used in agriculture can be modified according to the type of plant or soil to which they will be applied, hence offering strategies for intelligent release and delivery of active agents (Lowry et al., 2019). The application of nanotechnology and the introduction of nanomaterials in agriculture can contribute to sustainable development and maximize global food production (Fraceto et al., 2016; Kah et al., 2019).

Nanotechnology is a field of knowledge that is advancing terms of basic research, the development of new techniques, and the production of novel materials. Formulations based on nanotechnology have several objectives: i) to increase dispersibility of the active compounds; (ii) to release them slowly; iii) to protect them against premature degradation caused by environmental factors; iv) to direct delivery of the active ingredients more effectively, enabling reductions in the amounts used (Chhipa, 2019). In this way, using smaller amounts of active agents and releasing them in a controlled way, these formulations enable the agents to remain available at the target sites for extended periods, at the concentrations required for effective action. This increases efficiency, reduces toxicity, and helps to avoid environmental contamination. Furthermore, these formulations also help to

reduce the level of exposure of rural workers to agrochemicals, hence decreasing undesirable health effects (He et al., 2019).

Various atrazine-loaded nanoformulations (polymeric nanocapsules, nanospheres, and solid lipid nanoparticles) have been developed with the aim of reducing both environmental impacts and the amounts of this herbicide applied in the field (Clemente et al., 2013; Grillo et al., 2012; Kah et al., 2014; Oliveira et al., 2015a; Oliveira et al., 2015b; Oliveira et al., 2015c; Pereira et al., 2014; Souza et al., 2012). These effects are achieved because the use of encapsulation techniques provides protection of the active agent against physico-chemical and microbiological degradation (Pereira et al., 2014).

Triazine herbicides are commonly used in combinations, in order to increase the effectiveness of weed control. For this purpose, a system consisting of nanocapsules of poly(epsilon-caprolactone) (PCL) containing atrazine, ametryn, and simazine was developed, which proved to be less toxic, compared to the free herbicides, in genotoxicity tests with human lymphocyte cells at concentration 100 $mg.L^{-1}$ and *Allium cepa* at concentrations 1, 10 and 100 $mg.L^{-1}$ (Grillo et al., 2012). Clemente et al. (2013) also evaluated PCL nanocapsules loaded with atrazine and amethrin (at concentrations 1.5 – 30 $mg.L^{-1}$), employing cytogenetic analyses and bioassays with the aquatic organisms *Pseudokirchneriella subcapitata* (alga) and *Daphnia similis* (microcrustacean). It was observed that the encapsulation reduced cell damage in tests with lymphocyte cultures, while use of the formulation led to lower toxicity towards the alga, but higher toxicity towards the microcrustacean, which could have been due to the presence of other compounds in the formulation, such as surfactants.

In the work of Pereira et al. (2014), it was found that in comparison to the free herbicide, atrazine contained in PCL nanoparticles presented greater efficiency in control of the target organism (*Brassica* sp.), did not cause damage to the nontarget organism (*Zea mays*), reduced the mobility of atrazine in the soil, and decreased the genotoxicity of this

pesticide. In herbicidal activity assays and in soil column experiments was applied a formulation with the concentration of the active principle equivalent to 2.5 $kg.ha^{-1}$, already in *Allium cepa* chromosome aberration assays the seeds were germinated in the presence of different concentrations (0.7, 2.1, 6.3, 18, and 54 $\mu g.mL^{-1}$) (Pereira et al., 2014).

In bioassays with *Brassica juncea* (the target organism), Oliveira et al. (2015a) found that atrazine-loaded PCL nanocapsules at concentrations 2000 and 200 $g.ha^{-1}$ decreased overall photosynthesis and the maximum quantum yield of photosystem II, induced leaf lipid peroxidation, and caused inhibition of growth of the aerial part. The development of severe symptoms demonstrated the highly effective action of this system applied post-emergence. In tests with a nontarget organism (*Zea mays* L.), empty and atrazine-loaded PCL nanocapsules (at concentration 2000 and 200 $g.ha^{-1}$) did not cause any persistent deleterious effects, indicating that this nanosystem could be used as a safe tool in weed control, without affecting the development of the crop (Oliveira et al., 2015c).

In tests using atrazine-loaded PCL nanocapsules (at concentrations of atrazine of 0, 1, 5, 10, 50, 100 and 200 $mg.kg^{-1}$ of dry weight soil) against the soil invertebrate *Enchytraeus crypticus*, it was observed that empty nanocapsules did not affect this species, while the atrazine-loaded nanocapsules presented effects that differed from those of the free herbicide, which could be explained by different mechanisms of absorption, differential release of atrazine following nanoencapsulation, or the combination of these two factors (Gomes et al., 2019).

Andrade et al. (2019) used the fish *Prochilodus lineatus* to test the effect of atrazine-loaded PCL nanoparticless (at concentration of 2 and 20 $\mu g.L^{-1}$) and obtained satisfactory results, with integrated biomarker analysis demonstrating that the nanoencapsulation of the herbicide protected this fish species against the effects of the chemical.

Oliveira et al. (2015b) developed solid lipid nanoparticles (SLN) loaded with atrazine in combination with simazine, which proved to be effective against the target pest *Raphanus raphanistrum* (at concentration 3 and 0.3 $kg.ha^{-1}$), while presenting decreased toxicity towards nontarget organisms. Use of the system reduced both cytotoxicity towards rat 3T3 fibroblast cells (at concentrations 15.6, 31.25, and 62.5 $\mu g.mL^{-1}$) and phytotoxicity towards *Zea mays* plants (at concentration 3 and 0.3 $kg.ha^{-1}$). Jacques et al. (2017) evaluated the effect of these nanoparticles on the nematode *Caenorhabditis elegans* (at concentrations 0.1 – 0.5 $mg.mL^{-1}$), observing toxicity for the SLN alone and loaded with atrazine and simazine, while the herbicides themselves did not cause mortality of the worms. The authors suggested that the activity could have been associated with the composition of the nanoparticles, indicating the need for further ecotoxicological studies.

In recent work by Xiao-Ting and Wang (2019), atrazine-loaded nanoparticles of poly (lactic acid-co-glycolic acid) were developed and characterized (considering morphology, size, encapsulation efficiency, and release profile). According to the authors, the herbicide delivery system developed is promising in order to reduce the impact on the environment and minimize possible damage to farmers, although more work is needed to evaluate its toxicity in different matrices.

Taverna et al. (2018) prepared lignin microspheres for the release of atrazine, with the microparticles presenting efficient release of the herbicide, as well as a lower rate of leaching in the soil, compared to free atrazine. Although this system showed potential for use, it remains necessary to carry out ecotoxicological bioassays.

The studies presented in the literature indicate that encapsulated atrazine has the potential to be used as a more environmentally friendly alternative, because it promotes a reduction in toxicity due to the characteristics of the release method. The nano/microencapsulation of active principle result in effective action against target

organisms, while showing low toxicity towards nontarget species. However, despite this potential, further ecotoxicological studies with nano-formulated atrazine are needed, considering other species representative of aquatic and terrestrial environments. In addition, it is important that these nanosystems should be tested in bioassays that simulate different soil conditions, humidity, and temperature.

5. Conclusions and perspectives

The effects of atrazine are not restricted to the target organism, because the herbicide comes into contact with the wider environment and can interfere in the life cycles of many species. Atrazine is considered to be an effective herbicide in the control of target organisms, but may cause widely varying harm to nontarget species, due to its high potential to contaminate aquatic and terrestrial ecosystems. At concentrations that are environmentally relevant, this herbicide can cause sublethal effects in aquatic organisms.

Nano-formulated atrazine may potentially be an alternative that could be used to reduce the impacts of atrazine, since studies have shown that nanoformulations containing this herbicide improved the release of the active agent, allowed the application of lower doses (ten times smaller than conventional doses), and provided efficient herbicidal activity in tests with target organisms (Clemente et al., 2013; Grillo et al., 2012; Oliveira et al., 2015a; Oliveira et al., 2015b; Oliveira et al., 2015c; Pereira et al., 2014; Souza et al., 2012). These published results indicate the importance of performing ecotoxicological tests using these nanomaterials with different species, in order to identify formulations that do not present lethal and/or sublethal effects towards nontarget organisms.

The introduction of nanomaterials in the agricultural area can contribute to a shift towards more sustainable practices, reducing damage to human health and the environment, while ensuring more efficient utilization of pesticides and fertilizers (Damalas and

Eleftherohorinos, 2011; Fraceto et al., 2016; Grillo et al., 2012; Jacques et al., 2017; Oliveira et al., 2015a).

Nanoformulations need to be studied and evaluated in terms of their efficiency, toxicity, production scale, composition, degradation behavior, and cost-benefit, preferably in comparison with the conventional analogue (Andrade et al., 2019; Fraceto et al., 2016; Jacques et al., 2017; Kah et al., 2018). These studies may contribute to the establishment of regulatory frameworks for the use of nanotechnology in agriculture. However, the successful implementation of nanotechnological interventions will require collaboration among the areas of scientific research, policy development, and industrial technology (Kah et al., 2019; Mahawar and Prasanna, 2018).

Acknowledgments

This study was financed in part by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil, finance code 001). L.F.F. would like to thank the São Paulo State Research Foundation (FAPESP, grant #2017/21004-5) for financial support for nanopesticide development.

6. References

Abdulelah, S.A., Crile, K.G., Almouseli, A., Awali, S., Tutwiler, A.Y., Tien, E.A., Manzo, V.J., Hadeed, M.N., Belanger, R.M., 2020. Environmentally relevant atrazine exposures cause DNA damage in cells of the lateral antennules of crayfish (*Faxonius virilis*). Chemosphere 239, 1–6. https://doi.org/10.1016/j.chemosphere.2019.124786.

Ackerman, F., 2007. The Economics of Atrazine. Int. J. Occup. Environ. Health 13, 437–445. https://doi.org/10.1179/oeh.2007.13.4.437.

304 Adeyemi, J.A., Martins-Junior, A.C., Barbosa Jr., F., 2015. Teratogenicity, genotoxicity and
305 oxidative stress in zebrafish embryos (*Danio rerio*) co-exposed to arsenic and atrazine.
306 Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol. 172, 7-12.
307 https://dx.doi.org/10.1016/j.cbpc.2015.04.001.
308 Álvarez, N.B., Avigliano, L., Loughlin, C.M., Rodríguez, E.M., 2015. The adverse effect of
309 the herbicide atrazine on the reproduction in the intertidal varunid crab *Neohelice granulata*
310 (Dana, 1851). Reg. Stud. Mar. Sci. 1, 1–6. https://dx.doi.org/10.1016/j.rsma.2014.12.001.
311 Andrade, L.L., Pereira, A.E.S., Fraceto, L.F., Martinez, C.B.R., 2019. Can atrazine loaded
312 nanocapsules reduce the toxic effects of this herbicide on the fish *Prochilodus lineatus*? A
313 multibiomarker approach. Sci. Total Environ. 663, 548–559.
314 https://doi.org/10.1016/j.scitotenv.2019.01.380.
315 Araújo, C.V.M., Silva, D.C.V.R., Gomes, L.E.T., Acayaba, R.D., Montagner, C.C., Moreira-
316 Santos, M., Ribeiro, R., Pompeo, M.L.M., 2018. Habitat fragmentation caused by
317 contaminants: Atrazine as a chemical barrier isolating fish populations. Chemosphere, 193,
318 24–31. https://doi.org/10.1016/j.chemosphere.2017.11.014.
319 Balakrishnan, L., Athilakshmi, N., 2016. Degradation of atrazine by *Pseudomonas* spp and
320 *Bacillus* spp from *Saccharum officinarum* (sugar cane) fields of southern india and its
321 potential application in bioremediation. AJST 7, 2903–2911.
322 Balci, B., Oturan, N., Cherrier, R., Oturan, M.A., 2009. Degradation of atrazine in aqueous
323 medium by electrocatalytically generated hydroxyl radicals. A kinetic and mechanistic study.
324 Water Res. 43, 1924–1934. https://doi.org/10.1016/j.watres.2009.01.021.
325 Baranowska, I., Barchanska, H., Abuknesha, R.A., Price, R.G., Stalmach, A., 2008. ELISA
326 and HPLC methods for atrazine and simazine determination in trophic chains samples.
327 Ecotoxicol. Environ. Saf. 70, 341–348. https://doi.org/10.1016/j.ecoenv.2007.06.012.

328 Bautista, F.E.A., Varela Jr., A.S., Corcini, C.D., Acosta, I.B., Caldas, S.S., Primel,
329 E.G., Zanette, J., 2018. The herbicide atrazine affects sperm quality and the expression of
330 antioxidant and spermatogenesis genes in zebrafish testes. Comp. Biochem. Physiol., Part C:
331 Toxicol. Pharmacol. 206, 17–22. https://doi.org/10.1016/j.cbpc.2018.02.003.
332 Brasil, 2019. IBAMA – Instituto Brasileiro do Meio Ambiente. Available:
333 <http://www.ibama.gov.br/agrotoxicos/relatorios-de-comercializacao-de-agrotoxicos>. 21
334 jun. 2019.
335 Brain, R.A., Schneider, S.Z., Anderson, J.C., Knopper, L.D., Wolf, J.C., Hanson, M.L., 2018.
336 Extended fish short term reproduction assays with the fathead
337 minnow and Japanese medaka: No evidence of impaired fecundity
338 from exposure to atrazine. Chemosphere 205, 126–136.
339 https://doi.org/10.1016/j.chemosphere.2018.04.068.
340 Baxter, L., Brain, R.A., Hosmer, A.J., Nema, M., Müller, K.M., Solomon, K.R., Hanson,
341 M.L., 2015. Effects of atrazine on egg masses of the yellow-spotted salamander (*Ambystoma*
342 *maculatum*) and its endosymbiotic alga (*Oophila amblystomatis*). Environ. Pollut. 206, 324–
343 331. https://dx.doi.org/10.1016/j.envpol.2015.07.017.
344 Baxter, L., Brain, R.A., Lissemore, L., Solomon, K.R., Hanson, M.L., Prosser, R.S., 2016.
345 Influence of light, nutrients, and temperature on the toxicity of
346 atrazine to the algal species *Raphidocelis subcapitata*: Implications for the risk assessment of
347 herbicides. Ecotoxicol. Environ. Saf. 132, 250–259.
348 https://dx.doi.org/10.1016/j.ecoenv.2016.06.022.
349 Botelho, R.G., Monteiro, S.H., Christofoletti, C.A., Moura-Andrade, G.C.R., Tornisielo,
350 V.L., 2015. Environmentally relevant concentrations of atrazine and ametrine induce
351 micronuclei formation and nuclear abnormalities in erythrocytes of fish.
352 Arch. Environ. Contam. Toxicol. 69, 577–585. https://doi.org/10.1007/s00244-015-0171-6.

353 Camuel, A., Guieysse, B., Alcántara, C., Béchet, Q., 2017. Fast algal eco-toxicity assessment:
354 Influence of light intensity and exposure time on Chlorella vulgaris inhibition by atrazine and
355 DCMU. Ecotoxicol. Environ. Saf. 140, 141–147.
356 https://dx.doi.org/10.1016/j.ecoenv.2017.02.013.
357 Carmo, D.A., Carmo, A.P.B., Pires, J.M.B., Oliveira, J.L.M., 2013. Comportamento
358 ambiental e toxicidade dos herbicidas atrazina e simazina. Ambi-Agua 8, 133–143.
359 https://dx.doi.org/10.4136/ambi-agua.1073.
360 Chhipa, H. Chapter 6 - Applications of nanotechnology in agriculture. In: GURTLER,
361 Volker; BALL, Andrew S.; SONI, Sarvesh (Orgs.). Methods in Microbiology.
362 Nanotechnology. [S.l.]: Academic Press, 2019. 46 v. p. 115–142. Available:
363 <http://www.sciencedirect.com/science/article/pii/S0580951719300029>. 16 jun. 2019.
364 Chevrier, C., Limon, G., Monfort, C., Rouget, F., Garlantézec, R., Petit, C., Durand, G.,
365 Cordier, S., 2011. Urinary biomarkers of prenatal atrazine exposure and adverse birth
366 outcomes in the pelagie birth cohort. Environ. Health Perspect. 119, 1034–1041.
367 https://dx.doi.org/10.1289/ehp.1002775.
368 Cleary, J.A., Tillitt, D.E., Saal, F.S.V., Nicks, D.K., Claunch, R.A., Bhandari, R.K., 2019.
369 Atrazine induced transgenerational reproductive effects in medaka (*Oryzias latipes*). Environ.
370 Pollut. 251, 639–650. https://doi.org/10.1016/j.envpol.2019.05.013.
371 Clemente, Z., Grillo, R., Jonsson, M., Santos, N.Z.P., Feitosa, L.O.; Lima, R., Fraceto, L.F.,
372 2013. Ecotoxicological evaluation of poly (epsilon-caprolactone) nanocapsules containing
373 triazine herbicides. J. Nanosci. Nanotechnol. 13, 1–7. https://doi.org/10.1166/jnn.2013.8681.
374 CONAMA, 2005. Resolução n° 357, de 17 de março de 2005. Conselho Nacional do Meio
375 Ambiente, Brasil.

376 Damalas, C.A., Eleftherohorinos, I.G., 2011. Pesticide exposure, safety issues, and risk
377 assessment indicators. Int. J. Environ. Res. Public Health 8, 1402–1419.
378 https://doi.org/10.3390/ijerph8051402.
379 Dias, A.C.L., Santos, J.M.B., Santos, A.S.P., Bottrel, S.E.C.; Pereira, R.O., 2018. Ocorrência
380 de Atrazina em águas no Brasil e remoção no tratamento da água: revisão sistemática. RIC 8,
381 234–253. https://dx.doi.org/10.12957/ric.2018.34202.
382 Dores, E.F.G.C.; De-Lamonica-Freire, E.M., 2001. Contaminação do ambiente aquático por
383 pesticidas. estudo de caso: águas usadas para consumo humano em Primavera do Leste, Mato
384 Grosso – análise preliminar. Quim. Nova 24, 27–36. https://dx.doi.org/10.1590/S0100-
385 40422001000100007.
386 Dornelles, M.F., Oliveira, G.T., 2014. Effect of atrazine, glyphosate and quinclorac on
387 biochemical parameters, lipid peroxidation and survival in bullfrog tadpoles (*Lithobates*
388 *catesbeianus*). Arch. Environ. Contam. Toxicol. 66, 415–429.
389 https://dx.doi.org/10.1007/s00244-013-9967-4.
390 Ebert, E., Dumford, S.W., 1976. Effects of triazine herbicides on the physiology of plants.
391 Residue Rev. 65, 1–103. https://doi.org/10.1007/978-1-4613-9413-6_1.
392 Esperanza, M., Houde, M., Seoane, M., Cid, A., Rioboo, C., 2017. Does a short-term
393 exposure to atrazine provoke cellular senescence in *Chlamydomonas reinhardtii*? Aquat.
394 Toxicol. 189, 184–193. https://dx.doi.org/10.1016/j.aquatox.2017.06.015.
395 Fraceto, L.F., Grillo, R., Medeiros, G.A., Scognamiglio, V., Rea, G., Bartolucci, C., 2016.
396 Nanotechnology in agriculture: which innovation potential does it have? Front. Environ. Sci.
397 4, 1–5. https://dx.doi.org/10.3389/fenvs.2016.00020.
398 Forney, D.R., Davis, D.E., 1981. Effects of low concentrations of herbicides on submersed
399 aquatic plants. Weed Sci. 29, 677–685. https://doi.org/10.1017/S0043174500040261.

400 Gao, Y., Fang, J., Li, W., Wang, X., Li, F., Du, M., Fang, J., Lin, F., Jiang, W., Jiang, Z.,
401 2019. Effects of atrazine on the physiology, sexual reproduction, and metabolism of eelgrass
402 (*Zostera marina* L.). Aquat. Bot. 153, 8–14. https://doi.org/10.1016/j.aquabot.2018.10.002.
403 Garcia, F.P., Ascencio, S.Y.C., Oyarzun, J.C.G., Hernandez, A. C., Alavarado, P.V., 2012.
404 Pesticides: classification, uses and toxicity. Measures of exposure and genotoxic risks. J. Res.
405 Environ. Sci. Toxicol. 1, 279–293.
406 Graymore, M., Stagnitti, F., Allinson, G., 2001. Impacts of atrazine in aquatic ecosystems.
407 Environ. Int. 26, 483–495. https://doi.org/10.1016/S0160-4120(01)00031-9.
408 Grillo, R., Dos Santos, N.Z.P., Maruyama, C.R., Rosa, A.H., Lima, R., Fraceto, L.F., 2012.
409 Poly (epsilon-caprolactone) nanocapsules as carrier systems for herbicides: Physico-chemical
410 characterization and genotoxicity evaluation. J. Hazard. Mater. 231, 1–9.
411 https://dx.doi.org/10.1016/j.jhazmat.2012.06.019.
412 Gonçalves, M.W., Campos, C.B.M., Batista, V.G., Cruz, A.D., Marco Junior, P., Bastos,
413 R.P., Silva, D.M., 2017. Genotoxic and mutagenic effects of Atrazine Atanor 50 SC on
414 *Dendropsophus minutus* Peters, 1872 (Anura: Hylidae) developmental larval stages.
415 Chemosphere 182, 730–737. https://dx.doi.org/10.1016/j.chemosphere.2017.05.078.
416 Gomes, S.I.L., Scott-Fordsmand, J.J., Campos, E.V.R., Grillo, R., Fraceto, L.F., Amorim,
417 M.J.B., 2019. On the safety of nanoformulations to non-target soil invertebrates – an atrazine
418 case study. Enviro. Sci.: Nano 6, 1950–1958. https://dx.doi.org/10.1039/c9en00242a.
419 Guan, Ying-Hong, Ma, J., Ren, Yue-Ming, Liu, Yu-Lei; Xiao, Jia-Yue, Lin, Ling-Qiang,
420 Zhang, C., 2013. Efficient degradation of atrazine by magnetic porous copper ferrite
421 catalyzed peroxymonosulfate oxidation via the formation of hydroxyl and sulfate radicals.
422 Water Res. 47, 5431–5438. https://dx.doi.org/10.1016/j.watres.2013.06.023.
423 He, X., Deng, H., Hwang, Huey-Min, 2019. The current application of nanotechnology in
424 food and agriculture. J. Food Drug Anal. 27, 1–21. https://doi.org/10.1016/j.jfda.2018.12.002.

425 Hénault-Ethier, L., 2016. Backgrounder: Atrazine: Banned in Europe, Common in Canada.
426 CAPE. https://doi.org/10.13140/RG.2.1.2462.9365.
427 Hirano, L.Q.L., Alves, L.S., Menezes-Reis, L.T., Mendonça, J.S., Simões, K., Santos,
428 A.L.Q., Vieira, L.G., 2019. Effects of egg exposure to atrazine and/or glyphosate on bone
429 development in *Podocnemis uniflis* (Testudines, Podocnemididae). Ecotoxicol. Environ. Saf.
430 182, 1–6. https://doi.org/10.1016/j.ecoenv.2019.109400.
431 Hoskins, T.D., Dellapina, M., Papoulias, D.M., BOONE, M.D., 2019. Effects of larval
432 atrazine exposure in mesocosms on Blanchard's cricket frogs (*Acris blanchardi*) reared
433 through overwintering and to reproductive age. Chemosphere 220, 845–857.
434 https://doi.org/10.1016/j.chemosphere.2018.12.112.
435 Jablonowski, N.D., Schäffer, A., Burauel, P., 2011. Still present after all these years:
436 persistence plus potential toxicity raise questions about the use of atrazine. Environ. Sci.
437 Pollut. Res. 18, 328–331. https://dx.doi.org/10.1007/s11356-010-0431-y.
438 Jacques, M.T., Oliveira, J.L., Campos, E.V.R., Fraceto, L.F., Ávila, D.S., 2017. Safety
439 assessment of nanopesticides using the roundworm *Caenorhabditis elegans*. Ecotoxicol.
440 Environ. Saf. 139, 245–253. https://dx.doi.org/10.1016/j.ecoenv.2017.01.045.
441 Jin, R., Ke, J., 2002. Impact of atrazine disposal on the water resources of the Yang river in
442 Zhangjiakou area in China. Bull. Environ. Contam. Toxicol. 68, 893–900.
443 https://dx.doi.org/10.1007/s00128-002-0038-1.
444 Kah, M., Machinski, P., Koerner, P., Tiede, K., Grillo, R., Fraceto, L.F., Hofmann, T., 2014.
445 Analysing the fate of nanopesticides in soil and the applicability
446 of regulatory protocols using a polymer-based nanoformulation of atrazine. Environ. Sci.
447 Pollut. Res. 21, 11699–11707. https://doi.org/10.1007/s11356-014-2523-6.

448 Kah, M., Kookana, R.S., Gogos, A., Bucheli, T.D.A, 2018. critical evaluation of
449 nanopesticides and nanofertilizers against their conventional analogues. Nat. Nanotechnol.
450 13, 677–684. https://doi.org/10.1038/s41565-018-0131-1.
451 Kah, M., Tufenkji, N., White, J.C., 2019. Nano-enabled strategies to enhance crop nutrition
452 and protection. Nat. Nanotechnol. 14, 532–540. https://doi.org/10.1038/s41565-019-0439-5.
453 Kadian, N., Gupta, A., Satya, S., Mehta, R.K., Malik, A., 2008. Biodegradation of herbicide
454 (atrazine) in contaminated soil using various bioprocessed materials. Bioresour. Technol. 99,
455 4642–4647. https://doi.org/10.1016/j.biortech.2007.06.064.
456 Khan, A., Shah, N., Muhammad, Khan, M.S., Ahmad, M.S., Farooq, M., Adnan, M., Jawad,
457 S.M., Ullah, H., Yousafzai, A.M., 2016. Quantitative determination of lethal concentration Lc
458 50 of atrazine on biochemical parameters; total protein and serum albumin of freshwater fish
459 grass carp (*Ctenopharyngodon idella*). Pol. J. Environ. Stud. 25, 1555–1561.
460 https://dx.doi.org/10.15244/pjoes/61849.
461 Khoshnood, Z., Jamili, S., Khodabandeh, S., 2015. Histopathological effects of atrazine on
462 gills of Caspian kutum *Rutilus frisii kutum* fingerlings. Dis. Aquat. Org. 113, 227–234.
463 https://dx.doi.org/10.3354/dao02850.
464 Kim, Ki-Hyun, Kabir, E., Jahan, S.A., 2017. Exposure to pesticides and the associated human
465 health effects. Sci. Total Environ. 575, 525–535.
466 https://dx.doi.org/10.1016/j.scitotenv.2016.09.009.
467 Lee, D.H., Rhee, Y.J., Choi, K.S., Nam, S.E., Eom, H.J., Rhee, J.S., 2017.
468 Sublethal concentrations of atrazine promote molecular and biochemical changes in the
469 digestive gland of the Pacific oyster *Crassostrea gigas*. J. Toxicol. Environ. Health Sci. 9,
470 50–58. https://dx.doi.org/10.1007/s13530-017-0303-7.

471 Liu, Z., Wang, Y., Zhu, Z., Yang, E., Feng, X., Fu, Z., Jin, Y., 2016. Atrazine and its main
472 metabolites alter the locomotor activity of larval zebrafish (*Danio rerio*). Chemosphere, 148,
473 163–170. https://dx.doi.org/10.1016/j.chemosphere.2016.01.007.
474 Liu, Z., Fu, Z., Jin, Y., 2017. Immunotoxic effects of atrazine and its main metabolites at
475 environmental relevant concentrations on larval zebrafish (*Danio rerio*). Chemosphere 166,
476 212–220. https://dx.doi.org/10.1016/j.chemosphere.2016.09.100.
477 Londoño, D.K., Siegfried, B.D., Lydy, M.J., 2004. Atrazine induction of a family 4
478 cytochrome P450 gene in *Chironomus tentans* (Diptera: Chironomidae). Chemosphere 56,
479 701–706. https://dx.doi.org/10.1016/j.chemosphere.2003.12.001.
480 Lorber-Pascal, S., Laurent, F., 2011. Phytoremediation Techniques for Pesticide
481 Contaminations. – In: Lichtfouse, E. (ed.) Alternative Farming Systems, Biotechnology,
482 Drought Stress and Ecological Fertilization., Sustainable Agriculture Reviews 6. Berlin:
483 Springer, 77–105.
484 Loughlin, C.M., Canosa, I.S., Silveyra, G.R., Greco, L.S.L., Rodríguez, E.M., 2016. Effects
485 of atrazine on growth and sex differentiation, in juveniles of the freshwater crayfish *Cherax*
486 *quadricarinatus*. Ecotoxicol. Environ. Saf. 131, 96–103.
487 https://dx.doi.org/10.1016/j.ecoenv.2016.05.009.
488 Lowry, G.V., Avellan, A., Gilbertson, L.M., 2019. Opportunities and challenges for
489 nanotechnology in the agri-tech revolution. Nat. Nanotechnol. 14, 517–522.
490 https://dx.doi.org/10.1038/s41565-019-0461-7.
491 Lushchak, V.I., Matviishyna, T.M., Husaka, V.V., Storey, J.M., Storey, K.B., 2018. Pesticide
492 toxicity: a mechanistic approach. Experimental and Clinical Sciences 17, 1101–1136.
493 https://dx.doi.org/10.17179/excli2018-1710.

494 Mahawar, H., Prasanna, R., 2018. Prospecting the interactions of nanoparticles with
495 beneficial microorganisms for developing green technologies for agriculture. Environ.
496 Nanotechnol. Monit. Manage. 10, 477–485. https://doi.org/10.1016/j.enmm.2018.09.004.
497 Marchi, G., Marchi, E.C.S., Guimarães, T.G. Herbicidas: mecanismos de ação e uso.
498 Embrapa Cerrados, Planaltina, DF, 2008.
499 Mendas, G., Vuletic, M., Galic, N., Drevenkar, V., 2012. Urinary metabolites as biomarkers
500 of human exposure to atrazine: Atrazine mercapturate in agricultural workers. Toxicol. Lett.
501 210, 174–181. https://dx.doi.org/10.1016/j.toxlet.2011.11.023.
502 NHMRC, NRMMC, 2011. Australian Drinking Water Guidelines. Paper 6 National Water
503 Quality Management Strategy. National Health and Medical Research Council, National
504 Resource Management Ministerial Council, Common wealth of Australia, Canberra.
505 Nödler, K., Licha, T., Voutsa, D., 2013. Twenty years later – Atrazine concentrations in
506 selected coastal waters of the Mediterranean and the Baltic Sea. Mar. Pollut. Bull. 70, 112–
507 118. https://dx.doi.org/10.1016/j.marpolbul.2013.02.018.
508 Nwani, C.D., Lakra, W.S., Nagpure, N.S., Kumar, R., Kushwaha, B., Srivastava, S. K., 2010.
509 Toxicity of the herbicide atrazine: effects on lipid peroxidation and activities of antioxidant
510 enzymes in the freshwater fish *Channa punctatus* (bloch). Int. J. Environ. Res. Public Health
511 7, 3298–3312. https://dx.doi.org/10.3390/ijerph7083298.
512 Oliveira, H.C., Stolf-Moreira, R., Martinez, C.B.R., Grillo, R., Jesus, M.B., Fraceto, L.F.,
513 2015a. Nanoencapsulation enhances the post-emergence herbicidal activity of atrazina
514 against mustard plants. Plos One 10, 1–12. https://dx.doi.org/10.1371/journal.pone.0132971.
515 Oliveira, J.L., Campos, E.V.R., Silva, C.M.G., Pasquoto, T., Lima R., Fraceto, L.F., 2015b.
516 Solid lipid nanoparticles co-loaded with simazine and atrazine: preparation, characterization,
517 and evaluation of herbicidal activity. J. Agric. Food Chem. 63, 422–432.
518 https://dx.doi.org/10.1021/jf5059045.

519 Oliveira, H. C., Stolf-Moreira, R., Martinez, C.B.R., Sousa, G.F.M., Grillo, R., Jesus, M.B.,
520 Fraceto, L.F., 2015c. Evaluation of the side effects of poly(epsilon-caprolactone)
521 nanocapsules containing atrazine toward maize plants. Front. Chem. 3, 1–9.
522 https://dx.doi.org/10.3389/fchem.2015.00061.
523 Oliveira, S.E., Costa, P.M., Nascimento, S.B., Castro, W.V., Ribeiro, R.I.M.A.; Santos, H.B.,
524 Thomé, R.G., 2018. Atrazine promotes immunomodulation by melanomacrophage centre
525 alterations in spleen and vascular disorders in gills from *Oreochromis niloticus*. Aquat.
526 Toxicol. 202, 57–64. https://doi.org/10.1016/j.aquatox.2018.06.018.
527 Owolabi, O.D., Omotosho, J.S., 2017. Atrazine-mediated oxidative stress responses and lipid
528 peroxidation in the tissues of *Clarias gariepinus*. IJT 11, 29–38.
529 https://doi.org/10.29252/arakmu.11.2.29.
530 Pereira, A.E.S., Grillo, R., Mello, N.F.S., Rosa, A.H., Fraceto, L.F., 2014. Application of
531 poly (epsilon-caprolactone) nanoparticles containing atrazine herbicide as an alternative
532 technique to control weeds and reduce damage to the environment. J. Hazard. Mater. 268,
533 207–215. https://dx.doi.org/10.1016/j.jhazmat.2014.01.025.
534 Pérez, J., Monteiro, M.S., Quintaneiro, C., Soares, A.M.V.M., Loureiro, S., 2013.
535 Characterization of cholinesterases in *Chironomus riparius* and the effects of three herbicides
536 on chlorpyrifos toxicity. Aquat. Toxicol. 144, 296–302.
537 https://doi.org/10.1016/j.aquatox.2013.10.014.
538 Pérez-Iglesias, J.M., Franco-Belussi, L., Natale, G.S., Oliveira, C., 2019. Biomarkers at
539 different levels of organisation after atrazine formulation (SIPTRAN 500SC®) exposure in
540 *Rhinella schineideri* (Anura: Bufonidae) Neotropical tadpoles. Environ. Pollut. 244, 733–746.
541 https://doi.org/10.1016/j.envpol.2018.10.073.
542 Religia, P., Kato, Y., Fukushima, E.O., Matsuura, T., Muranaka, T., Watanabe, H., 2019.
543 Atrazine exposed phytoplankton causes the production of non-viable offspring on *Daphnia*

544 *magna*. Marine Environ. Res. 145, 177–183.
545 https://doi.org/10.1016/j.marenvres.2019.02.007.
546 Rimayi, C., Odusanya, D., Weiss, J.M., Boer, J., Chimuka, L., Mbajiorgu, F., 2018. Effects of
547 environmentally relevant sub-chronic atrazine concentrations on African clawed frog
548 (*Xenopus laevis*) survival, growth and male gonad development. Aquat. Toxicol. 199, 1–11.
549 https://doi.org/10.1016/j.aquatox.2018.03.028.
550 Rocha, T.L., Gomes, T., Sousa, V.S., Mestre, N.C., Bebianno, M.J., 2015. Ecotoxicological
551 impact of engineered nanomaterials in bivalve molluscs: An overview. Mar. Environ. Res.
552 111, 74–88. https://doi.org/10.1016/j.marenvres.2015.06.013.
553 Roman, E.S.; Vargas, L., Rizzardi, M.A., Hall, L., Beckie, H., Wolf, T.M. Como funcionam
554 os herbicidas – da biologia a aplicação. Berthier: Passo Fundo, 2007.
555 Rusiecki, J.A., Roos, A., Lee, W.J., Dosemeci, M., Lubin, J.H., Hoppin, J.A., Blair, A.,
556 Alavanja, M.C.R., 2004. Cancer Incidence Among Pesticide Applicators Exposed to Atrazine
557 in the Agricultural Health Study. J. Natl. Cancer Inst. 96, 1375–1382.
558 https://doi.org/10.1093/jnci/djh264.
559 Santos, T.G., Martinez, C.B.R., 2012. Atrazine promotes biochemical changes and DNA
560 damage in a Neotropical fish species. Chemosphere 89, 1118–1125.
561 https://doi.org/10.1016/j.chemosphere.2012.05.096.
562 Sass, J.B., Colangelo, A., 2006. European Union Bans Atrazine, While the United States
563 Negotiates Continued Use. Int. J. Occup. Environ. Health 12, 260–267.
564 https://doi.org/10.1179/oeh.2006.12.3.260.
565 Schmidt, A.M., Sengupta, N., Saski, C.A., Noorai, R.E., Baldwin, W.S., 2017. RNA
566 sequencing indicates that atrazine induces multiple detoxification genes in *Daphnia magna*
567 and this is a potential source of its mixture interactions with other chemicals. Chemosphere
568 189, 699–708. https://doi.org/10.1016/j.chemosphere.2017.09.107.

569 Sengupta, N., Litoff, E.J., Baldwin, W.S., 2015. The HR96 activator, atrazine, reduces
570 sensitivity of *D. magna* to triclosan and DHA. Chemosphere 128, 299–306.
571 http://dx.doi.org/10.1016/j.chemosphere.2015.02.027.
572 Shabana, E.F., 1987. Use of batch assays to assess the toxicity of atrazine to some selected
573 cyanobacteria. I. Influence of atrazine on the growth, pigmentation and carbohydrate contents
574 of *Aulosira fertilissima, Anabaena oryzae, Nostoc muscorum* and *Tolypothrix tenuis, Nostoc*
575 *muscorum* and *Tolypothyrix tenius*. J. Basic Microbiol. 2, 113–119.
576 https://doi.org/10.1002/jobm.3620270214.
577 Silveyra, G.R, Canosa, I.S., Rodríguez, E.M., Medesani D.A., 2017. Effects of atrazine on
578 ovarian growth, in the estuarine crab Neohelice granulata. Comp. Biochem. Physiol, Part C:
579 Toxicol. Pharmacol. 192, 1–6. https://dx.doi.org/10.1016/j.cbpc.2016.10.011.
580 Singh, S., Kumar, V., Chauhan, A., Datta, S., Wani, A.B., Singh, N., Singh, J., 2018.
581 Toxicity, degradation and analysis of the herbicide atrazin. Environ. Chem. Lett. 16, 211–
582 237. https://doi.org/10.1007/s10311-017-0665-8.
583 Snyder, M.N., Henderson, W.M., Glinski, D.A., Purucker, S.T., 2017. Biomarker analysis of
584 American toad (*Anaxyrus americanus*) and grey tree frog (*Hyla versicolor*) tadpoles
585 following exposure to atrazine. Aquat. Toxicol. 182, 184–193.
586 https://doi.org/10.1016/j.aquatox.2016.11.018.
587 Solomon, K.R., Baker, D.B., Richards, R.P., Dixon, K.R., Klaine, S.J., La Point, T.W.,
588 Kendall, R.J., Weisskopf, C.P., Giddings, J.M., Giesy, J.P., Hall, L.W.Jr., Williams, W.M.,
589 1996. Risk assessment of atrazine in North American surface waters. Environ. Toxicol.
590 Chem. 15, 31–76. https://doi.org/10.1002/etc.2050.
591 Soltanian, S., 2016. Effect of atrazine on immunocompetence of redeared slider turtle
592 (*Trachemys scripta*). J. Immunotoxicol. 13, 804–809.
593 https://dx.doi.org/10.1080/1547691X.2016.1195463.

Souza, P.M.S., Lobo, F.A., Rosa, A.H., Fraceto, L.F., 2012. Desenvolvimento de nanocápsulas de poli-e-caprolactona contendo o herbicida atrazina. Quim. Nova 35, 132–137. https://dx.doi.org/10.1590/S0100-40422012000100024.

Stara, A., Kouba, A., Velisek, J., 2018. Biochemical and histological effects of sub-chronic exposure to atrazine in crayfish *Cherax destructor*. Chem. Biol. Interact. 291, 95–102. https://doi.org/10.1016/j.cbi.2018.06.012.

Sun, J.T., Pan, L.L., Zhan, Y., Tsang, D.C.W., Zhu, L.Z., LI, X.D., 2017. Atrazine contamination in agricultural soils from the Yangtze River Delta of China and associated health risks. Environ. Geochem. Health 39, 369–378. https://doi.org/10.1007/s10653-016-9853-x.

Tang, G., Yao, J., Li, D., He, Y., Zhu, Y.C., Zhang, X., Zhu, K.Y., 2017. Cytochrome P450 genes from the aquatic midge *Chironomus tentans*: atrazine-induced up-regulation of CtCYP6EX3 enhanced the toxicity of chlorpyrifos. Chemosphere 186, 68–77. https://doi.org/10.1016/j.chemosphere.2017.07.137.

Taverna, M.E., Busatto, C.A., Lescano, M.R., Nicolau, V.V., Zalazar, C.S., Meira, G.R., Estenoz, D.A., 2018. Microparticles based on ionic and organosolv lignins for the controlled release of atrazine. J. Hazard. Mater. 359, 139–147. https://doi.org/10.1016/j.jhazmat.2018.07.010.

Toughan, H., Khalil, S.R., El-Ghoneimy, A.A., Awadd, A., Seddek, A.SH., 2018. Effect of dietary supplementation with Spirulina platensis on Atrazineinduced oxidative stress-mediated hepatic damage and inflammation in thecommon carp (*Cyprinus carpio* L.). Ecotoxicol. Environ. Saf. 149, 135–142. https://doi.org/10.1016/j.ecoenv.2017.11.018.

USEPA, 2009. United States Environmental Protection Agency. National Primary Drinking Water Regulations.

618 Vasanth, S., Vijayakumar, T.S., Bupesh, G., Subramanian, P., 2017. Dose dependent effect of
619 synthetic herbicide (atrazine) on the morphological parameters in *Poecilia sphenops*.
620 European J. Biomed. Pharm. Sci. 4, 455–457.
621 Xiao-Ting, C., Wang, T., 2019. Preparation and characterization of atrazine-loaded
622 biodegradable PLGA nanospheres. J. Integr. Agric. 18, 1035–1041.
623 https://doi.org/10.1016/S2095-3119(19)62613-4.
624 Zadeh, A.K., Dadolahi-Sohrab, A, Alishahi, M., Khazaei, S.H., Asgari, H.M., 2016.
625 Evaluation of acute and sub-lethal toxicity of herbicide, atrazine,
626 on hematological parameters of *Tor grypus*. J. Vet. Res. 71, 295–301.
627 https://doi.org/10.22059/JVR.2016.58727.
628 Zhao, F., Li, Y., Huang, L., Gu, Y., Zhang, H., Zeng, D., Tan, H., 2018. Individual and
629 combined toxicity of atrazine, butachlor, halosulfuronmethyl and mesotrione on the
630 microalga *Selenastrum capricornutum*. Ecotoxicol. Environ. Saf. 148, 969–975.
631 https://doi.org/10.1016/j.ecoenv.2017.11.069.
632 Zhao, R., Hu, Y., Li, B., Chen, M., Ren, Z., 2020. Potential effects of internal physio-
633 ecological changes on the online biomonitoring of water quality: The behavior responses with
634 circadian rhythms of zebrafish (*Danio rerio)* to different chemicals. Chemosphere 239, 1–9.
635 https://doi.org/10.1016/j.chemosphere.2019.124752.
636 Zhu, X., Sun, Y., Zhang, X., Heng, H., Nan, H., Zhang, L., Huang, Y., Yang, Z., 2016.
637 Herbicides interfere with antigrazer defenses in *Scenedesmus obliquus*. Chemosphere 162
638 243–251. https://doi.org/10.1016/j.chemosphere.2016.07.087.
639 Yoon, D.S., Park, J.C., Park, H.G., Lee, J.S., Han, J., 2019 Effects of atrazine on life
640 parameters, oxidative stress, and ecdysteroid biosynthetic pathway in the marine copepod
641 *Tigriopus japonicus*. Aquat. Toxicol. 213, 1–10.
642 https://doi.org/10.1016/j.aquatox.2019.05.015.

643 Wang, S., Zhang, Q., Zheng, S., Chen, M., Zhao, F., Xu. S., 2019. Atrazine exposure triggers
644 common carp neutrophil apoptosis via the CYP450s/ROS pathway. Fish Shellfish Immunol.
645 89, 551–557. https://doi.org/10.1016/j.fsi.2018.10.029.
646 WHO, 2017. World Health Organization. Guidelines for drinking water quality. 1.
647 Available:<http://apps.who.int/iris/bitstream/handle/10665/254637/9789241549950eng.pdf;js
648 essionid=3FCA37069207D6A3E9B5117CCD3A1AE9?sequence=1>. 11 jun. 2019.
649 Wirbisky, S.E., Weber, G.J., Schlotman, K.E., Sepúlveda, M.S.,
650 Freeman, J.L., 2016. Embryonic atrazine exposure alters zebrafish and human miRNAs
651 associated with angiogenesis, cancer, and neurodevelopment. Food Chem. Toxicol. 98, 25–
652 33. https://dx.doi.org/10.1016/j.fct.2016.03.027.

Table 1

Adverse effects of atrazine on aquatic organisms.

Group	Scientific name	Concentration	Exposure	Observed effects	Reference
Plant	*Zostera marina* L.	1, 3, and 10 µg.L^{-1}	30 hours	Inhibited photosynthesis and reduced sugar levels in the aerial part.	Gao et al. (2019)
Algae	*Oophila amblystomatis*	3, 10, 30, 100, 300, and 600 µg.L^{-1}	96 hours	Reduced the yield of photosystem II.	Baxter et al. (2015)
	Raphidocelis subcapitata	10, 25, 50, 100, and 250 µg.L^{-1}	96 hours	Affected photosystem II.	Baxter et al. (2016)
	Chlamydomonas reinhardtii	0.25 µM	24 hours	Increased intracellular calcium level. Alteration of cellular morphology, as well as the activities of biochemical and molecular markers.	Esperanza et al. (2017)
	Chlorella vulgaris	1, 2, 5, and 10 µM	<1 hour	Affected the electron transfer chain.	Camuel et al. (2017)
	Selenastrum capricornutum	0.076 and 0.023 mg.L^{-1}	48 and 72 hours	Inhibited growth.	Zhao et al. (2018)
	Raphidocelis subcapitata	50, 100, 150, 300, 450, and 600 µg.L^{-1}	72 hours	Presented lower carbon:nitrogen ratio.	Religia et al. (2019)
Invertebrate					
Crustacean	*Neohelice granulata*	2.5, 5, and 15 mg.L^{-1}	32 days	Altered ovarian maturation; caused hydrops; hyperpigmentation of the body; atrophy of spines, bristles, and eyes.	Álvarez et al. (2015)
	Daphnia magna	40 µM	16 hours	Increased antioxidant activities.	Sengupta et al. (2015)
	Cherax quadricarinatus	0.1, 0.5, and 2.5 mg.L^{-1}	28 days	Dysregulated the endocrine system, increasing the proportion of females.	Loughlin et al. (2016)

	Neohelice granulata	0.03, 0.3, and 3 mg.L^{-1}	90 days and 24 hours	Decreased glycogen in the muscles and potentiated the accumulation of vitellogenin in the hepatopancreas.	Silveyra et al. (2017)
	Daphnia magna	20 and 40 μM	48 hours	Activated HR96 and induced detoxification genes of phase I and III.	Schmidt et al. (2017)
	Cherax destructor	6.86 μg.L^{-1} and 1.21 mg.L^{-1}	7 and 14 days	Changed biochemical parameters of hemolymph, the activity of antioxidant biomarkers, and the histology of the gill tissue.	Stara et al. (2018)
	Daphnia magna	150 μg.L^{-1}	21 days	Increased the number of non-viable offspring; morphological abnormalities.	Religia et al. (2019)
	Tigriopus japonicus	5, 10, and 20 mg.L^{-1}	1 and 20 days	Induced oxidative stress; altered the expression of genes related to the ecdysteroid biosynthetic pathway, delaying metamorphosis.	Yoon et al. (2019)
	Faxonius virilis	10, 40, 80, 100 and 300 μg.L^{-1}	10 days	Caused DNA damage in cells of the lateral antennules, including olfactory sensory neurons, thus leading impairments in chemosensory cells and chemoreception.	Abdulelah et al. (2020)
Insect	*Chironomus tentans*	5000 μg.L^{-1}	48 hours	Regulated the transcription of four genes (CtCYP6EX3, CtCYP6EV3, CYP9AT1, and CtCYPEX1).	Tang et al. (2017)
Mollusc	*Crassostrea gigas*	1, 10, and 50 μg.L^{-1}	96 hours	Harmed the digestive gland by modulating important molecular and biochemical parameters.	Lee et al. (2017)
Vertebrate					
Fish	*Danio rerio*	1.0, 1.5, and 2.0 μg.L^{-1}	96 hours	Induced the formation of micronuclei.	Botelho et al. (2015)
	Danio rerio	0.1 mM	96 hours	Presented teratogenic and genotoxic potential; oxidative stress.	Adeyemi et al. (2015)
	Rutilus frisii kutum	12.47 mg.L^{-1}	96 hours	Damaged the tissues of the gill, affecting respiration and the ionic regulation function.	Khoshnood et al. (2015)

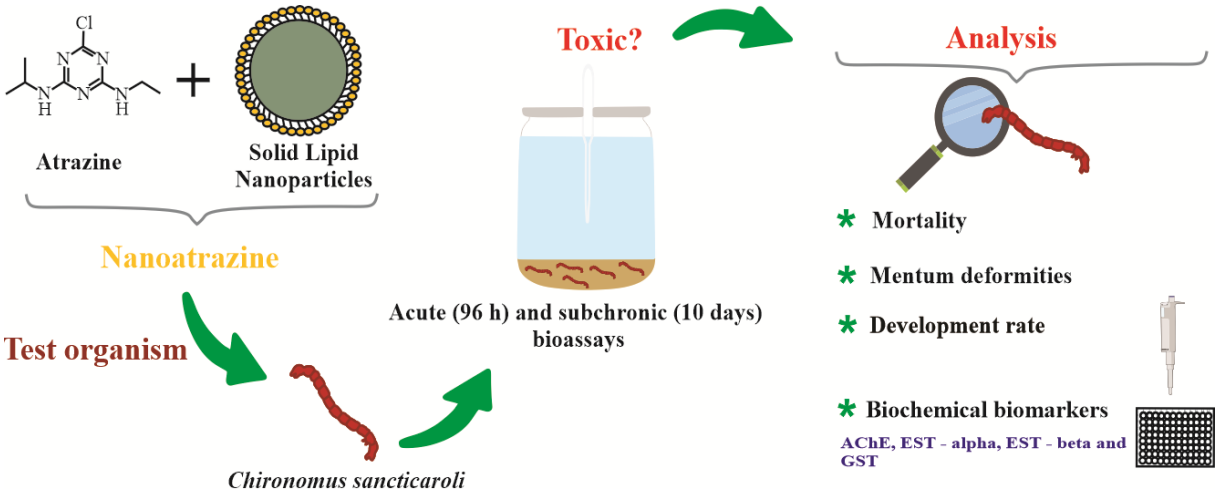
Danio rerio	30, 100, and 300 µg.L^{-1}	5 days	Neuroendocrine disruptor activity, affecting the expression of genes related to neurotoxicity; alteration of swimming behavior; inhibition of acetylcholinesterase activity.	Liu et al. (2016)
Danio rerio	0.3, 3, and 30 ppb	72 hours	Deregulated microRNAs.	Wirbisky et al. (2016)
Ctenopharyngodon idella	15, 13, 10, 0.8, 0.6, 0.4, and 0.2 µl.L^{-1}	4 and 25 days	Decreased levels of total protein and serum albumin.	Khan et al. (2016)
Barbus grypus	3.25, 6.5, and 13 mg.L^{-1}	21 days	Reduced levels of hemoglobin, hematocrits, and erythrocytes.	Zadeh et al. (2016)
Danio rerio	30, 100, and 300 µg.L^{-1}	96 hours	Possible induction of immunotoxicity in larvae.	Liu et al. (2017)
Clarias gariepinus	7, 7.5, 8, 8.5, 28, 30, 32, and 34 µg.L^{-1}	96 hours and 28 days	Caused oxidative stress; enzymatic and metabolic changes.	Owolabi and Omotosho (2017)
Poecilia sphenops	0.83, 1.25, and 2.5 ppm	100 days	Influenced the sexual ratio, increasing feminization; reduced the gonadosomatic index; increased the hepatosomatic index and the weight of the liver.	Vasanth et al. (2017)
Danio rerio	2, 10, and 100 µg.L^{-1}	11 days	Affected the quality of sperm, resulting in reduced fertility rates.	Bautista et al. (2018)
Poecilia reticulata	0.02, 0.3, 0.065, 1.9, 11.2, 105, 150, and 1058 µg.L^{-1}	3 hours	Formed a chemical barrier and reduced the migratory potential of fish.	Araújo et al. (2018)
Oreochromis niloticus	1 and 2 ppm	7 and 15 days	Caused an immunomodulatory effect in the spleen; vascular and structural changes in the gills.	Oliveira et al. (2018)
Pimephales promelas	1, 10, 26, 52, and 105 µg.L^{-1}	28 days	No significant effect was observed.	Brain et al. (2018)
Oryzias latipes	9.4, 48, 74, 97, and 244 µg.L^{-1}	28 or 29 days	No significant effect was observed.	Brain et al. (2018)

	Cyprinus carpio L.	428 µg.L^{-1}	48 days	Caused liver damage, induced by oxidative stress.	Toughan et al. (2018)
	Oryzias latipes	5 and 50 µg.L^{-1}	12 days	Altered sperm parameters and fertilization efficiency.	Cleary et al. (2019)
	Cyprinus carpio L	25 µg.ml^{-1}	1, 2 and 3 hours	Altered biomarkers, causing oxidative stress and mitochondrial damage.	Wang et al. (2019)
	Danio rerio	3 and 30 µg.L^{-1}	15 days	Changed the circadian rhythm.	Zhao et al. (2020)
Amphibian	*Lithobates catesbeianus*	5, 10, and 20 µg.L^{-1}	7 days	Induced significant changes in biochemical parameters and increased levels of lipid peroxidation.	Dornelles and Oliveira (2014)
	Ambystoma maculatum	3, 10, 30, 100, and 300 µg.L^{-1}	66 days	No significant effect was observed.	Baxter et al. (2015)
	Anaxyrus americanos and *Hyla versicolor*	10, 50, 250, and 1250 µg.L^{-1}	48 hours	Impacted the metabolic pathways associated with energy and purine metabolism.	Snyder et al. (2017)
	Dendropsophus minutus	2.25, 4.5, 9, and 18 µg.L^{-1}	96 hours	Caused genotoxic and mutagenic effects.	Gonçalves et al. (2017)
	Xenopus laevis	0.01, 200, and 500 µg.L^{-1}	90 days	Affected development and caused mortality.	Rimayi et al. (2018)
	Acris blanchardi	1, 10, 100, and 200 µg.L^{-1}	51 days	Increased the prevalence of testicular eggs in metamorphosis, which were also observed in the emergent off spring and at reproductive age.	Hoskins et al. (2019)
	Rhinella schneideri	1.5, 3, 6, 12.5, 19, and 25 mg.L^{-1}	48 and 96 hours	Induced sublethal effects, causing morphological abnormalities and changes in swimming and growth.	Pérez-Iglesias et al. (2019)
Reptile	*Trachemys scripta*	0.01, 0.1, and 1.0 ng/g	14 days	Induced immunosuppression.	Soltanian (2016)
	Podocnemis uniflis	2 and 200 µg.L^{-1}	30 and 50 days	Induced malformations in embryos.	Hirano et al. (2019)

CHAPTER II

Use of nontarget organism *Chironomus sancticaroli* to study the toxic effects of nanoatrazine

Submitted to Journal of Hazardous Materials



Use of nontarget organism *Chironomus sancticaroli* to study the toxic effects of nanoatrazine

Felícia Pereira de Albuquerque[a], Jhones Luiz de Oliveira[a], Leila dos Santos Machado[a], Vinicius Sobrinho Richardi[b], Mario Antônio Navarro da Silva[b], Marcelo Luiz Martins Pompêo[a, c], Leonardo Fernandes Fraceto[a], Viviane Moschini Carlos[a]

[a]São Paulo State University (UNESP), Institute of Science and Technology of Sorocaba, Av. Três de março, 511, Alto da Boa Vista, 18087-180, Sorocaba, Brazil

[b]Department of Zoology, Federal University of Paraná, Curitiba, Paraná, Brazil

[c]Department of Ecology, University of São Paulo, São Paulo, Brazil

Corresponding authors at: São Paulo State University (UNESP), Institute of Science and Technology of Sorocaba, Av. Três de março, 511, Alto da Boa Vista, 18087-180, Sorocaba, Brazil

E-mail address: felicia.pa@hotmail.com (F.P. de Albuquerque); leonardo.fraceto@unesp.br (L.F. Fraceto).

Abstract

Nanoatrazine was developed as a carrier system and have been considered efficient in weed control. The aim of the present study was to investigate ecotoxicological effects of solid lipid nanoparticles (empty and loaded with atrazine) and atrazine on *Chironomus sancticaroli* larvae, evaluating the endpoints: mortality, mentum deformity, development rate and biochemical biomarkers. The contaminant concentrations used were 0.002, 0.47, 0.95, and 1.9 $mg.L^{-1}$ in acute (96 h) and 0.002 $mg.L^{-1}$ in subchronic (10 days) bioassays. An environmentally relevant concentration of atrazine presented toxic and lethal effects towards the larvae. The nanoparticles loaded with atrazine showed toxic effects similar to free atrazine, causing mortality and biochemical alterations on the larvae. The nanoparticle without atrazine caused biochemical alterations and mortality, indicating a possible toxic effect of the formulation on the larvae. In the acute bioassay, most concentrations of nanoparticles loaded with atrazine were not dose dependent for the endpoint mortality. Only the atrazine concentration of 0.47 $mg.L^{-1}$ was statistically significant to endpoint mentum deformity. The atrazine and nanoparticles (with and without atrazine) did not affect larval development. The results indicate that *Chironomus sancticaroli* was sensitive to monitor nanoatrazine, presenting potential to be used in studies of toxicity of nanopesticides.

Keywords: Aquatic organism; Atrazine; Ecotoxicology; Invertebrate; Nanotechnology.

1. Introduction

Atrazine (2-chloro-4-ethylamine-6-isopropylamine-triazine) is an herbicide used in several countries (Tillitt et al., 2010) for controlling annual broadleaf and grass weeds in crops such as maize, sorghum, sugar cane, and pineapple (Chevrier et al., 2011; Graziano et al., 2006; Nwani et al., 2010; Solomon et al., 1996; Yue et al., 2017). Due to its widespread use and high persistence in the environment, it is possible to detect it in water bodies

including groundwater, streams, lakes, rivers, reservoirs used for public water supply and seas (Albuquerque et al., 2020; Carmo et al., 2013; Choung et al., 2013; Hénault-Ethier, 2016; Nödler et al., 2013; Santos et al., 2015).

This herbicide is considered to be resistant to hydrolysis and photolysis, presents low soil adsorption, and has strong potential for infiltration in groundwater (Hénault-Ethier, 2016). In addition, atrazine half-life varies from 14 days to 4 years in soil and from 6 months to several years in water (Guan et al., 2013; Stara et al., 2018). These factors contribute to its entry into the environment and the food chain (Graziano et al., 2006; Garcia et al., 2012).

The presence of atrazine in aquatic environments is of concern, since studies have found that the compound presents serious risks to humans and ecosystems (Araújo et al., 2018; Albuquerque et al., 2020; Carmo et al., 2013; Chevrier et al., 2011; Gilden et al., 2010; Hénault-Ethier, 2016; Ralston-Hooper et al., 2011; Rusiecki et al., 2004).

In agriculture, it is essential to develop new technologies that minimize the negative effects of pesticides (Oliveira et al., 2015a). Following advances made in the area of nanotechnology and nanomaterials, it has been found that the use of nanocarriers can help to reduce the applications of fertilizers and pesticides, as well as decrease losses due to leaching, degradation, and volatilization of chemicals, hence contributing to sustainability (Duhan et al., 2017; Fraceto et al., 2016; Oliveira et al., 2018).

Effective systems for the release of atrazine have been developed (Clemente et al., 2013; Grillo et al., 2010; Grillo et al., 2012; Kah et al., 2014; Oliveira et al., 2015a, b, c; Pereira et al., 2014; Souza et al., 2012; Taverna et al., 2018; Xiao-Ting and Wang, 2019). An example is the solid lipid nanoparticle (SLN) formulation loaded simultaneously with atrazine and simazine, reported by Oliveira et al. (2015b), who used bioassays to show that the systems prepared were less toxic and more efficient than a commercial formulation. However,

Jacques et al. (2017) reported toxic effect of this formulation on the soil organism *Caenorhabditis elegans*, indicating the need for further studies.

In-depth study and knowledge of the interactions of nanoparticles with aquatic as well as soil organisms are essential for understanding their toxic effects, especially considering the potential toxic effects of nanopesticides in nontarget organisms (Sigg et al., 2014). In addition, the chemical compositions of these substances enable them to be easily adsorbed in the sediment, hence affecting benthic organisms (Oberholster et al., 2011; Silva et al., 2016). Among the benthic organisms, stands out some species of family Chironomidae that are probably exposed to nanomaterials due have the habit of ingesting sediment particles (Oberholster et al., 2011; Strixino and Strixino, 1981, 1982).

In the Neotropical region it is possible to use of the aquatic insect *Chironomus sancticaroli* Strixino and Strixino, 1981 to evaluate the toxic effects of nanopesticides in nontarget organisms, since this species has provided excellent results in ecotoxicology bioassays, including the evaluation of biochemical biomarkers, DNA strand damage, histology, mortality, development rate and mentum deformities (Beghelli et al., 2018; Dornfeld et al., 2006; Janke et al., 2011; Moreira-Santos et al., 2005; Novelli et al., 2012; Palacio-Cortés et al., 2017; Printes et al., 2007; Printes et al., 2011; Rebechi et al., 2014; Rebechi-Baggio et al., 2016; Richardi et al., 2018; Santos et al., 2007; Silvério et al., 2005; Sotero-Santos et al., 2007; Vicentini et al., 2017). Therefore, *Chironomus sancticaroli* it is considered a good bioindicator of sediment pollution in South America (Beghelli et al., 2018).

So far, there is not reported studies using a benthic organism to evaluate the effects of atrazine encapsulated in solid lipid nanoparticles. Therefore, the aim of the present study was to investigate the ecotoxicological effects of SLN (empty and loaded with atrazine) on *Chironomus sancticaroli* larvae (nontarget organism), in acute and subchronic bioassays, in order to obtain the potential toxic effects of this nanosystem and compare them with atrazine

(active ingredient) ones, evaluating biochemical biomarkers, mortality, mentum deformity and larval development. This study is important to better understand nanoatrazine effects on benthic organisms and it contributes to check sensitivity of this specie as a marker in bioassays that evaluates nanopesticides toxicity in aquatic environments.

2. Materials and Methods

2.1 Biological material

Larvae of *Chironomus sancticaroli* were obtained from a culture maintained at the Limnology Laboratory of São Paulo State University (Sorocaba, São Paulo State). Controlled climate chambers were used for the culture and the bioassays, following the protocol of Maier et al. (1990) and the OECD (2004a, b), with modifications in terms of temperature (25 ± 2 ºC), photoperiod (12 h), and relative humidity (80 ± 10%).

2.2 Sediment and water conditions for bioassays

The preparation of artificial sediment followed the protocol of the OECD (2004a, b, 2010), using sand calcined as described by Araújo et al. (2006). The chlorine-free mineral water used had the following chemical composition (in mg L^{-1}): calcium 15.04, fluoride 0.070, bicarbonate 80.30, magnesium 1.826, potassium 1.476, sodium 5.468, sulfate 2.780, phosphate 0.800, and chloride 0.060 (López-Doval et al., 2012; OECD, 2004a, b, 2010). The hardness of the water was determined by titration with EDTA (ethylenediamine tetraacetic acid). Measurements were made of the pH, conductivity, dissolved oxygen content, and temperature of the water, at the beginning and end of each bioassay (Table S1) (Dornfeld et al., 2006; OECD, 2004a, b).

125 **2.3 Acute and subchronic bioassays**

126 The bioassays were carried out in 250 mL glass containers containing 40 g (wet
127 weight) of artificial sediment and 160 mL of chlorine-free mineral water, doses of 5 mg of
128 TetraMin® feed (diluted) were added at the beginning of the bioassay and then after every 48
129 h (OECD, 2004a, b, 2010), and the system was kept under constant aeration (Richardi et al.,
130 2015; Vicentini et al., 2017). The treatments of bioassays (acute and subchronic) were
131 performed in quadruplicate, with each replicate employing 20 larvae (n = 80), and the
132 experiments were repeated two times (OECD, 2004a, b). Fourth instar larvae were used in the
133 acute toxicity bioassay, while the subchronic toxicity bioassay employed first instar larvae.
134 The controls used in the atrazine bioassay were water and acetone 1:100 (v:v). The durations
135 of the acute and subchronic bioassays were 96 hours (h) and 10 days, respectively. Larvae that
136 had normal or low mobility were considered alive.

137 Four concentrations of atrazine (Sigma-Aldrich) were used in the acute toxicity
138 bioassays. The value of 0.002 $mg.L^{-1}$ was defined according to Brazilian National
139 Environmental Council (CONAMA, Resolution 357, updated and modified by Resolution
140 430/2011) for freshwater (surface waters intended for human consumption and the protection
141 of aquatic life). This concentration can be considered environmentally relevant to aquatic life,
142 since it was detected in aquatic environments (Armas et al., 2007; Botelho et al., 2015). The
143 other concentrations were adapted from the average of the LC_{50} values reported by Phyu et al.
144 (2005) Taylor et al. (1991) for species of the genus *Chironomus*. A value of 1.9 $mg.L^{-1}$,
145 representing the NOEC (No Observed Effect Concentration) for the species *Chironomus*
146 *riparius* and *Chironomus tepperi*, was obtained by dividing the LC_{50} by ten (Zagatto and
147 Bertoletti, 2008). Values of 0.95 and 0.47 $mg.L^{-1}$ were obtained by dividing the NOEC value
148 by two and four, respectively. A concentration of 0.002 $mg.L^{-1}$ was used in the subchronic
149 toxicity bioassay, because it presented the lowest mortality rate during the acute bioassay.

150 **2.4 Preparation and characterization of nanoparticles**

151 The SLN were produced according to the methodology of Oliveira et al. (2015b),
152 using the emulsification/solvent evaporation technique. Daily size distribution measurements
153 were made in order to evaluate SLN stability during the bioassays, using two different
154 techniques. Dynamic light scattering (DLS) analyses were performed with a ZetaSizer Nano
155 ZS 90 instrument (Malvern Instruments). Nanoparticle tracking analysis (NTA) employed a
156 NanoSight LM 10 analyzer (green laser, 532 nm) and a CMOS camera, operated with
157 NanoSight v. 2.3 software. The results were expressed as the means of triplicates.

158

159 **2.5 Stability of atrazine in water**

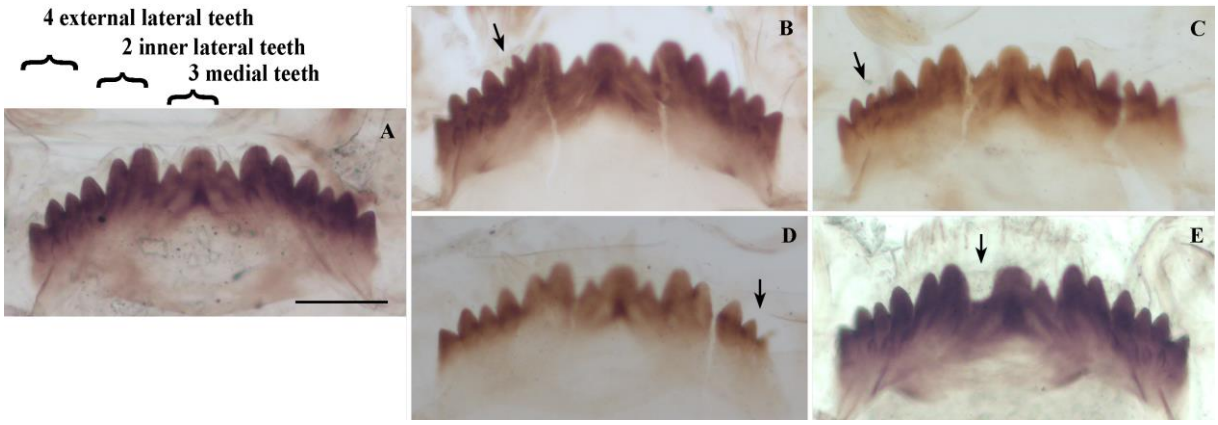
160 To verify the stability of atrazine in the test system used in this study, acute (96 h) and
161 subchronic (10 days) bioassays were performed. During ten days aliquots of three samples of
162 each treatment were collected daily and quantified using high performance liquid
163 chromatography (HPLC), as described by Grillo et al. (2012).

164

165 **2.6 Morphological biomarkers**

166 For analyze the mentum deformity and alteration on the development stage of
167 *Chironomus sancticaroli* larvae were separated fifteen specimens by treatment, of the acute
168 and subchronic bioassays. The larvae were stored in vials which were labeled, in 70% alcohol
169 (Richardi et al., 2013). Head capsules were removed and treated according the methodology
170 described by Epler (2001) with adaptations in the mounting. The slides were mounted with
171 the ventral side facing up, with glycerin and absolute ethanol in a 3:1 (v:v) ratio and sealed
172 with nail polish (Richardi et al., 2013). Morphological analysis and the photographs were
173 performed under a light microscope (Zeiss Axio Scope.A1) at 100x and 200x magnification.
174 Linear measurements from fixed points were taken through the software Zen version 3.0. The

175 mentum was considered as deformed if it had bifid tooth, extra tooth, missing tooth, fused
176 teeth and Köhn Gap (Figure 1B–E), according to Arambourou et al. (2012, 2014).



177

178 **Figure 1A–E** Mentum deformity of *Chironomus sancticaroli*. A: Normal mentum. B: Bifid tooth. C: Extra
179 tooth. D: Missing tooth. E: Köhn gap. Arrows indicate morphological abnormality. Scale 0.50 µm.

180

181 **2.7 Biochemical biomarkers**

182 For determination of the biochemical effects, 30 larvae from each treatment (acute and
183 subchronic) were transferred to Eppendorf tubes and kept at -80 ºC in a freezer, prior to use.
184 The organisms were separated into six groups, each with five larvae, and each group was
185 homogenized following the addition of 620 µL of deionized water.

186 Total proteins were quantified according to the Bradford (1976) protocol, using bovine
187 serum albumin as a standard. The analysis of acetylcholinesterase (AChE) was performed
188 according to the methodology proposed by Ellman et al. (1961), modified for microplates by
189 Silva de Assis (1998). The other biomarkers and methods used were as follows: esterases
190 alpha (EST-α) and beta (EST-β) (Valle et al., 2006), glutathione S-transferase (GST) (Keen et
191 al., 1976). All the absorbance readings, whether kinetic (AChE and GST) or endpoint (EST-α
192 and EST-β), were performed using a spectrophotometer (Spectra Max 190, Molecular
193 Devices).

194

195

196 **2.8 Statistical analysis**

197 Homogeneity analysis (Bartlett's test) and normality evaluation (Shapiro-Wilk's test)
198 were performed for all statistical tests. The statistical analyses of the stability (nanoparticles
199 and atrazine), mortality and biochemical biomarkers activity were performed through one-
200 way ANOVA followed by the Tukey's test ($p < 0.05$). To evaluate the mentum deformities
201 was used Kruskal-Wallis followed by the Dunn's test ($p < 0.05$). All statistical analysis and
202 graph construction were performed using the free software RStudio v. 3.4.1 (R Development
203 Core Team, 2011).

204

205 **3. Results**

206 **3.1 Physico-chemical stability of the nanoparticles and atrazine**

207 Daily evaluation was made to measure the stability of atrazine concentration in water
208 and the nanoparticles stability in the sediment-water exposure system under laboratory
209 conditions with controlled physico-chemical parameters, reproducing environmental
210 conditions. The variation of atrazine concentration in water during the experiments did not
211 show significant difference (p value = 0.177) during bioassays (Figure S1).

212 For the nanoparticles, the mean particle diameter of the formulations of SLN +
213 atrazine and SLN without atrazine remained stable during the bioassays (acute and
214 subchronic), for all the concentrations tested (Figures S2–S3). In the acute bioassay
215 (concentration of 0.002 $mg.L^{-1}$), the SLN formulation with atrazine presented an initial (0 h)
216 mean diameter of 292 ± 7 nm (DLS) and a final (96 h) value of 318 ± 3 nm (DLS) (Figure
217 S2A). The SLN control (without the active ingredient), at a concentration of 0.002 $mg.L^{-1}$,
218 presented the same stability profile, with an initial (0 h) mean diameter of 241 ± 5 nm (DLS)
219 and a final (96 h) value of 262 ± 10 nm (DLS) (Figure S2B). Similar results were observed for

220 the other concentrations and to the subchronic bioassay, with no significant difference (p
221 value = 0.087 and 0.998, respectively) in the variation of the mean diameter values (Figures
222 S2–S3). In the subchronic bioassay, even with a longer exposure time (240 h), no particle
223 aggregations were observed in the formulations.
224
225 **3.2 Lethal effect**
226 The mortality of the *Chironomus sancticaroli* larvae in the water and solvent control
227 bioassays did not exceed 30% (Figure 2 A–F), the maximum value permitted by the OECD
228 (2004a, b). Significant difference was observed between all concentrations and the control,
229 except to atrazine in the 10 days exposure (Figure 2A–F). In the 96 h and 10 days exposures,
230 the treatment with SLN + atrazine caused a greater lethal effect on the larvae (Figure 2A–F).
231 In the SLN + atrazine and SLN bioassays, the concentration of 1.9 $mg.L^{-1}$ caused 100%
232 mortality of the larvae (Figure 2B–C). The mortality rates were also obtained for the atrazine
233 concentration of 0.002 $mg.L^{-1}$ permitted by environmental legislation (Figure 2A–F). At 96 h
234 exposure, it was observed that some concentrations of the treatments with atrazine (0.47, 0.95
235 and 1.9), SLN + atrazine (0.002, 0.47 and 0.95) and SLN (0.47 and 0.95) no significantly
236 differences between them (Figure 2A–C).

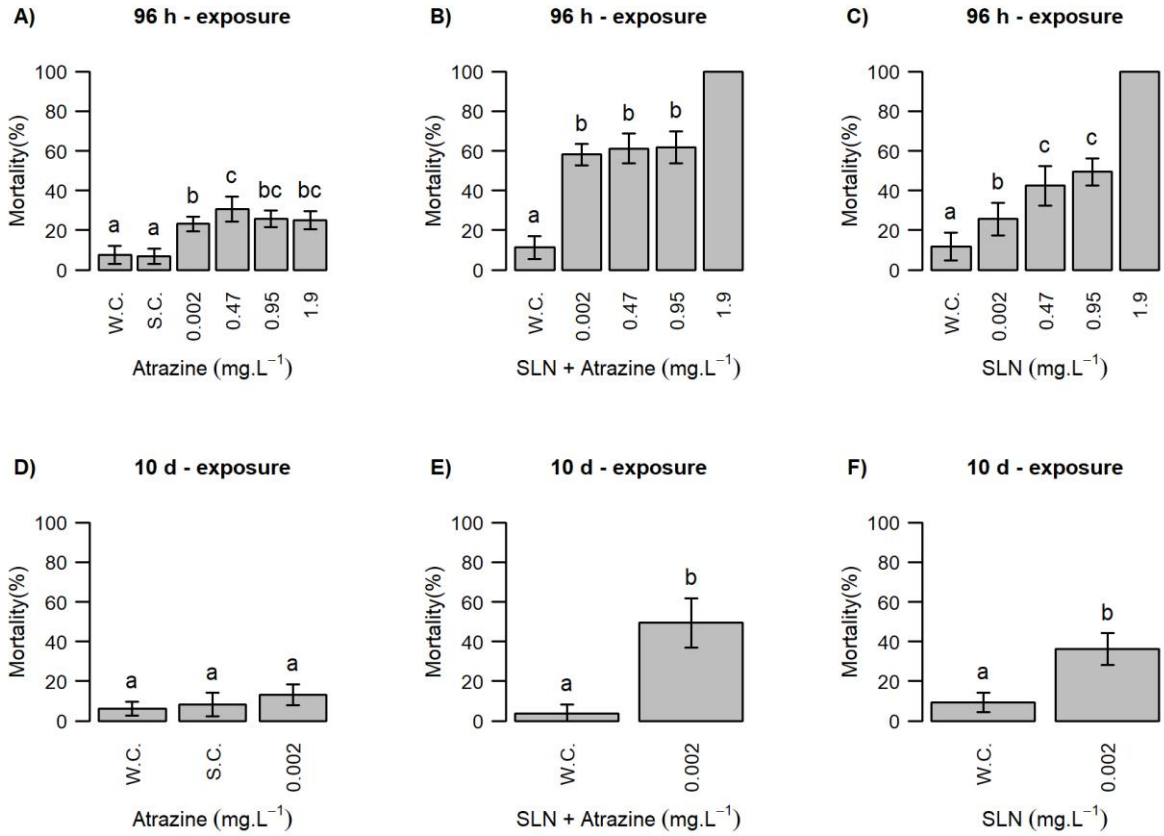


Figure 2 Mortality percentages of *Chironomus sancticaroli* larvae in the acute (96 h) and subchronic (10 days) bioassays. A) Larvae exposed to atrazine in acute bioassay. B) Larvae exposed to solid lipid nanoparticles loaded with atrazine in acute bioassay. C) Larvae exposed to solid lipid nanoparticles in acute bioassay. D) Larvae exposed to atrazine in subchronic bioassay. E) Larvae exposed to solid lipid nanoparticles loaded with atrazine in subchronic bioassay. F) Larvae exposed to solid lipid nanoparticles in subchronic bioassay. SLN + ATZ = solid lipid nanoparticles loaded with atrazine; SLN = solid lipid nanoparticles; W.C. = water control; S.C. = solvent control. Different letters indicate significant differences at $p < 0.05$ (one-way ANOVA and Tukey's test).

3.3 Mentum deformities

The control and the treatment with nanoparticles without atrazine in the subchronic bioassay did not cause mentum deformity of the larvae *Chironomus sancticaroli* (Table 1). In the acute bioassay were observed mentum deformities in most treatments (Table 1). However, only the atrazine in the acute bioassay in the concentration 0.47 mg.L^{-1} was significant difference (Table 1). Teeth with Köhn Gap was the type of mentum deformity that

predominated among the larvae, followed by missing teeth, extra teeth and bifid tooth (Table 1; Figure 1).

Table 1 Mean percentage of mentum deformities and proportion of each type of deformities in the acute (96 h) and subchronic (10 days) bioassays. Fifteen larvae were analyzed per treatment. W.C. = water control; S.C. = solvent control; ATZ = atrazine; SLN+ATZ solid lipid nanoparticle loaded with atrazine; SLN = solid lipid nanoparticle. Different letters in the group indicate significant difference at $p < 0.05$ (Kruskal-Wallis and Dunn's test).

Treatments (mg.L^{-1})	Deformities (%)	Bifid (%)	Extra (%)	Missing (%)	Köhn Gap (%)	Group
Acute bioassay 96 h						
W.C.	0	0	0	0	0	a
S.C.	0	0	0	0	0	a
0.002 ATZ	6.67	0	0	0	6.67	a
0.47 ATZ	33.33	0	13.33	6.67	13.33	b
0.95 ATZ	6.67	0	0	0	6.67	a
1.9 ATZ	6.67	6.67	0	0	0	a
W.C.	0	0	0	0	0	a
0.002 SLN+ATZ	6.67	0	0	0	6.67	a
0.47 SLN+ATZ	13.34	0	6.67	0	6.67	a
0.95 SLN+ATZ	20	0	0	6.67	13.33	a
W.C.	0	0	0	0	0	a
0.002 SLN	0	0	0	0	0	a
0.47 SLN	13.33	0	0	0	13.33	a
0.95 SLN	13.33	0	0	13.33	0	a
Subchronic bioassay 10 days						
W.C.	0	0	0	0	0	a
S.C.	0	0	0	0	0	a
0.002 ATZ	6.67	0	0	0	6.67	a
W.C.	0	0	0	0	0	a
0.002 SLN+ATZ	6.67	0	0	0	6.67	a
W.C.	0	0	0	0	0	a
0.002 SLN	0	0	0	0	0	a

3.4 Development rate

In the acute and subchronic bioassays, the treatments with atrazine and nanoparticles (with and without atrazine) did not affect the larvae developmental stage, because at the end of the experiments all the larvae of *Chironomus sancticaroli* were in the IV instar (Table 2).

Table 2 Ventral length of head capsule of larvae of *Chironomus sancticaroli* in acute (96 h) and subchronic (10 days) bioassays. Fifteen larvae were measured per treatment. W.C. = water control; S.C. = solvent control; ATZ = atrazine; SLN = solid lipid nanoparticle; SLN+ATZ solid lipid nanoparticle loaded with atrazine. The values are shown as the minimum, maximum, mean, standard deviation (SD) and confidence interval for each treatment. Measures in millimeters.

Treatments (mg.L^{-1})	Instar	Minimum	Maximum	Mean	SD	Confidence interval
Acute bioassay 96 h						
W.C.	IV	0.279	0.315	0.297	0.015	0.271–0.323
S.C.	IV	0.272	0.320	0.298	0.013	0.265–0.313
0.002 ATZ	IV	0.267	0.309	0.291	0.015	0.259–0.317
0.002 SLN	IV	0.279	0.318	0.300	0.013	0.272–0.325
0.002 SLN+ATZ	IV	0.271	0.322	0.300	0.016	0.263–0.330
0.47 ATZ	IV	0.283	0.307	0.297	0.011	0.277–0.313
0.47 SLN	IV	0.269	0.319	0.300	0.015	0.261–0.326
0.47 SLN+ATZ	IV	0.265	0.321	0.289	0.024	0.253–0.333
0.95 ATZ	IV	0.264	0.307	0.293	0.014	0.257–0.314
0.95 SLN	IV	0.264	0.319	0.291	0.017	0.255–0.328
0.95 SLN+ATZ	IV	0.277	0.323	0.306	0.012	0.271–0.329
1.9 ATZ	IV	0.275	0.315	0.299	0.013	0.268–0.322
Subchronic bioassay 10 days						
W.C.	IV	0.264	0.296	0.284	0.010	0,259–0.301
S.C.	IV	0.273	0.302	0.287	0.012	0.267–0.308
0.002 ATZ	IV	0.265	0.302	0.281	0.011	0.259–0.308
0.002 SLN	IV	0.266	0.311	0.289	0.015	0.258–0.319
0.002 SLN+ATZ	IV	0.264	0.310	0.293	0.010	0.259–0.315

3.5 Biochemical biomarkers activity

The AChE activity in the *Chironomus sancticaroli* larvae showed 9% inhibition at 10 days exposure to SLN + atrazine at a concentration of 0.002 mg.L^{-1} (Figure 3E). The activity of this enzyme was not affected by the other concentrations of the contaminants.

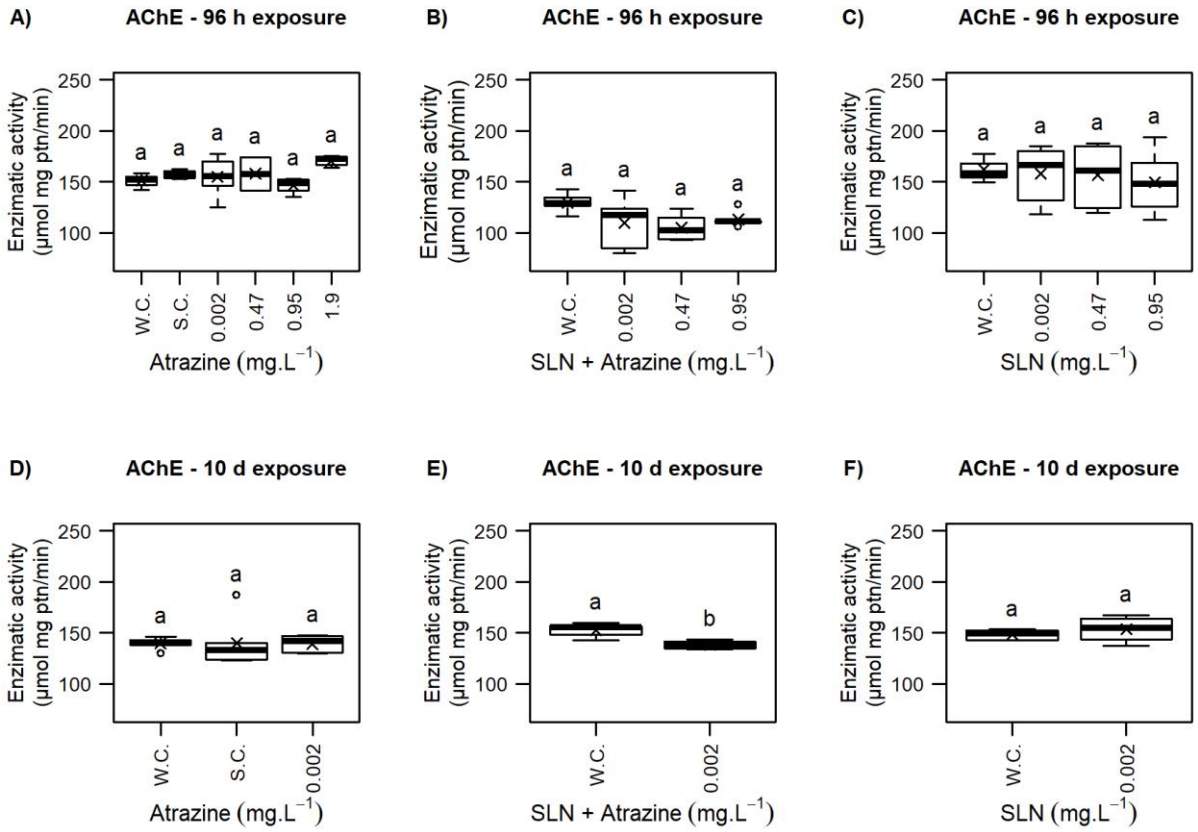


Figure 3 Acetylcholinesterase (AChE) activity in larvae of *Chironomus sancticaroli*. A) AChE activity in larvae exposed to atrazine in the acute bioassay (96 h). B) AChE activity in larvae exposed to solid lipid nanoparticles loaded with atrazine in acute bioassay (96 h). C) AChE activity in larvae exposed to solid lipid nanoparticles in acute bioassay (96 h). D) AChE activity in larvae exposed to atrazine in subchronic bioassay (10 days). E) AChE activity in larvae exposed to solid lipid nanoparticles loaded with atrazine in subchronic bioassay (10 days). F) AChE activity in larvae exposed to solid lipid nanoparticles in subchronic bioassay (10 days). The values are expressed as the median enzymatic activity ± upper and lower quartiles (n = 30). The + symbol indicates the average and the dots represent outliers. Different letters indicate significant differences at $p < 0.05$ (one-way ANOVA and Tukey's test).

The EST-α response increased by 47% at 96 h exposure to atrazine at 1.9 mg.L^{-1}, while exposure to SLN + atrazine at concentrations of 0.47 and 0.95 mg.L^{-1} resulted in 47 and 26% decreases of the activity of this enzyme, respectively (Figure 4A–B). In the case of the 10 days exposures, the EST-α activity showed a 97% increase in the bioassay with atrazine at 0.002 mg.L^{-1}, while the use of SLN + atrazine at 0.002 mg.L^{-1} resulted in a 20% decrease of EST-α activity (Figure 4D–E).

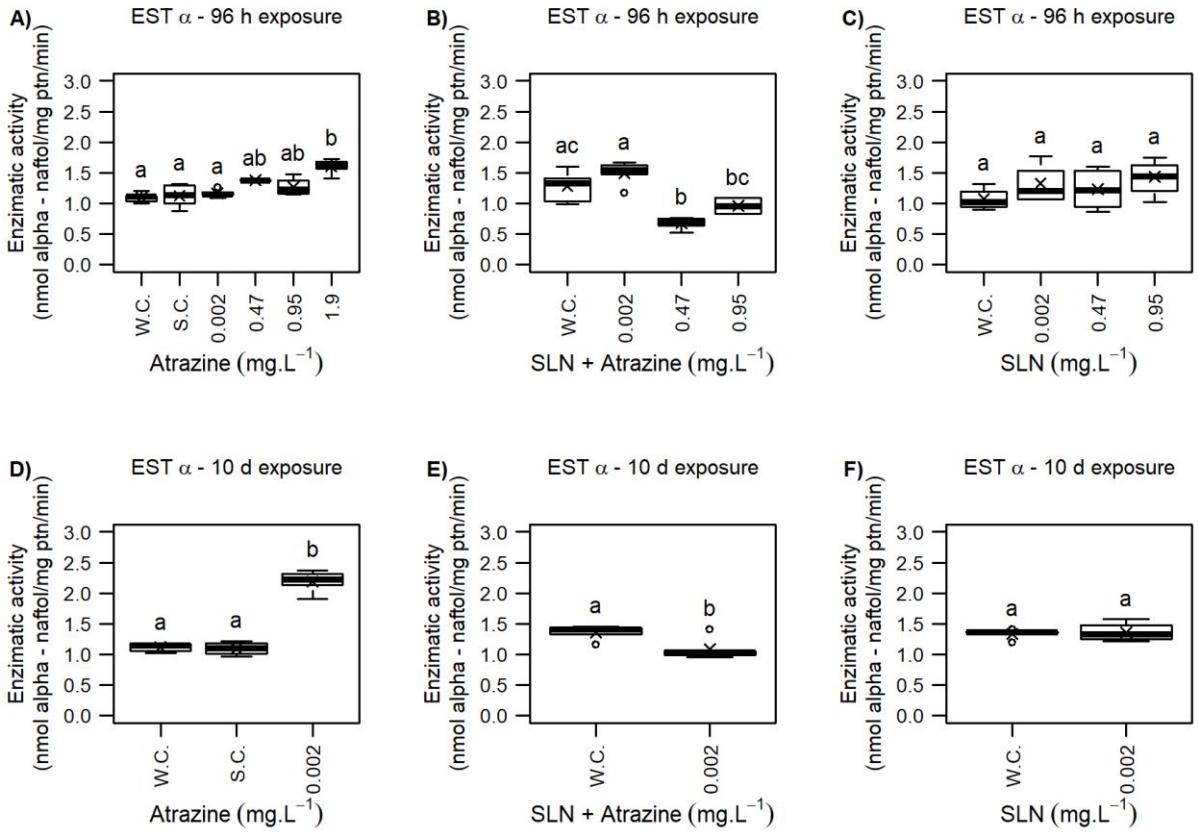


Figure 4 Alpha esterase (EST-α) activity in larvae of *Chironomus sancticaroli*. A) EST-α activity in larvae exposed to atrazine in the acute bioassay (96 h). B) EST-α activity in larvae exposed to solid lipid nanoparticles loaded with atrazine in acute bioassay (96 h). C) EST-α activity in larvae exposed to solid lipid nanoparticles in acute bioassay (96 h). D) EST-α activity in larvae exposed to atrazine in subchronic bioassay (10 days). E) EST-α activity in larvae exposed to solid lipid nanoparticles loaded with atrazine in subchronic bioassay (10 days). F) EST-α activity in larvae exposed to solid lipid nanoparticles in subchronic bioassay (10 days). The values are expressed as the median enzymatic activity ± upper and lower quartiles (n = 30). The + symbol indicates the

average and the dots represent outliers. Different letters indicate significant differences at $p < 0.05$ (one-way ANOVA and Tukey's test).

The EST-β response only changed in the 96 h exposure, where the treatments with atrazine at concentrations of 0.002, 0.47, and 1.9 $mg.L^{-1}$ increased the activity of this enzyme by 34%, while the use of SLN + atrazine at a concentration of 0.002 $mg.L^{-1}$ increased the activity by 17% (Figure 5A–B). The use of SLN + atrazine at concentrations of 0.47 and 0.95 $mg.L^{-1}$ resulted in decreases of 42 and 63%, respectively (Figure 5B). In the SLN bioassay, the activity of this enzyme decreased to 27 and 57% for concentrations of 0.47 and 0.95 $mg.L^{-1}$ (Figure 5C).

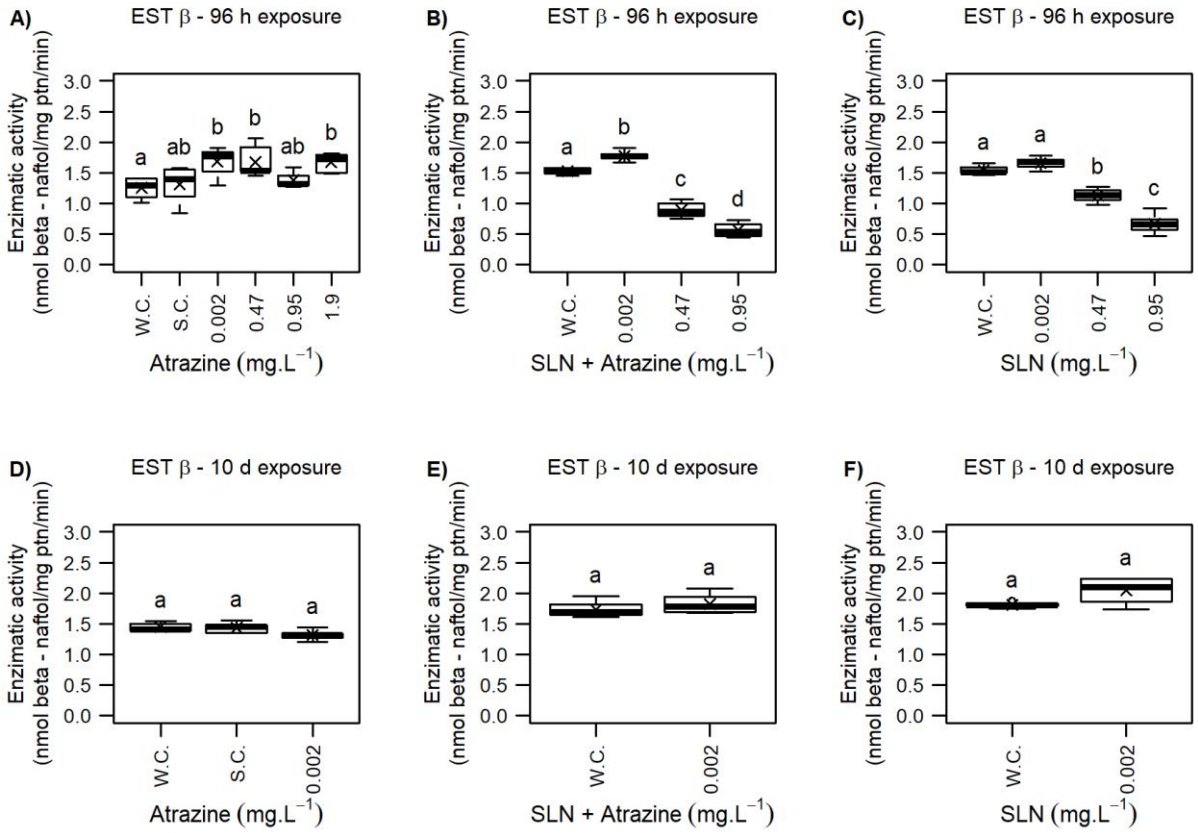


Figure 5 Beta esterase (EST-β) activity in larvae of *Chironomus sancticaroli*. A) EST- β activity in larvae exposed to atrazine in the acute bioassay (96 h). B) EST-β activity in larvae exposed to solid lipid nanoparticles loaded with atrazine in acute bioassay (96 h). C) EST-β activity in larvae exposed to solid lipid nanoparticles in

acute bioassay (96 h). D) EST-β activity in larvae exposed to atrazine in subchronic bioassay (10 days). E) EST-β activity in larvae exposed to solid lipid nanoparticles loaded with atrazine in subchronic bioassay (10 days). F) EST-β activity in larvae exposed to solid lipid nanoparticles in subchronic bioassay (10 days). The values are expressed as the median enzymatic activity ± upper and lower quartiles (n = 30). The + symbol indicates the average and the dots represent outliers. Different letters indicate significant differences at $p < 0.05$ (one-way ANOVA and Tukey's test).

In the 96 h exposure, the activity of GST increased by 32% for the treatment with atrazine at 0.47 $mg.L^{-1}$, while an increase of 33% was obtained for SLN + atrazine at a concentration of 0.002 $mg.L^{-1}$ (Figure 6A–B).

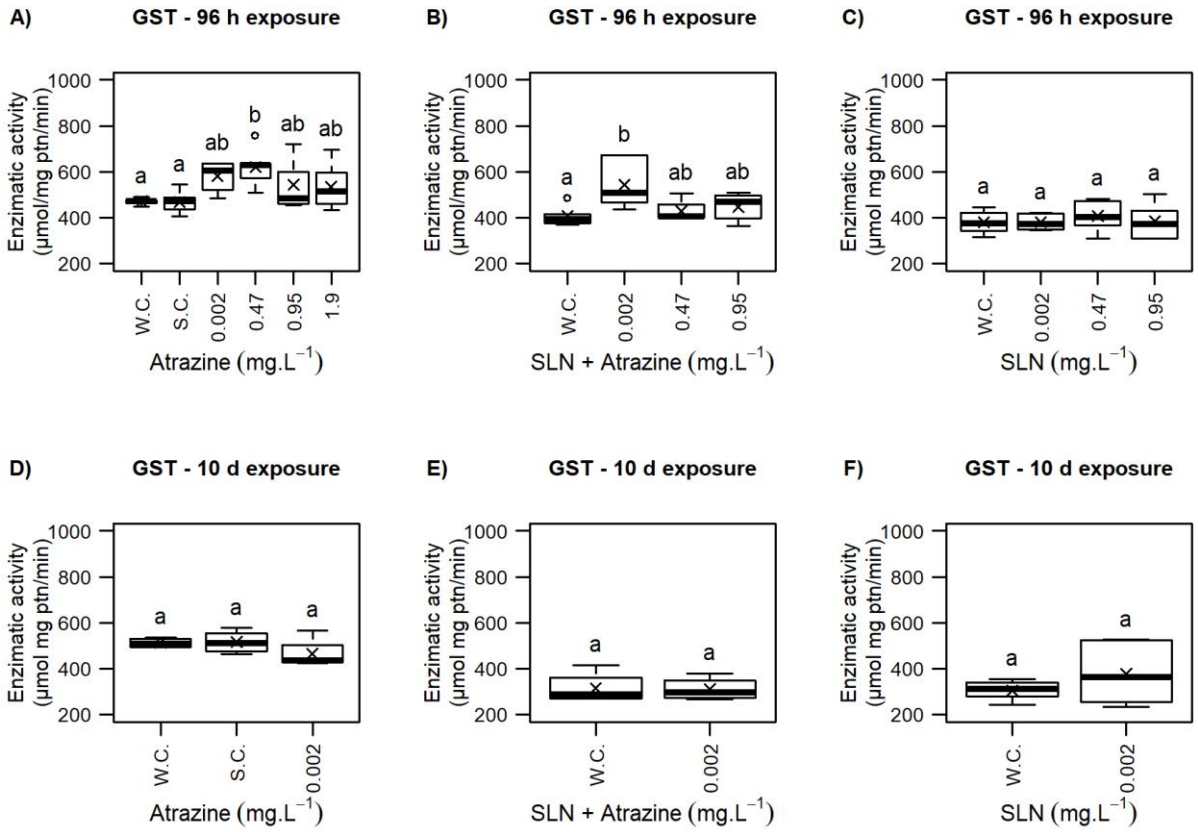


Figure 6 Glutathione S-transferase (GST) activity in larvae of *Chironomus sancticaroli*. A) GST activity in larvae exposed to atrazine in the acute bioassay (96 h). B) GST activity in larvae exposed to solid lipid nanoparticles loaded with atrazine in acute bioassay (96 h). C) GST activity in larvae exposed to solid lipid nanoparticles in acute bioassay (96 h). D) GST activity in larvae exposed to atrazine in subchronic bioassay (10

337 days). E) GST activity in larvae exposed to solid lipid nanoparticles loaded with atrazine in subchronic bioassay
338 (10 days). F) GST activity in larvae exposed to solid lipid nanoparticles in subchronic bioassay (10 days). The
339 values are expressed as the median enzymatic activity ± upper and lower quartiles (n = 30). The + symbol
340 indicates the average and the dots represent outliers. Different letters indicate significant differences at $p < 0.05$
341 (one-way ANOVA and Tukey's test).
342
343 Exposure to the SLN for 10 days did not cause any biochemical effects in the larvae of
344 *Chironomus sancticaroli*, since the responses for AChE, CAT, EST-α, EST-β and GST were
345 not altered. In the case of the 96 h exposure, changes were only observed for EST-β at 0.47
346 and 0.95 $mg.L^{-1}$ (Figures 3–6). The EST-β was the biochemical marker in the larvae of
347 *Chironomus sancticaroli* that was most sensitive to atrazine, SLN + atrazine and SLN. The
348 atrazine and SLN + atrazine were the contaminants that more caused alteration of the
349 biomarkers (Table 3).
350
351 **Table 3** Alteration of biochemical biomarkers activity in *Chironomus sancticaroli* larvae exposed to atrazine
352 (ATZ), solid lipid nanoparticles loaded with atrazine (SLN+ATZ) and solid lipid nanoparticles without atrazine
353 (SLN). AChE: Acetylcholinesterase. EST-α: Alpha esterase. EST-β: Beta esterase. GST: Glutathione S-
354 transferase. Table adapted from graphics of biochemical biomarkers activity (Figures 3–6).

Treatments ($mg.L^{-1}$)	**AChE**	**EST-α**	**EST-β**	**GST**
Acute bioassay				
0.002 ATZ	No significative	No significative	**Significative**	No significative
0.47 ATZ	No significative	No significative	**Significative**	**Significative**
0.95 ATZ	No significative	No significative	No significative	No significative
1.9 ATZ	No significative	**Significative**	**Significative**	No significative
0.002 SLN+ATZ	No significative	No significative	**Significative**	**Significative**
0.47 SLN+ATZ	No significative	**Significative**	**Significative**	No significative
0.95 SLN+ATZ	No significative	**Significative**	**Significative**	No significative
0.002 SLN	No significative	No significative	No significative	No significative
0.47 SLN	No significative	No significative	**Significative**	No significative
0.95 SLN	No significative	No significative	**Significative**	No significative
Subchronic bioassay				
0.002 ATZ	No significative	**Significative**	No significative	No significative
0.002 SLN+ATZ	**Significative**	**Significative**	No significative	No significative

0.002 SLN	No significative	No significative	No significative	No significative

355

356 **4. Discussion**

357 Benthic organisms differ in their tolerance to a diversity of pollutants, while their low
358 mobility and relatively short life cycles are characteristics that make these macroinvertebrates
359 suitable for use in various types of environmental studies. In aquatic ecosystems, the largest
360 sink of nanomaterials can be considered to be the sediment, which once contaminated directly
361 affects benthic organisms, so it is extremely important to evaluate and manage the
362 ecotoxicological risks of nanoparticles in this type of environment (Lee et al., 2016; Park et
363 al., 2015; Silva et al., 2016).

364 The variations of the physico-chemical parameters were in accordance with the
365 conditions established by the OECD (2004a, b) (Table S1), ensuring that under laboratory
366 conditions, the effects observed on *Chironomus sancticaroli* larvae were not associated with
367 these variables.

368 The highest concentration of atrazine (1.9 $mg.L^{-1}$) here tested represents NOEC for the
369 species *Chironomus riparius* and *Chironomus tepperi* (Phyu et al., 2005; Taylor et al., 1991).
370 However, in the present study it was observed a sublethal and lethal effect in the larvae of
371 *Chironomus sancticaroli* exposed at 1.9, 0.95, 0.47 and 0.002 $mg.L^{-1}$ of atrazine, indicating a
372 higher sensitivity of this species to the contaminant.

373 The SLN tested in this study were prepared previously by Oliveira et al. (2015b), who
374 observed that SLN loaded with atrazine had high encapsulation efficiency, as well as
375 performed a sustained release of active compound. The authors evaluated the active kinetic
376 release of the active through SLN using a compartment system by cellulose membrane under
377 “sink” conditions and observed that under these conditions the release of atrazine by SLN was
378 21.16 times slower than the herbicide not encapsulated, since SLN released 50% of atrazine in
379 52.9 hours.

According to Oliveira et al. (2015b) the SLN loaded with a combination of atrazine + simazine acted to reduce the cytotoxicity to nontarget organisms (3T3 rat fibroblast cells) and phytotoxicity to corn plants (*Zea mays*), as well as improved biological effects in the target pest (*Raphanus raphanistrum*).

In this study with larvae of *Chironomus sancticaroli*, the nanoparticles loaded with atrazine did not reduce the toxic effect of the herbicide, since mortality and biochemical marker results were similar to free atrazine. In addition, empty nanoparticles in all concentrations also caused mortality statistically significant in relation to control, indicating a possible toxic effect of the formulation on the larvae. However, the empty nanoparticles in the subchronic bioassay did not change the activity of the tested biomarkers.

The toxic effects of nanoformulations may have occurred because the larvae exposed the nanoparticles remained for a longer time in contact with the herbicide, for which the release was modified by the nanosystem. Another possibility is that the particles were incorporated by the organism, resulting in higher amounts of atrazine within the larvae. The formulation may have caused a toxic effect on the organism used. Further investigations will be needed in order to elucidate this effect.

Jacques et al. (2017) evaluated the toxicity of the same formulation described in this study in bioassays with the soil organism *Caenorhabditis elegans*. It was found that the mortality results for the loaded SLN formulation and the control were similar, suggesting that the toxicity could be mainly caused by the composition of the nanoparticles, since no mortality was observed when the worms were exposed to the herbicide.

Larvae of *Chironomus sancticaroli* have the habit of ingesting sediment particles and build tubes with silk and mud (Araújo et al., 2006; Fonseca and Rocha, 2004), so it is possible that they could have ingested the SLN that deposited in the sediment, increasing its toxicity. Previous studies have reported the absorption of lipid nucleus nanoparticles by

Caenorhabditis elegans via the oral route (Charão et al., 2015; Jacques et al., 2017; Moraes et al., 2016), this can be an efficient method for controlling the target organism, but hazardous for nontarget species.

The concentrations (0.002, 0.47 and 0.95 $mg.L^{-1}$) of solid lipid nanoparticles loaded with atrazine did not show significant differences between them to endpoint mortality. As demonstrated by another carrier (PCL nanocapsules containing atrazine) that had the atrazine concentration reduced up to ten times (200 $g\ ha^{-1}$) and still remained with the same efficiency (Bombo et al., 2019; Oliveira et al., 2015a; Souza et al., 2018).

The nanoparticles (with and without atrazine) and atrazine caused mentum deformities of *Chironomus sancticaroli* larvae, but only atrazine at concentration 0.47 $mg.L^{-1}$ was statistically significant. Beghelli et al. (2018) performed *in situ* bioassays with third instar larvae of *Chironomus sancticaroli* to assess the sediment toxicity of the Paiva Castro reservoir, Brazil. In this study, it was report mouthpart alterations of this species, however the alterations were not considered statistically significant. The authors also hypothesize that the toxicity of sediments is the main environmental factor driving the proportion mouthpart alterations and the occurrence of deaths in chironomid larvae.

The larval development stage was not affected by treatments with atrazine and nanoparticles (with and without atrazine), because the measures of ventral length of head capsule of larvae coincide with values recorded by Richardi et al. (2013) and Strixino And Strixino (1982).

Several studies have reported that atrazine alone exerted no toxic effects in *Chironomus tentans* and *Chironomus tepperi*, but that this herbicide enhanced the toxicity of some organophosphorus pesticides (Anderson and Zhu, 2004; Belden and Lydy, 2000, 2001; Jin-Clark et al., 2002; Mehler et al., 2008; Pape-Lindstrom and Lydy, 1997; Pérez et al., 2013; Schuler et al., 2005). Atrazine in combination with organophosphorus has been reported to

inhibit AChE activity in chironomid larvae (Anderson and Zhu, 2004; Belden and Lydy, 2001; Jin-Clark et al., 2002; Pérez et al., 2013). However, in the present case, when the atrazine was encapsulated in SLN, it inhibited the AChE activity in the 10 days bioassay.

The alpha and beta esterases are phase I biotransformation enzymes that are of great importance in the metabolization of various classes of endogenous and exogenous compounds (Montella et al., 2012; Vicentini et al., 2017). According to Vicentini et al. (2017), enzymatic activity of the esterases during phase I is indicative of metabolic alterations in the larvae of *Chironomus sancticaroli*.

GST is a phase II biotransformation enzyme that can also be considered an antioxidant, acting in the process of metabolic detoxification (Frova, 2006; Shi et al., 2012; Xie et al., 2019) and the induction of GST in larvae of *Chironomus sancticaroli* may indicate an initial detoxification process for cell protection (Palacio-Cortés et al., 2017).

Variations of temperature and food supply in bioassays with *Chironomus sancticaroli* and *Chironomus riparius* have been found to affect the activities of the enzymes AChE, EST-α, EST-β, and GST, with each enzyme having an optimal activity temperature (Callaghan et al., 2002; Rebechi-Baggio et al., 2016). The temperature and food supply were therefore kept constant, in order to ensure that the biochemical changes were not related to these factors.

The use of atrazine was banned by the European Union in 2004, due to its potential to contaminate groundwater (Ackerman, 2007). Nevertheless, the prohibition of this herbicide has generated much debate and research, and it is still used in other countries (Hénault-Ethier, 2016; Sass and Colangelo, 2006).

The atrazine concentration of 0.002 $mg.L^{-1}$ permitted by Brazilian legislation for freshwater, considering use of the water for human consumption and the protection of aquatic life (CONAMA, 2005) is lower than stipulated by the World Health Organization (0.1 $mg.L^{-1}$) (WHO, 2017), Australia (0.02 $mg.L^{-1}$) (NHMRC/NRMMC, 2011) e United State (0.003

455 mg.L^{-1}) (USEPA, 2009). The results obtained in the present study also indicate the need to
456 review the maximum concentrations of atrazine currently established by environmental
457 agencies, since atrazine at a concentration of 0.002 mg.L^{-1} caused lethal and sublethal effects
458 in the larvae of *Chironomus sancticaroli*.

459 In a study carried by Armas et al. (2007) in the Corumbataí River (Mato Grosso do
460 Sul, Brazil), concentrations of atrazine were detected which ranged from 0.0006-0.0027 mg.L^{-}
461 ^{1}, including levels near the standard established by Brazilian legislation (0.002 mg.L^{-1}). The
462 toxicity to aquatic organisms of atrazine at environmentally relevant concentrations also was
463 reported by Botelho et al. (2015), who detected the herbicide in water samples from the
464 Piracicaba River (São Paulo State, Brazil) and in Bioassays with *Danio rerio* revealed that
465 concentrations between 0.001 and 0.002 mg.L^{-1} induced the formation of micronuclei,
466 indicative of genotoxicity potential of this pesticide.

467 Based on 78 published articles, Kah et al. (2018) reported that nanoagrochemicals are
468 about 20-30% more efficient than the conventional analogues. However, it is essential to
469 evaluate their toxicity and environmental bioavailability, due to concerns about the potential
470 adverse impacts that nanoparticles can cause for human health and the environment.

471

472 **5. Conclusions**

473 The concentration of atrazine considered acceptable according to regulatory agencies
474 (as example from Brazil) was toxic to the larvae of *Chironomus sancticaroli*, leading to
475 mortality and biochemical changes. It is suggested that environmental agencies should review
476 the concentration of atrazine allowed, because the current limit value for this contaminant
477 represents a risk for aquatic species, highlight by *Chironomus sancticaroli*. Under the
478 experimental conditions tested the nanoparticles loaded with atrazine showed results similar
479 to free atrazine, causing mortality and biochemical alterations on *Chironomus sancticaroli*

larvae. The nanoparticle control (without the active ingredient) caused biochemical alterations and mortality, indicating a possible toxic effect of the formulation on the larvae. However, the nanoparticles did not affect the larvae developmental stage of *Chironomus sancticaroli* and also did not cause statistically significant mentum deformities. In the acute bioassay the concentrations (0.002, 0.47 and 0.95 $mg.L^{-1}$) of formulation loaded with atrazine were not dose dependent for the endpoint mortality. The toxicities of solid lipid nanoparticles (with or without an active agent) should be evaluated using different routes of exposure. In addition, as a safer strategy for nanopesticides, it is important to separately test the compounds present in nanoformulation. This is the first study to report the effects of atrazine, as well as a nanoparticle formulation, using the benthic organism *Chironomus sancticaroli*. The results showed that this organism could be a useful model in investigations of the potential toxic effects of nanopesticides in the Neotropical region.

Acknowledgments

This study was financed in part by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil, finance code 001). L.F.F. and J.L.O. would like to thank the São Paulo State Research Foundation (FAPESP, grants #2017/21004-5 and #2018/21142-1) for financial support for nanopesticide development.

6. References

Ackerman, F., 2007. The Economics of Atrazine. Int. J. Occup. Environ. Health 3, 437–445. https://doi.org/10.1179/oeh.2007.13.4.437.

Albuquerque, F.P., Oliveira, J.L., Moschini-Carlos, V., Fraceto, L.F., 2020. An overview of the potential impacts of atrazine in aquatic environments: Perspectives for tailored solutions

504 based on nanotechnology. Sci Total Environ 700, 1–9.
505 https://doi.org/10.1016/j.scitotenv.2019.134868.
506 Anderson, T.D., Zhu, K.Y., 2004. Synergistic and antagonistic effects of atrazine on the
507 toxicity of organophosphorodithioate and organophosphorothioate insecticides to *Chironomus*
508 *tentans* (Diptera: Chironomidae). Pestic. Biochem. Physiol. 80, 54–64.
509 https://doi.org/10.1016/j.pestbp.2004.06.003.
510 Arambourou, H., Beisel, J.N., Branchu, P., Debat, V., (2012). Patterns of fluctuating
511 asymmetry and shape variation in *Chironomus riparius* (Diptera, Chironomidae) exposed to
512 nonylphenol or lead. Plos One 7, 1–12.
513 Arambourou, H., Beisel, J.N., Branchu, P., Debat, V., (2014). Exposure to sediments from
514 polluted rivers has limited phenotypic effects on larvae and adults of *Chironomus riparius*.
515 Sci. Total Environ. 484, 92–101. https://doi.org/10.1016/j.scitotenv.2014.03.010.
516 Araújo, R.P.A., Shimizu, G.Y., Almeida, C.A., Rocha, O., 2006. Testes com invertebrados
517 para avaliar a toxicidade de contaminantes associados ao sedimento de ecossistemas de água
518 doce. in: Mozeto, A.A., Umbuzeiro, G.A., Jardim, W.F. (Eds.), Métodos de coleta, análises
519 físico-químicas e ensaios biológicos e ecotoxicológicos de sedimentos de água doce. São
520 Carlos, pp. 111–133.
521 Araújo, C.V.M., Silva, D.C.V.R., Gomes, L.E.T., Acayaba, R.D., Montagner, C.C., Moreira-
522 Santos, M., Ribeiro, R., Pompeo, M.L.M., 2018. Habitat fragmentation caused by
523 contaminants: Atrazine as a chemical barrier isolating fish populations. Chemosphere 193,
524 24–31. https://doi.org/10.1016/j.chemosphere.2017.11.014.
525 Armas, E.D., Monteiro, R.T.R., Antunes, P.M., Santos, M.A.P.F., Camargo, P.B., 2007.
526 Diagnóstico espaço-temporal da ocorrência de herbicidas nas águas superficiais e sedimentos
527 do Rio Corumbataí e principais afluentes. Quim. Nova 30, 1119–1127.
528 https://doi.org/10.1590/S0100-40422007000500013.

529 Belden, J.B., Lydy, M.J., 2000. Impact of atrazine on organophosphate insecticide toxicity.
530 Environ. Toxicol. Chem. 19, 2266–2274. https://doi.org/10.1002/etc.5620190917.
531 Belden, J.B., Lydy, M.J., 2001. Effects of atrazine on acetycholinesterase activity in midges
532 (*Chironomus tentans*) exposed to organophosphorus insecticides. Chemosphere 44, 1685–
533 1689. https://doi.org/10.1016/S0045-6535(00)00519-1.
534 Beghelli, F.G.S., Lopez-Dovál, J.C., Rosa, A.H., Pompêo, M., Moschini-Carlos, V., 2018.
535 Lethal and sublethal effects of metal-polluted sediments on *Chironomus sancticaroli* Strixino
536 and Strixino, 1981. Ecotoxicol. 27, 286–299.
537 Bombo, A.B., Pereira, A.E.S., Lusa, M.G., Oliveira, E.M., Oliveira, J.L., Campos, E.V.R.,
538 Jesus, M.B., Oliveira, H.C., Fraceto, L.F., Mayer, J.L.S., 2019. A mechanistic view of
539 interactions of a nanoherbicide with target organism. J. Agric. Food Chem. 67, 4453-4462.
540 https://doi.org/10.1021/acs.jafc.9b00806.
541 Bradford, M., 1976. A rapid and sensitive method for the quantification of microgram
542 quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–
543 254. https://doi.org/10.1016/0003-2697(76)90527-3.
544 Botelho, R.G., Monteiro, S.H., Christofoletti, C.A., Moura-Andrade, G.C.R., Tornisielo, V.L.,
545 2015. Environmentally relevant concentrations of atrazine and ametrine induce micronuclei
546 formation and nuclear abnormalities in erythrocytes of fish. Arch. Environ. Contam. Toxicol.
547 69, 577–585. https://doi.org/10.1007/s00244-015-0171-6.
548 Carmo, D.A., Carmo, A.P.B., Pires, J.M.B., Oliveira, J.L.M., 2013. Comportamento
549 ambiental e toxicidade dos herbicidas atrazina e simazina. Rev. Ambient. Água 8, 133–143.
550 http://doi.org/10.4136/ambi-agua.1073.
551 Charão, M.F., Souto, C., Brucker, N., Barth, A., Jornada, D.S., Fagundez, D., Ávila, D.S.,
552 Eifler-Lima, V.L., Guterres, S.S., Pohlmann, A.R., Garcia, S.C., 2015. *Caenorhabditis*
553 *elegans* as an alternative in vivo model to determine oral uptake, nanotoxicity, and efficacy of

554 melatonin-loaded lipid-core nanocapsules on paraquat damage. Int. J. Nanomedicine 10,
555 5093–5106. https://doi.org/10.2147/IJN.S84909.
556 Callaghan, A., Fisher, T.C., Grosso, A., Holloway, G.J., Crane, M., 2002. Effect of
557 temperature and pirimiphos methyl on biochemical biomarkers in *Chironomus riparius*
558 Meigen. Ecotoxicol. Environ. Saf. 52, 128–133. https://doi.org/10.1006/eesa.2002.2160.
559 Chevrier, C., Limon, G., Monfort, C., Rouget, F., Garlantézec, R., Petit, C., Durand, G.,
560 Cordier, S., 2011. Urinary biomarkers of prenatal atrazine exposure and adverse birth
561 outcomes in the pelagie birth cohort. Environ. Health Perspect. 119, 1034–1041.
562 https://doi.org/10.1289/ehp.1002775.
563 Choung, C.B., Hyne, R.V., Stevens, M.M., Hose, G.C., 2013. The ecological effects of a
564 herbicide insecticide mixture on an experimental freshwater ecosystem. Environ. Pollut. 172,
565 264–274. https://doi.org/10.1016/j.envpol.2012.09.002.
566 Clemente, Z., Grillo, R., Jonsson, M., Santos, N.Z.P., Feitosa, L.O., Lima, R., Fraceto, L.F.,
567 2013. Ecotoxicological evaluation of poly (epsilon-caprolactone) nanocapsules containing
568 triazine herbicides. J. Nanosci. Nanotechnol. 13, 1–7. https://doi.org/10.1166/jnn.2013.8681.
569 CONAMA, 2005. Resolução nº 357, de 17 de março de 2005. Conselho Nacional do
570 Meio Ambiente, Brasil.
571 Dornfeld, C.B., Espíndola, E.L.G., Fracácio, R.; Rodrigues, B.K., Novelli, A., 2006.
572 Comparison between laboratory and “in situ” bioassays using *Chironomus xanthus* in the
573 assessment of sediment toxicity in the Monjolinho river (São Carlos, SP). J. Braz. Soc.
574 Ecotoxicol. 1, 161–165. https://doi.org/10.5132/jbse.2006.02.014.
575 Duhan, J.G., Kumar, R., Kumar, N., Kaur, P., Kiran, N., Duhan, S., 2017. Nanotechnology:
576 The new perspective in precision agriculture. Biotechnol. Rep. 15, 11–23.
577 https://doi.org/10.1016/j.btre.2017.03.002.

578 Ellman, G.L., Courtney, K.D., Andres-Jr, V., Featherstone, R.M., 1961. A nem and rapid
579 colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88–95.
580 https://doi.org/10.1016/0006-2952(61)90145-9.
581 Epler J.H., (2001). Identification manual for the larval Chironomidae (Diptera) of North and
582 South Carolina. North Carolina Department of Environment and Natural Resources.
583 Fraceto, L.F., Grillo, R., Medeiros, G.A., Scognamiglio, V., REA, G., Bartolucci, G., 2016.
584 Nanotechnology in agriculture: which innovation potential does it have? Front. Environ. Sci.
585 4, 1–5. https://doi.org/10.3389/fenvs.2016.00020.
586 Frova, C., 2006. Glutathione transferases in the genomics era: New insights and perspectives.
587 Biomol. Eng. 23, 149–169. https://doi.org/10.1016/j.bioeng.2006.05.020.
588 Fonseca, A.L., Rocha, O., 2004. Laboratory cultures of the native species *Chironomus*
589 *Xanthus* Rempel, 1939 (Diptera-Chironomidae). Acta Limnol. Bras. 16, 153–161.
590 Garcia, F.P., Ascencio, S.Y.C., Oyarzun, J.C.G., Hernandez, A.C., Alavarado, P.V., 2012.
591 Pesticides: classification, uses and toxicity. Measures of exposure and genotoxic risks. J. Res.
592 Environ. Sci. Toxicol. 1, 279–293.
593 Gilden, R.C., Huffling, K., Sattler, B., 2010. Pesticides and Health Risks. J. Obstet. Gynecol.
594 Neonatal Nurs. 39, 103–110. https://doi.org/10.1111/j.1552-6909.2009.01092.x.
595 Graziano, N., Mcguire, M.J., Roberson, A., Adams, C., Jiang, H., Blute, N., 2006. National
596 atrazine occurrence monitoring program using the abraxis ELISA method. Environ. Sci.
597 Technol. 40, 1163–1171. https://doi.org/10.1021/es051586y.
598 Grillo, R., Melo, N.F.S., Lima, R., Lourenço, R.W., Rosa, A.H., Fraceto, L.F., 2010.
599 Characterization of atrazine-loaded biodegradable poly (hydroxybutyrate-co-hydroxyvalerate)
600 microspheres. J. Polym. Environ. 18, 26–32. https://doi.org/10.1007/s10924-009-0153-8.
601 Grillo, R., Santos, N.Z.P., Maruyama, C.R., Rosa, A.H., Lima, R., Fraceto, L.F., 2012. Poly (-
602 caprolactone) nanocapsules as carrier systems for herbicides: Physico-chemical

603 characterization and genotoxicity evaluation. J. Hazard. Mater. 231, 1–9.
604 https://doi.org/10.1016/j.jhazmat.2012.06.019.
605 Guan, Ying-Hong, Ma, J., Ren, Yue-Ming, Liu, Yu-Lei, Xiao, Jia-Yue, Lin, Ling-Qiang,
606 Zhang, C., 2013. Efficient degradation of atrazine by magnetic porous copper ferrite catalyzed
607 peroxymonosulfate oxidation via the formation of hydroxyl and sulfate radicals. Water Res.
608 47, 5431–5438. https://doi.org/10.1016/j.watres.2013.06.023.
609 Hénault-Ethier, L., 2016. Backgrounder: Atrazine: Banned in Europe, Common in Canada.
610 CAPE. https://doi.org/10.13140/RG.2.1.2462.9365.
611 Jacques, M.T., Oliveira, J.L., Campos, E.V.R., Fraceto, L.F., Ávila, D.S., 2017. Safety
612 assessment of nanopesticides using the roundworm *Caenorhabditis elegans*. Ecotoxicol.
613 Environ. Saf. 139, 245–253. https://doi.org/10.1016/j.ecoenv.2017.01.045.
614 Janke, H., Yamada, T.M., Beraldo, D.A.S., Botta, C.M.R., Nascimento, M.R.L., Mozeto,
615 A.A., 2011. Assessment of the acute toxicity of eutrophic sediments after the addition of
616 calcium nitrate (Ibirité reservoir, Minas Gerais-SE Brazil): initial laboratory experiments.
617 Braz. J. Biol. 71, 903–914. https://doi.org/10.1590/S1519-69842011000500011.
618 Jin-Clark, Y., Lydy, M..J., Zhu, K.Y., 2002. Effects of atrazine and cyanazine on chlorpyrifos
619 toxicity in *Chironomus tentans* (Diptera: Chironomidae). Environ. Toxicol. Chem. 21, 598–
620 603. https://doi.org/10.1002/etc.5620210319.
621 Kah, M., Machinski, P., Koerner, P., Tiede, K., Grillo, R., Fraceto, L.F., Hofmann, T., 2014.
622 Analysing the fate of nanopesticides in soil and the applicability of regulatory protocols using
623 a polymer-based nanoformulation of atrazine. Environ. Sci. Pollut. Res. 21, 11699–11707.
624 https://doi.org/10.1007/s11356-014-2523-6.
625 Kah, M., Kookana, R.S., Gogos, A., Bucheli, T.D., 2018. A critical evaluation of
626 nanopesticides and nanofertilizers against their conventional analogues. Nat. Nanotechnol. 13,
627 677–684. https://doi.org/10.1038/s41565-018-0131-1.

628 Keen, J.H., Habig, W.H., Jakoby, W.B., 1976. Mechanism for the several activities of the
629 glutathione S-transferases. J. Biol. Chem**.** 251, 6183–6188.
630 Lee, S.W., Park, S.Y., Kim, Y., IM, H., Choi, J., 2016. Effect of sulfidation and dissolved
631 organic matters on toxicity of silver nanoparticles in sediment dwelling organism,
632 *Chironomus riparius*. Sci. Total Environ. 553, 565–573.
633 https://doi.org/10.1016/j.scitotenv.2016.02.064.
634 López-Doval, J.C., Kukkonen, J.V.K., Rodrigo, P., Muñoz, I., 2012. Effects of indomethacin
635 and propranolol *on Chironomus riparius* and *Physella (Costatella) acuta*. Ecotoxicol.
636 Environ. Saf. 78, 110–115. https://doi.org/doi:10.1016/j.ecoenv.2011.11.004.
637 Maier, K. J., Kosalwat, P., Knight, A. W., 1990. Culture of *Chironomus decorus* (Diptera:
638 Chironomidae) and the effect of temperature on its life history. Environ. Entomol. 19, 1681–
639 1688. https://doi.org/10.1093/ee/19.6.1681.
640 Mehler, W.T., Schuler, L.J., Lydy, M.J., 2008. Examining the joint toxicity of chlorpyrifos
641 and atrazine in the aquatic species: *Lepomis macrochirus*, *Pimephales promelas* and
642 *Chironomus tentans*. Environ. Pollut. 152, 217–224.
643 https://doi.org/10.1016/j.envpol.2007.04.028.
644 Montella, I.R., Schama, R., Valle, D., 2012. The classification of esterases:
645 an important gene family involved in insecticide resistance - A Review. Mem. Inst. Oswaldo
646 Cruz 107, 437–449. https://doi.org/10.1590/S0074-02762012000400001.
647 Moraes, B.S., Vieira, S.M., Salgueiro, W.G., Michels, L.R., Colomé, L.M., Ávila, D.S., Hass,
648 S.E., 2016. Clozapine-Loaded Polysorbate-Coated Polymeric Nanocapsules: Physico-
649 Chemical Characterization and Toxicity Evaluation in *Caenorhabditis elegans* Model. J.
650 Nanosci. Nanotechnol. 16, 1257–1264. https://doi.org/10.1166/jnn.2016.11668.
651 Moreira-Santos, M., Fonseca, A.L., Moreira, S.M., Osten, J.R. V., Silva, E. M., Soares,
652 A.M.V.M., Guilhermino, L., Ribeiro, R., 2005. Short-term sublethal (sediment and aquatic

653 roots of floating macrophytes) assays with a tropical chironomid based on postexposure
654 feeding and biomarkers. Environ. Toxicol. Chem. 24, 2234–2242.
655 https://doi.org/10.1897/04207R.1.
656 NHMRC/NRMMC, 2011. Australian Drinking Water Guidelines. Paper 6 National
657 Water Quality Management Strategy. National Health and Medical Research
658 Council, National Resource Management Ministerial Council, Common wealth
659 of Australia, Canberra.
660 Nödler, K., Licha, T., Voutsa, D., 2013. Twenty years later – Atrazine concentrations in
661 selected coastal waters of the Mediterranean and the Baltic Sea. Mar. Pollut. Bull. 70, 112–
662 118. https://doi.org/10.1016/j.marpolbul.2013.02.018.
663 Novelli, A., Vieira, B.H., Cordeiro, D., Cappelini, L.T.D., Vieira, E.M., Espíndola, E.L.G.,
664 2012. Lethal effects of abamectin on the aquatic organisms *Daphia similis*, *Chironomus*
665 *xanthus* and *Danio rerio*. Chemosphere 86, 36–40.
666 https://doi.org/10.1016/j.chemosphere.2011.08.047.
667 Nwani, C.D., Lakra, W.S., Nagpure, N.S., Kumar, R., Kushwaha, B., Srivastava, S.K., 2010.
668 Toxicity of the herbicide atrazine: effects on lipid peroxidation and activities of antioxidant
669 enzymes in the freshwater fish *Channa punctatus* (bloch). Int. J. Environ. Res. Public Health
670 7, 3298–3312. https://doi.org/10.3390/ijerph7083298.
671 Oberholster, P.J., Musee, N., Botha, A.M., Chelule, P.K., Focke, W.W., Ashton, P.J., 2011.
672 Assessment of the effect of nanomaterials on sediment-dwelling invertebrate *Chironomus*
673 *tentans* larvae. Ecotoxicol. Environ. Saf. 74, 416–423.
674 https://doi.org/10.1016/j.ecoenv.2010.12.012.
675 OECD/OCDE - ORGANISATION FOR ECONOMIC CO-OPERATION AND
676 DEVELOPMENT, 2004a. Sediment-water chironomid toxicity test using spiked water, Paris,

677 Test Guideline n. 219, Guidelines for the testing of chemicals. 21.
678 https://doi.org/10.1787/9789264070288-en.
679 OECD/OCDE - ORGANISATION FOR ECONOMIC CO-OPERATION AND
680 DEVELOPMENT, 2004b. Sediment-water chironomid toxicity test using spiked sediment,
681 Paris, Test Guideline n. 218, Guidelines for the Testing of Chemicals. 17p.
682 https://doi.org/10.1787/9789264070264-en.
683 OECD - ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT,
684 2010. Sediment-Water Chironomid Life-Cycle Toxicity Test Using Spiked Water or Spiked
685 Sediment, Paris, Test Guideline n. 233, Guidelines for the Testing of Chemicals. 29.
686 https://doi.org/10.1787/9789264090910-en.
687 Oliveira, H.C., Stolf-Moreira, R., Martinez, C.B.R., Grillo, R., Jesus, M. B., Fraceto, L.F.,
688 2015a. Nanoencapsulation enhances the post-emergence herbicidal activity of atrazine against
689 mustard plants. Plos One 10, 1–12. https://doi.org/10.1371/journal.pone.0132971.
690 Oliveira, J.L., Campos, E.V.R., Silva, C.M.G., Pasquoto, T., Lima R., Fraceto, L. F., 2015b.
691 Solid lipid nanoparticles co-loaded with simazine and atrazine: preparation, characterization,
692 and evaluation of herbicidal activity. J. Agric. Food Chem. 63, 422–432.
693 https://doi.org/10.1021/jf5059045.
694 Oliveira, H.C., Stolf-Moreira, R., Martinez, C.B.R., Sousa, G.F.M., Grillo, R., Jesus, M.B.,
695 Fraceto, L.F., 2015c. Evaluation of the side effects of poly(epsilon-caprolactone)
696 nanocapsules containing atrazine toward maize plants. Front. Chem. 3, 1–9.
697 https://doi.org/10.3389/fchem.2015.00061.
698 Oliveira, J.L., Campos, E.V.R., Fraceto, L.F., 2018. Recent developments and challenges for
699 nanoscale formulation of botanical pesticides for use in sustainable agriculture. J. Agric. Food
700 Chem. 66, 8898-8913. https://doi.org/10.1021/acs.jafc.8b03183.

701 Palacio-Cortés, A.M., Signorini-Souza, I.L., Hara, E.L.Y., Disner, R.G., Rebechi, D., Grassi,
702 M.T., Cestari, M.M., Navarro-Silva, M.A., 2017. Polybrominated diphenyl ethers (PBDEs)
703 effects on *Chironomus sancticaroli* larvae after short-term exposure. Ecotoxicol. Environ.
704 Saf. 139, 308–315. https://doi.org/10.1016/j.ecoenv.2017.01.052.
705 Pape-Lindstrom, P.A., Lydy, M.J. 1997. Synergistic toxicity of atrazine and organophosphate
706 insecticides contravenes the response addition mixture model. Environ. Toxicol. Chem. 16,
707 2415–2420. https://doi.org/10.1002/etc.5620161130.
708 Park, S.Y., Chung, J., Colman, B.P., Matson, C.W., Kim, Y., Lee, B.C., Kim, P.J., Choi, K.,
709 Choi, J., 2015. Ecotoxicity of bare and coated silver nanoparticles in the aquatic midge,
710 *Chironomus riparius*. Environ. Toxicol. Chem. 34, 2023–2032.
711 https://doi.org/10.1002/etc.3019.
712 Pereira, A.E.S., Grillo, R., Mello, N.F.S., Rosa, A.H., Fraceto, L.F., 2014. Application of poly
713 (epsilon-caprolactone) nanoparticles containing atrazine herbicide as an alternative technique
714 to control weeds and reduce damage to the environment. J. Hazard. Mater. 268, 207–215.
715 https://doi.org/10.1016/j.jhazmat.2014.01.025.
716 Pérez, J., Monteiro, M.S., Quintaneiro, C., Soares, A.M.V.M., Loureiro, S., 2013.
717 Characterization of cholinesterases in *Chironomus riparius* and the effects of three herbicides
718 on chlorpyrifos toxicity. Aquat. Toxicol. 144, 296–302.
719 https://doi.org/10.1016/j.aquatox.2013.10.014.
720 Phyu, Y.L., Warne, M.St.J., Lim, R.P., 2005. The toxicity and bioavailability of atrazine and
721 molinate to *Chironomus tepperi* larvae in laboratory and river water in the presence and
722 absence of sediment. Chemosphere 58, 1231–1239.
723 https://doi.org/10.1016/j.chemosphere.2004.10.035.

724 Printes, L.B., Espíndola, E.L.G., Fernandes, M.N., 2007. Biochemical biomarkers in
725 individual larvae of *Chironomus Xanthus* (Rempel, 1939) (Diptera, Chironomidae**)**. J. Braz.
726 Soc. Ecotoxicol. 2, 53–60. https://doi.org/10.5132/jbse.2007.01.008.
727 Printes, L.B., Fernandes, M.N., Espíndola, E.L.G., 2011. Laboratory measurements of
728 biomarkers and individual performances in *Chironomus xanthus* to evaluate pesticide
729 contamination of sediments in a river of southeastern Brazil. Ecotoxicol. Environ. Saf. 74,
730 424–430. https://doi.org/10.1016/j.ecoenv.2010.10.033.
731 R Development Core Team, 2011. R: A Language and Environment for Statistical
732 Computing. R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-
733 900051-07-0.
734 Ralston-Hooper, K., Sanchez, B.C., Adamec, J., Sepúlveda, M.S., 2011. Proteomics in aquatic
735 amphipods: can it be used to determine mechanisms of toxicity and interspecies responses
736 after exposure to atrazine? Environ. Toxicol. Chem. 30, 1197–1203.
737 https://doi.org/10.1002/etc.475.
738 Rebechi, D., Richardi, V.S., Vicentini, M., Guiloski, I.C., Assis, H.C.S., Navarro-Silva, M.A.,
739 2014. Low malathion concentrations influence metabolism in *Chironomus sancticaroli*
740 (Diptera, Chironomidae) in acute and chronic toxicity tests. Rev. Bras. Entomol. 58, 296–301.
741 https://doi.org/10.1590/S0085-56262014000300012.
742 Rebechi-Baggio, D., Richardi, V.S., Vicentini, M., Guiloski, I.C., Assis, H.C.S., Navarro-
743 Silva, M.A., 2016. Factors that alter the biochemical biomarkers of environmental
744 contamination in *Chironomus sancticaroli* (Diptera, Chironomidae). Rev. Bras. Entomol. 60,
745 341–346. https://doi.org/10.1016/j.rbe.2016.07.002.
746 Richardi, V.S., Rebechi, D., Aranha, J.M.R., Navarro-Silva, M.A., 2013. Determination of
747 larval instars in *Chironomus sancticaroli* (Diptera: Chironomidae) using novel head capsule
748 structures. Zoologia 30, 211–216. https://doi.org/10.1590/S1984-46702013000200011.

749 Richardi, V.S., Vicentini, M., Rebechi, D., Fávaro, L.F., Navarro-Silva, M.A., 2015. Morpho-
750 histological characterization of immature of the bioindicator midge *Chironomus sancticaroli*
751 Strixino and Strixino (Diptera, Chironomidae). Rev. Bras. Entomol. 59, 240–250.
752 https://doi.org/10.1016/j.rbe.2015.07.003.
753 Richardi, V.S., Vicentini, M., Morais, G.S., Rebechi, D., Silva, T.A., Favaro, L.F., Navarro-
754 Silva, M.A., 2018. Effects of phenanthrene on different levels of biological organization in
755 larvae of the sediment-dwelling invertebrate *Chironomus sancticaroli* (Diptera:
756 Chironomidae). Environ. Pollut. 242, 277–287. https://doi.org/10.1016/j.envpol.2018.06.091.
757 Rusiecki, J.A., De Roos, A., Lee, W.J., Dosemeci, M., Lubin, J.H., Hoppin, J.A., Blair, A.,
758 Alavanja, M.C.R., 2004. Cancer Incidence Among Pesticide Applicators Exposed to Atrazine
759 in the Agricultural Health Study. J. Natl. Cancer Inst. 96, 1375–1382.
760 https://doi.org/10.1093/jnci/djh264.
761 Santos, M.A.P.F., Vicensotti, J., Monteiro, R.T.R., 2007. Sensitivity of NaCl of four test
762 organisms: *Chironomus xantus*, *Daphnia magna*, *Hydra attenuata* and *Pseudokirchneriella*
763 *subcapitata*, as an alternative to reference toxicants. J. Braz. Soc. Ecotoxicol. 2, 229–236,
764 2007. https://doi.org/10.5132/jbse.2007.03.004.
765 Santos, E.A., Cruz, C., Carraschi, S.P., Silva, J.R.M., Botelho, R.G., Velini, E.D., Pitelli,
766 R.A., 2015. Atrazine levels in the Jaboticabal water stream (São Paulo State, Brazil) and its
767 toxicological effects on the pacu fish *Piaractus mesopotamicus*. Arh. Hig. Rada. Toksikol. 66,
768 73–82. https://doi.org/10.1515/aiht-2015-66-257.
769 Sass, J.B., Colangelo, A., 2006. European Union Bans Atrazine, While the United States
770 Negotiates Continued Use. Int. J. Occup. Environ. Health 12, 260-267.
771 https://doi.org/10.1179/oeh.2006.12.3.260.

772 Schuler, L.J., Trimble, A.J., Belden, J.B., Lydy, M.J., 2005. Joint toxicity of triazine
773 herbicides and organophosphate insecticides to the midge *Chironomus tentans*. Arch.
774 Environ. Contam. Toxicol. 49, 173–177. https://doi.org/10.1007/s00244-004-0224-8.
775 Shi, H., Pei, L., Gu, S., Zhu, S., Wang, Y., Zhang, Y., Li, B., 2012. Glutathione S-transferase
776 (GST) genes in the red flour beetle, *Tribolium castaneum*, and comparative analysis with five
777 additional insects. Genomics 100, 327–335. https://doi.org/10.1016/j.ygeno.2012.07.010.
778 Sigg, L., Behra, R., Groh, K., Isaacson, C., Odzak, N., Piccapietra, F., Röhder, L., Schug, H.,
779 Yue, Y., Schirmer, K., 2014. Chemical Aspects of Nanoparticle Ecotoxicology. Chimia 68,
780 806–811. https://doi.org/10.2533/chimia.2014.806.
781 Silva De Assis, H.C., 1998. Der einsatz von biomarkern zur summarischen erfassung vom
782 gewässerverschmutzungen. Ph.D. Thesis, University of Berlin, Berlin, Germany.
783 Silva, M.B., Abrantes, N., Nogueira, V., Gonçalves, F., Pereira, R., 2016. TiO2 nanoparticles
784 for the remediation of eutrophic shallow freshwater systems: Efficiency and impacts on
785 aquatic biota under a microcosm experiment. Aquat. Toxicol. 178, 58–71.
786 https://doi.org/10.1016/j.aquatox.2016.07.004.
787 Silvério, P.F., Fonseca, A.L., Botta-Paschoal, C.M.R., Mozeto, A.A., 2005. Release,
788 bioavailability and toxicity of metals in lacustrine sediments: A case study of reservoirs and
789 lakes in Southeast Brazil. Aquat. Ecosyst. Health Manag. 8, 313–322.
790 https://doi.org/10.1080/14634980500234735.
791 Solomon, K.R., Baker, D.B., Richards, R.P., Dixon, K.R., Klaine, S.J., La Point, T.W.,
792 Kendall, R.J., Weisskopf, C.P., Giddings, J.M., Giesy, J.P., Hall, L.W.Jr., Williams, W.M.,
793 1996. Ecological risk assessment of atrazine in North American surface waters. Environ.
794 Toxicol. Chem. 15, 31–76. https://doi.org/10.1002/etc.2050.

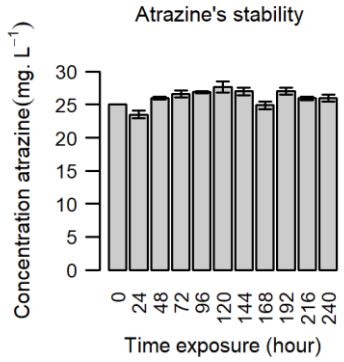
795 Sotero-Santos R.B., Rocha, O., Povinelli, J., 2007. Toxicity of ferric chloride sludge to
796 aquatic organisms. Chemosphere 68, 628–636.
797 https://doi.org/10.1016/j.chemosphere.2007.02.049.
798 Souza, P.M.S., Lobo, F.A., Rosa, A.H., Fraceto, L.F., 2012. Desenvolvimento de
799 nanocápsulas de poli-e-caprolactona contendo o herbicida atrazina. Quim. Nova 35, 132–137.
800 https://doi.org/10.1590/S0100-40422012000100024.
801 Sousa, G.F.M., Gomes, D.G., Campos, E.V.R., Oliveira, J.L., Fraceto, L.F., Stolf-Moreira, R.,
802 Oliveira, H.C., 2018. Post-emergence herbicidal activity of nanoatrazine against susceptible
803 weeds. Front. Environ. Sci. 6, 1-6. https://doi.org/10.3389/fenvs.2018.00012
804 Stara, A., Kouba, A., Velisek, J., 2018. Biochemical and histological effects of subchronic
805 exposure to atrazine in crayfish Cherax destructor. Chem. Biol. Interact. 291, 95–102.
806 https://doi.org/10.1016/j.cbi.2018.06.012.
807 Strixino, S.T., Strixino, G., 1981. Nova espécie do gênero *Chironomus* Meigen do sul do
808 Brasil (Diptera: Chironomidae). Rev. Bras. Entomol. 25, 333–340.
809 Strixino, S.T., Strixino, G., 1982. Ciclo de vida de *Chironomus sancticaroli* Strixino &
810 Strixino, (Diptera, Chironomidae). Rev. Bras. Entomol. 26, 183–189.
811 Taverna, M.E., Busatto, C.A., Lescano, M.R., Nicolau, V.V., Zalazar, C.S., Meira, G.R.,
812 Estenoz, D.A., 2018. Microparticles based on ionic and organosolv lignins for the
813 controlled release of atrazine. J. Hazard. Mater. 359, 139–147.
814 https://doi.org/10.1016/j.jhazmat.2018.07.010.
815 Taylor, E.J., Maund, S.J., Pascoe, D., 1991. Toxicity of four common pollutants to the
816 freshwater macroinvertebrates *Chironomus riparius* Meigen (Insecta: Diptera) and *Gammarus*
817 *pulex* (L.) (Crustacea: Amphipoda). Arch. Environ. Contam. Toxicol. 21, 371–376.
818 https://doi.org/10.1007/BF01060358.

819 Tillitt, D.E., Papoulias, D.M., Whyte, J.J., Richter, C.A., 2010. Atrazine reduces reproduction
820 in fathead minnow (*Pimephales promelas*). Aquat. Toxicol. 99, 149–159.
821 https://doi.org/10.1016/j.aquatox.2010.04.011.
822 USEPA, 2009. United States Environmental Protection Agency. National Primary Drinking
823 Water Regulations.
824 Valle, D., Montella, I.R., Ribeiro, R.A., Medeiros, P.F.V., Martins-Jr, A.J., Lima, J.B.P.,
825 2006. Quantification methodology for enzyme activity related to insecticide resistance in
826 *Aedes aegypti*. Fundação Oswaldo Cruz and Secretaria de Vigilância em Saúde, Ministério da
827 Saúde. Rio de Janeiro and Distrito Federal. 129.
828 Vicentini, M., Morais, G.S., Rebechi-Bagio, D., Richardi, V.S., Santos, G.S., Cestari, M.M.,
829 Navarro-Silva, M.A., 2017. Benzo(a) pyrene exposure causes genotoxic and biochemical
830 changes in the midge larvae of *Chironomus sancticaroli* Strixino & Strixino (Diptera:
831 Chironomidae). Neotrop. Entomol. 46, 658–665. https://doi.org/10.1007/s13744-017-0505-3.
832 WHO, 2017. In: World Health Organization. Guidelines for Drinking Water Quality, p. 1.
833 Available:<http://apps.who.int/iris/bitstream/handle/10665/254637/9789241549950eng.pdf;js
834 essionid=3FCA37069207D6A3E9B5117CCD3A1AE9?sequence=1> (17 jul. 2019).
835 Xiao-Ting, C., Wang, T., 2019. Preparation and characterization of atrazine-loaded
836 biodegradable PLGA nanospheres. J. Integr. Agric. 18, 1035–1041.
837 https://doi.org/10.1016/S2095-3119(19)62613-4.
838 Xie, Z., Tang, J., Wu, X., Li, X., Hua, R., 2019. Bioconcentration, metabolism and the effects
839 of tetracycline on multiple biomarkers in *Chironomus riparius* larvae. Sci. Total Environ.
840 649, 1590–1598. https://doi.org/10.1016/j.scitotenv.2018.08.371.
841 Zagatto, P.A., Bertoletti, E., 2008. Ecotoxicologia Aquática – Princípios e Aplicações. RiMa,
842 São Carlos.

Yue, L., Ge, C., Feng, D., Yu, H., Deng, H., Fu, B., 2017. Adsorption–desorption behavior of atrazine on agricultural soils in China. J. Environ. Sci. 57, 180–189. https://dx.doi.org/10.1016/j.jes.2016.11.002.

881 **Supplementary Material**

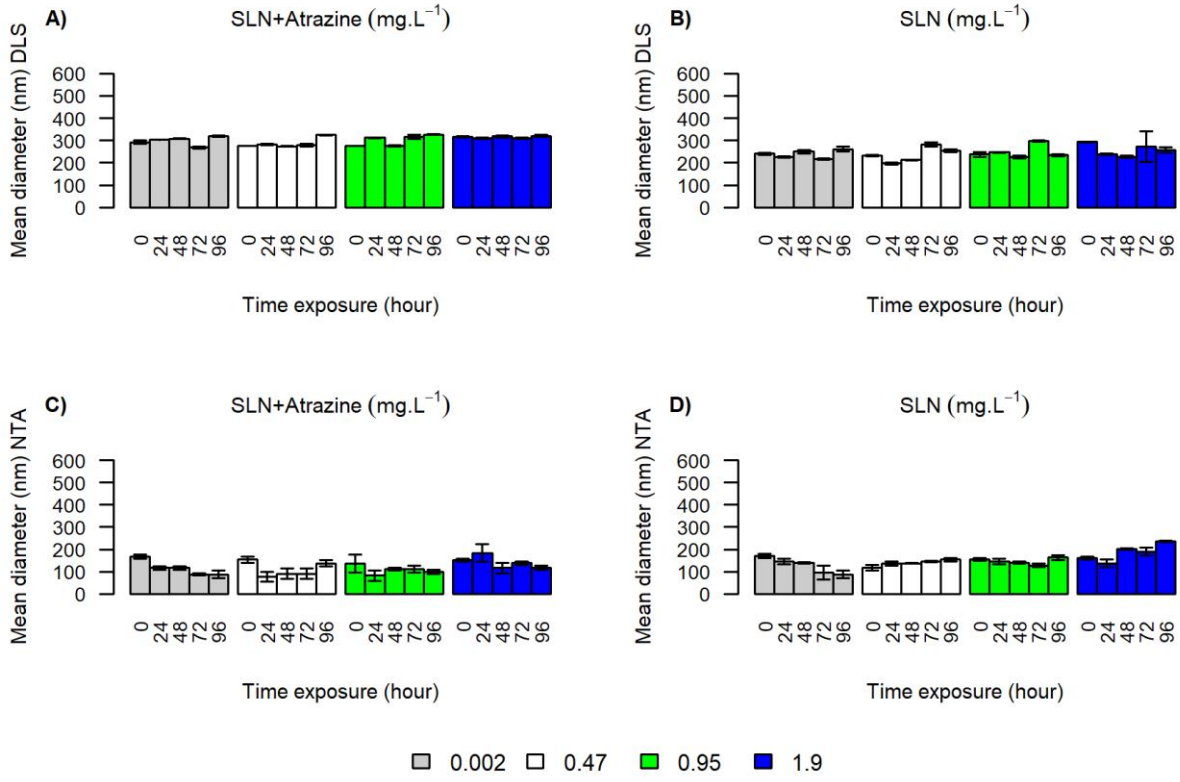
882



883

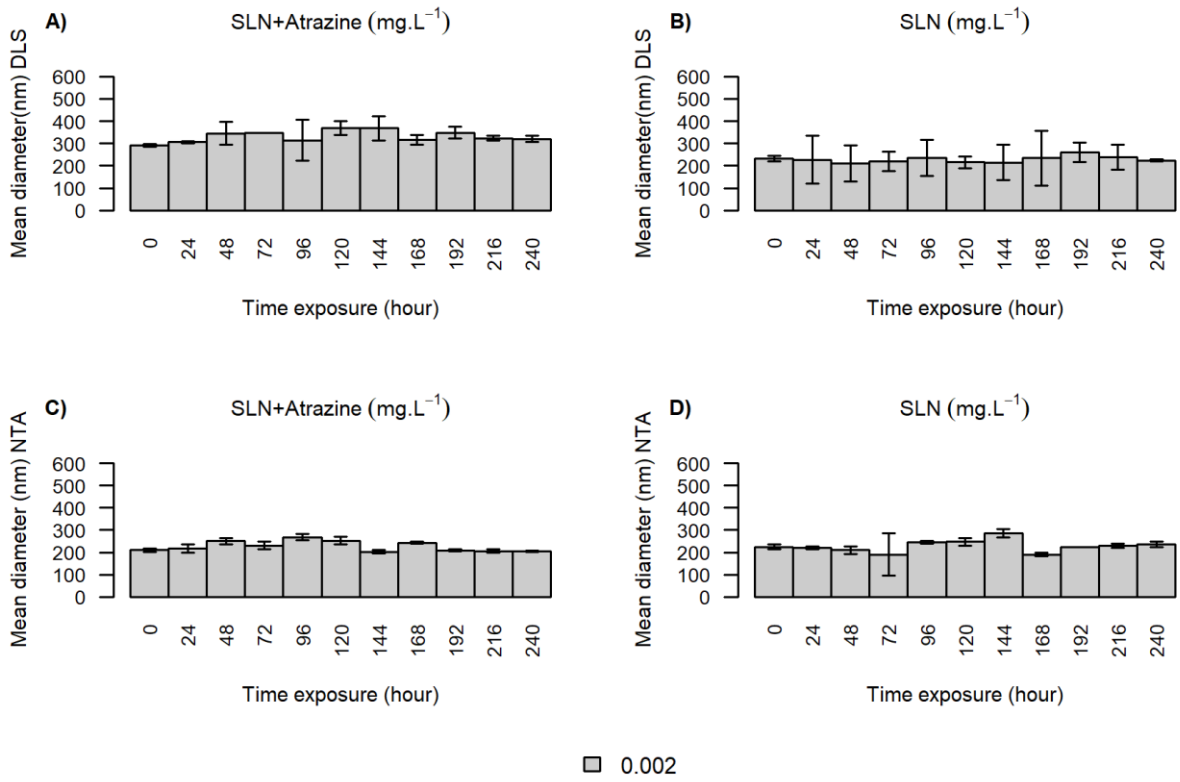
884 **Figure S1** Stability of atrazine in water in the sediment-water exposure system under laboratory conditions,
885 during ten days.

886



887

888 **Figure S2A–D** Daily analysis of the stability of the nanoparticles in the sediment-water exposure system under
889 laboratory conditions, during the acute bioassay (96 h). A–B Mean diameter by dynamic light scattering (DLS).
890 C–D Mean diameter by nanoparticle tracking analysis (NTA). SLN + ATZ = solid lipid nanoparticles loaded
891 with atrazine; SLN = solid lipid nanoparticles.



892

893 **Figure S3A–D** Daily analysis of the stability of the nanoparticles in the sediment-water exposure system under
894 laboratory conditions, during the subchronic bioassay (240 h). A–B Mean diameter by dynamic light scattering
895 (DLS). C–D Mean diameter by nanoparticle tracking analysis (NTA). SLN + ATZ = solid lipid nanoparticles
896 loaded with atrazine; SLN = solid lipid nanoparticles.

897

898

899

900

901

902

903

904

905

Table 1S Physical-chemical variables for the acute and subchronic bioassays using free atrazine, SLN loaded with atrazine, and SLN. pH = hydrogen potential; Cond. = conductivity; D.O. = dissolved oxygen; A. = acute bioassay; S. = subchronic bioassay; ATZ = atrazine; SLN+ATZ = solid lipid nanoparticle loaded with atrazine; SLN = solid lipid nanoparticle; W.C. = water control; S.C. = solvent control. The values are shown as the mean and standard deviation for each treatment.

Treatments	**pH**		**Cond. 20 ms**		**D. O. (mg.L^{-1})**	
A. ATZ	**Start**	**Final**	**Start**	**Final**	**Start**	**Final**
W.C.	7.20 ± 0.08	7.19 ± 0.06	0.15 ± 0.01	0.14 ± 0.01	6.59 ± 0.15	6.50 ± 0.16
S.C.	7.19 ± 0.06	7.25 ± 0.05	0.15 ± 0.01	0.14 ± 0.01	6.51 ± 0.16	6.53 ± 0.11
0.002 mg.L^{-1}	7.23 ± 0.05	7.25 ± 0.08	0.15 ± 0.00	0.15 ± 0.01	6.58 ± 0.15	6.48 ± 0.11
0.47 mg.L^{-1}	7.30 ± 0.08	7.28 ± 0.05	0.15 ± 0.00	0.15 ± 0.00	6.52 ± 0.11	6.47 ± 0.19
0.95 mg.L^{-1}	7.24 ± 0.07	7.21 ± 0.06	0.15 ± 0.00	0.15 ± 0.01	6.57 ± 0.14	6.66 ± 0.10
1.9 mg.L^{-1}	7.28 ± 0.07	7.24 ± 0.05	0.15 ± 0.00	0.14 ± 0.01	6.58 ± 0.10	6.63 ± 0.14
A. SLN+ATZ						
W.C.	7.38 ± 0.07	7.36 ± 0.05	0.16 ± 0.00	0.16 ± 0.00	6.47 ± 0.14	6.39 ± 0.12
0.002 mg.L^{-1}	7.38 ± 0.07	7.34 ± 0.05	0.17 ± 0.01	0.16 ± 0.01	6.58 ± 0.14	6.46 ± 0.09
0.47 mg.L^{-1}	7.35 ± 0.05	7.29 ± 0.12	0.16 ± 0.01	0.16 ± 0.00	6.42 ± 0.10	6.41 ± 0.10
0.95 mg.L^{-1}	7.35 ± 0.08	7.36 ± 0.05	0.16 ± 0.01	0.15 ± 0.01	6.45 ± 0.10	6.36 ± 0.09
1.9 mg.L^{-1}	7.33 ± 0.05	7.31 ± 0.04	0.16 ± 0.01	0.15 ± 0.01	6.51 ± 0.06	6.42 ± 0.12
A. SLN						
W.C.	7.33 ± 0.05	7.33 ± 0.05	0.16 ± 0.00	0.16 ± 0.01	6.49 ± 0.19	6.48 ± 0.11
0.002 mg.L^{-1}	7.34 ± 0.07	7.31 ± 0.04	0.16 ± 0.00	0.16 ± 0.01	6.48 ± 0,16	6.44 ± 0.14
0.47 mg.L^{-1}	7.34 ± 0.05	7.33 ± 0.05	0.16 ± 0.01	0.16 ± 0.01	6.45 ± 0.16	6.60 ± 0.18
0.95 mg.L^{-1}	7.31 ± 0.04	7.33 ± 0.05	0.16 ± 0.00	0.16 ± 0.01	6.39 ± 0.11	6.63 ± 0.14
1.9 mg.L^{-1}	7.33 ± 0.05	7.30 ± 0.00	0.16 ± 0.00	0.16 ± 0.01	6.48 ± 0.16	6.61 ± 0.16
S. ATZ						
W.C.	7.30 ± 0.08	7.30 ± 0.08	0.15 ± 0.01	0.16 ± 0.00	6.64 ± 0.12	6.69 ± 0.16
0.002 mg.L^{-1}	7.25 ± 0.05	7.35 ± 0.05	0.15 ± 0.00	0.16 ± 0.00	6.62 ± 0.15	6.65 ± 0.13
S. SLN+ATZ						
W.C.	7.30 ± 0.00	7.30 ± 0.00	0.16 ± 0.00	0.16 ± 0.01	6.49 ± 0.09	6.44 ± 0.17
0.002 mg.L^{-1}	7.35 ± 0.08	7.30 ± 0.00	0.16 ± 0.01	0.16 ± 0.00	6.45 ± 0.11	6.48 ± 0.09
S. SLN						
W.C.	7.31 ± 0.04	7.30 ± 0.00	0.16 ± 0.01	0.16 ± 0.01	6.48 ± 0.17	6.44 ± 0.15
0.002 mg.L^{-1}	7.29 ± 0.04	7.33 ± 0.05	0.16 ± 0.01	0.16 ± 0.01	6.42 ± 0.08	6.39 ± 0.15

Conclusão Geral

Atrazina é um herbicida pré e pós emergente que está disponível no mercado a mais de 50 anos, com amplo uso em diversos países. Devido sua persistência e mobilidade pode ser detectada em ambientes aquáticos e terrestres que vão além da sua área de aplicação. Em ecossistemas aquáticos este herbicida afeta o desenvolvimento, reprodução e comportamento de uma grande diversidade de espécies.

A nanotecnologia oferece alternativas para otimizar e melhorar o uso de pesticidas na área agrícola, melhorando as formulações existentes e criando novos nanosistemas. Estas nanoformulações possibilitam reduzir a contaminação ambiental e frequência de aplicações, diminuem a dose a ser aplicada e sua liberação modificada (mais lentamente) permite que o organismo alvo fique em contato com o ativo por maior tempo de exposição.

Neste estudo, sobre as condições testadas para os endpoints mortalidade e biomarcadores bioquímicos foi observado que as nanopartículas carregadas com atrazina não diferem da sua forma livre apresentando efeito tóxico e letal sobre as larvas de *Chironomus sancticaroli*. A nanopartícula controle (sem o ingrediente ativo) também causou alterações nos biomarcadores bioquímicos e mortalidade, indicando um possível efeito tóxico da nanoformulação. Entretanto, ambas nanoformulações não causaram deformidade no mento e nenhum atraso no desenvolvimento larval, bem como nanopartículas sem atrazina não causaram alterações dos biomarcadores bioquímicos no bioensaio de longa duração (10 dias), indicando que novos estudos precisam ser realizados com esta espécie avaliando outros marcadores.

Para que a nanoatrazina seja utilizada no campo, são necessários novos estudos para compreender os efeitos desta formulação sobre organismos não alvo, incluindo a análise de diferentes marcadores biológicos (morfológico, molecular, celular, genético e histológico).

Além disso, é de grande relevância que essa nanoformulação seja testada no campo e em bioensaios que simulem diferentes condições ambientais (solo, umidade e temperatura).

O organismo bentônico *Chironomus sancticaroli* demonstrou sensibilidade as nanopartículas, indicando potencial para ser utilizado na avaliação nanotoxicológica na região Neotropical.

Desta forma a presente tese pode contribuir para o uso do organismo *Chironomus sancticaroli* como um potencial marcador para estudos de segurança no uso de nanopesticidas.

ANEXO I

Teste com substância de referência

O teste com substância de referência deve ser realizado periodicamente para verificar a sensibilidade dos organismos teste e as condições da cultura (Araújo et al., 2006; OECD, 2004a, b). Este deve ser realizado após serem introduzidos novos organismos na cultura ou quando há mudanças significativas nas condições de criação (OECD, 2011).

A sensibilidade da cultura foi avaliada a cada seis meses, pois de acordo com a OECD (2011) deve ser realizado teste com substância de referência pelo menos duas vezes por ano.

Como substância de referência foi utilizado o cloreto de potássio (KCl) nas seguintes concentrações: 1,5; 2,25; 3,5; 5,0 e 7,5 $g.L^{-1}$ (Baggio, 2016; Rebechi, 2012; Richardi, 2013; Morais, 2014), este sal foi selecionado porque tem sido muito utilizado em testes de sensibilidade com espécies da família Chironomidae (OECD, 2004a, b, 2010, 2011).

O bioensaio agudo foi conduzido em frascos de vidro de 250 mL contendo: 200 mL de água mineral sem cloro, larvas de IV instar inicial (7 dias) de *C. sancticaroli*, concentrações de cloreto de potássio a serem testadas (Baggio, 2016; Rebechi, 2012; Richardi, 2013; Morais 2014) e 1,5 mg de ração Tetra Min adicionada no início e terceiro dia do teste, adaptado de OECD (2004a, b, 2010). Como controle foi utilizado água (Baggio, 2016; Morais, 2014; Rebechi, 2012; Richardi, 2013). Os bioensaios foram acondicionados em câmara do tipo BOD programada, com temperatura de 25ºC ± 2, fotoperíodo 12 horas luz e 12 horas escuro, umidade em torno de 80 ± 10% e aeração constante.

Os tratamentos foram realizados em triplicata, com cada réplica contendo 6 larvas (n = 18), totalizando 108 organismos por bioensaio (Baggio, 2016; Morais, 2014; Rebechi, 2012; Richardi, 2013). A duração do bioensaio agudo foi de 96 horas (Rebechi, 2012; Richardi, 2013). Foram consideradas larvas vivas as que apresentaram mobilidade normal ou baixa.

A solução padrão foi preparada com 30 g de KCl e 100 mL de água mineral sem cloro, da qual pegou-se uma alíquota referente as concentrações utilizadas no teste.

Para preparar o alimento foi utilizado 60 mg de ração Tetra Min® (macerada) e 20 mL de água mineral sem cloro, desta foi pipetado em cada frasco 0,5 mL = 1,5 mg. O cloreto de potássio e o alimento foram adicionados no experimento na forma líquida, assim descontou-se o valor de ambos no valor total a ser adicionado de água em cada béquer.

Avaliação da população de *Chironomus sancticaroli*

A população de *Chironomus sancticaroli* foi avaliada durante a realização de todos os bioensaios agudo e subcrônico, para isso realizou-se 3 testes de sensibilidade com cloreto de potássio, cada um com intervalo de 180 dias (Tabela 1).

Os resultados do teste de sensibilidade foram analisados através do programa Trimmed Spearman-Karber com a finalidade de obter a CL_{50} do cloreto de potássio para larvas de *Chironomus sancticarloli*. A faixa de sensibilidade foi estabelecida de acordo com a USEPA (2000) através do valor médio da CL_{50}, mais ou menos dois desvios padrão, para este modelo calculou-se a média (X), o desvio padrão (S) e o coeficiente de variação (CV) (Tabela 1). Assim foi estabelecido o limite superior (X + 2.S) e o limite inferior (X – 2.S) (Fig. 1). Valores da CL_{50} que permanecem dentro desta faixa são utilizados e os demais descartados (Fig. 1). O coeficiente de variação foi utilizado para avaliar a precisão analítica e a variabilidade dos testes de sensibilidade (Baggio, 2016; Morais, 2014; Rebechi, 2012; Richardi, 2013), através da seguinte equação 1.

$$\mathrm{CV} = \frac{S}{X} .100 \qquad \text{(Equação 1)}$$

Onde CV é o Coeficiente de variação; S é o Desvio padrão e X é a Média

Tabela 1 Valores da Concentração Letal (CL_{50}) durante o bioensaio agudo (96 horas) com cloreto de potássio e o intervalo de confiância (IC (95%)) de cada teste de sensibilidade com *Chironomus sancticaroli*. Coef. = coeficiente.

Número do teste	Data	CL_{50} (96 h) $g.L^{-1}$	IC (95%) $g.L^{-1}$
1	12/2016	3,71	2,90 – 4,74
2	06/2017	4,33	3,60 – 5,20
3	12/2017	3,18	2,03 – 4,97
Média (X)		3,74	
Variância (S^2)		0,33	
Desvio Padrão (S)		0,58	
Coef. variação em % (CV)		15,51	
Faixa de sensibilidade		4,90 – 2,58	

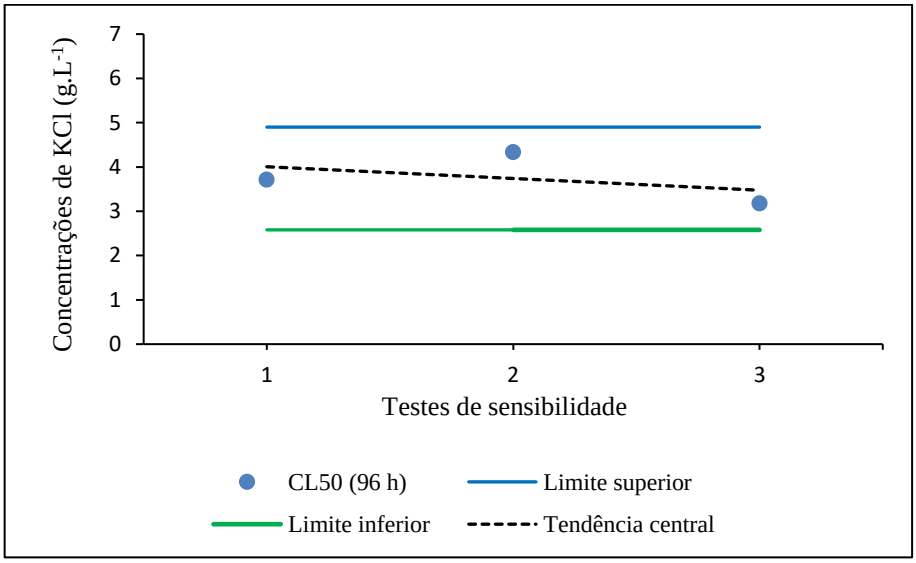


Figura 1 – Concentrações Letais CL_{50} ($g.L^{-1}$) por teste de sensibilidade realizado, com o limite superior e inferior da faixa sensibilidade de *Chironomus sancticaroli* ao cloreto de potássio (KCl).

Todos os testes apresentaram a CL_{50} dentro da faixa de sensibilidade estabelecida, sendo a média 3,74 $g.L^{-1}$, limite superior 4,90 e limite inferior 2,58 $g.L^{-1}$, com um coeficiente de varição de 15,51% (Tabela 1; Fig. 1). Os resultados do presente estudo permaneceram próximos dos obtidos por Baggio (2016), Dornfeld (2006), Morais (2014), Rebechi (2012) e Richardi (2013). Diante do exposto, é possível indicar que a população de *Chironomus*

sancticaroli mantida no Laboratório de Limnologia da Unesp de Sorocaba – SP, foi adequada para ser utilizada como organismo teste em bioensaios ecotoxicológicos.

Referências

Araújo, R.P.A., Shimizu, G.Y., Almeida, C.A., Rocha, O., 2006. Testes com invertebrados para avaliar a toxicidade de contaminantes associados ao sedimento de ecossistemas de água doce. in: Mozeto, A.A., Umbuzeiro, G.A., Jardim, W.F. (Eds.), Métodos de coleta, análises físico-químicas e ensaios biológicos e ecotoxicológicos de sedimentos de água doce. São Carlos, pp. 111–133.

Baggio, D.R. Respostas metabólicas e moleculares de *Chironomus sancticaroli* Strixino & Strixino, 1981 (Diptera: Chironomidae) exposta ao malathion, fenantreno e cádmio. Tese (Doutorado em Ciências Biológicas área de concentração Entomologia, Universidade Federal do Paraná), Curitiba, Paraná, 2016.

Dornfeld, C.B. Utilização de *Chironomus sp* (Diptera, Chiromidae) para a Avaliação da Qualidade de Sedimentos e Contaminação por Metais. Tese (Doutorado em Ciências da Engenharia Ambiental, Universidade Federal de São Carlos), São Carlos, São Paulo, 2006.

Morais, G.S. Efeitos genotóxicos e biológicos em *Chironomus sancticaroli* (Strixino & Strixino, 1981) causados pela exposição ao fenantreno. Dissertação (Mestrado em Ciências Biológicas área de concentração Entomologia, Universidade Federal do Paraná), Curitiba, Paraná, 2014.

OECD/OCDE - ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT, 2004a. Sediment-water chironomid toxicity test using spiked water, Paris, Test Guideline n. 219, Guidelines for the testing of chemicals. 21. https://doi.org/10.1787/9789264070288-en.

OECD/OCDE - ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT, 2004b. Sediment-water chironomid toxicity test using spiked sediment, Paris, Test Guideline n. 218, Guidelines for the Testing of Chemicals. 17p. https://doi.org/10.1787/9789264070264-en.

OECD - ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT, 2010. Sediment-Water Chironomid Life-Cycle Toxicity Test Using Spiked Water or Spiked Sediment, Paris, Test Guideline n. 233, Guidelines for the Testing of Chemicals. 29. https://doi.org/10.1787/9789264090910-en.

OECD - ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT, 2011. *Chironomus* sp., Acute Immobilisation Test, Paris, Test Guideline n. 235, Guidelines for the Testing of Chemicals. https://doi.org/10.1787/9789264122383-en.

Rebechi, D. Efeitos ecotoxicológicos em *Chironomus sancticaroli* Strixino & Strixino, 1981 (DIPTERA: CHIRONOMIDAE) expostos ao Malathion. Dissertação (Mestrado em Ciências Biológicas área de concentração Entomologia, Universidade Federal do Paraná), Curitiba, Paraná, 2012.

Richardi, V.S. Descrição histológica de imaturos de *Chironomus sancticaroli* Strixino & Strixino, 1981 (DIPTERA: CHIRONOMIDAE) e efeitos histopatológicos e biológicos após a exposição ao fenantreno. Dissertação (Mestrado em Ciências Biológicas área de concentração Entomologia, Universidade Federal do Paraná), Curitiba, Paraná, 2013.

USEPA, 2000. United States Environmental Protection Agency. Methods for measuring the toxicity and bioaccumulation of sedimentassociated contaminants with freshwater invertebrates.