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UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Instituto de Biociências
Câmpus do Litoral Paulista

ESTUDO ECOTOXICOLÓGICO DO BIOCIDA ANTIINCRUSTANTE DCOIT (SEA-NINE™) EM INVERTEBRADOS MARINHOS NEOTROPICAIS

Bruno Galvão de Campos

SÃO VICENTE – SP

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Bruno Galvão de Campos

Denis Moledo de Souza Abessa

Tese apresentada ao Instituto de Biociências, Câmpus do Litoral Paulista, UNESP, para obtenção do título de Doutor no Programa de Pós-Graduação em Biodiversidade de Ambientes Costeiros.

SÃO VICENTE – SP

2022

C198e

Campos, Bruno Galvão de
Estudo ecotoxicológico do biocida antiincrustante DCOIT (SEA-NINE™) em invertebrados marinhos neotropicais / Bruno Galvão de Campos. -- São Vicente, 2022
192 f. : il., tabs., mapas

Tese (doutorado) - Universidade Estadual Paulista (Unesp), Instituto de Biociências, São Vicente
Orientador: Denis Moledo de Souza Abessa

1. Ecotoxicologia. 2. Poluição Marinha. 3. Contaminantes. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca do Instituto de Biociências, São Vicente. Dados fornecidos pelo autor(a).

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Acknowledgments

First and foremost, I am extremely grateful to my supervisor, Prof. Dr. Denis Abessa for his invaluable advice, continuous support, and patience during my Ph.D. and academic life. His immense knowledge and plentiful experience have encouraged me in all the time of my academic research and daily life. I would also like to thank Dr. Roberto Martins for his invaluable supervision, support, and tutelage during my internship in Portugal. Roberto has become a true friend to me. During the first strike of the pandemic, he always looked after me and welcomed me in his family and in his home with open arms. I also would like to thank the *Coordenação e Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) and the São Paulo Research Foundation (FAPESP - *Fundação de Amparo à Pesquisa do Estado de São Paulo*) for the financial support under the grants FAPESP: 2019/19898-3 and FAPESP: 2017/10211-0. Additionally, I would like to express gratitude to Dr. Fernando Perina for his treasured friendship and support which was influential in shaping my professional life as an ecotoxicologist, and for introducing me to the coffee world. I also would like to express my sincere gratitude to my much-loved friend Dra. Paloma Choueri whose guidance, support, and encouragement have been invaluable throughout this study and my career, and also for the warm welcome on her lovely home in moments of need. I also would like to thank my inspiring colleagues Dr. Lucas Buruaem and Dra Ana Carolina Cruz for their support in providing invaluable feedback on my study, at times responding to emails late at night and early in the morning. I would like to thank all the members of the NEPEA for their collaborative support during all these years and for a cherished time spent together in the lab, and in social settings. It is their kind help and support that have made my study and life in the UNESP a wonderful time. For me, NEPEA is kind of a second home, a place where I've grown and learned so much. Lastly, my family deserves endless gratitude: To my parents, Regina and Werner, for their unconditional, unequivocal, and loving support. To my brother for always being there for me. And to my brilliant, supportive, and inspiring wife Dra. Mayana Fontes. Mayana, you were always there, wherever, and whenever that was. Each midnight draft, each early morning rant, each time I cried and smiled you were there supporting and inspiring me to keep up. Without my family's tremendous understanding and encouragement, it would be impossible for me to complete this journey. To my family, I give everything, including this.

Abstract

After the ban on the tributyltin-based antifouling paints, DCOIT (4,5-Dichloro-2-octylisothiazol-3(2H)-one) has become one of the most used antifouling biocide. Besides being considered a pseudo persistent contaminant in areas with Hight traffic of vessels and toxic to non-target species, the bioaccumulation and trophic transfer of DCOIT in marine organisms remains unknown. The present study is divided into three chapters which: I) presented, as a critical review, a comprehensive compilation of toxicological and environmental data of the more commons biocides, and further used such information in an ecological risk assessment (ERA) of the 11 EU approved antifouling biocides (PT21), which indicated that DCOIT, diuron, dichlofluanid, chlorothalonil, CuSCN, Cu2O, medetomidine, and zineb pose risk for the coastal ecosystems. II) evaluated the degradation of DCOIT, Irgarol, Diuron, and Dichlofluanid during a sediment spiking equilibrium phase of 24 hours in three different time points and concentrations through kinetic degradation models, resulting in the following half-lives: DCOIT and Diuron: < 5 h; dichlofluanid < 2 h; and Irgarol < 6h. The results also indicated that apart from dichlofluanid, the antifouling biocides have shown that in 6 hours of equilibrium the rate of degradation is reduced dramatically. III) Investigated the sublethal effects (biochemical, cellular, and histopathological) of environmentally relevant concentrations of DCOIT on the neotropical oyster *Crassostrea brasiliana* exposed to increasing concentrations of DCOIT. This study showed that DCOIT causes negative effects on *C. brasiliana* at all analyzed levels of biological organization. IV) Evaluated the water and whole sediment toxicity of DCOIT in the following species: *Perna perna* (bivalve), *Echinometra lucunter* (sea-urchin) *Artemia sp* (crustacean), *Nitrocra sp* (copepod) and *Tiburonella viscanna* (amphipod). The toxicity data were used to calculate endpoints of environmental hazard and risk which were compared to values obtained for temperate species, revealing that tropical pelagic organisms were in avarage 1.7-fold more sensitive to DCOIT compared to non-tropical species. For sediment, based on the environmental concentrations and toxic thresholds, DCOIT possibly presents environmental risk in Korea, Japan, Spain, Malaysia, Indonesia, Vietnam, and Brazil. V) Investigated the bioaccumulation, biomagnification, and trophic transfer of DCOIT and SiNC-DCOIT (a nanoengineered and environmentally friendly alternative of DCOIT) from the marine microalgae *Tetraselmis chuii* to the mussel *Mytilus galloprovincialis* during uptake of 24h and depuration of 72h, which showed that the mussels rapidly internalized and metabolized both DCOIT and SiNC-DCOIT, being considered non-bioaccumulative. Yet, food exposure treatment indicated that DCOIT and SiNC-DCOIT can transfer up a food chain with biomagnification capabilities. VI) assessed short and long-term sub-lethal effects of nanostructured and soluble forms of AF biocides (DCOIT; Ag; SiNC-DCOIT; SiNC-DCOIT-Ag) and the “empty” nanocapsule (SiNC) on juveniles of *Crassostrea gigas* after 96 h and 14 days of exposure, indicating that the SiNC-DCOIT presented a lower toxicity profile compared to the free biocide. Overall our results generated important ecotoxicological data for regulatory context that will enable more accurate predictions of risk to the marine environment. The results also indicated that coastal areas close to ports and marinas are hotspots of antifouling contamination being considered the most threatened locations, thus requiring rigorous control of the release rates and strict regulation on these areas.

Keywords: DCOIT, antifouliing, biocides, biaccumulation, toxiciy, ERA, hazzard

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Introduction

Marine organisms quickly colonize immersed substrates in a process known as bio-incrustation (Dobretsov et al., 2006). This process causes economic problems in ships and boats, such as increasing friction, fuel consumption, and overall maintenance (Demirel et al., 2017), thus costing approximately 3 billion dollars per year to this industrial sector (Jacobson & Willingham, 2000). In addition, bio-incrustation has biological implications as heavier boats increase their greenhouse gas emissions and can contribute to bio-invasion (Fernandes et al., 2016).

To prevent bio-incrustation, submerged structures have been coated with antifouling paints containing biocides in their composition, since ancient civilizations (Yebra et al., 2004). Romans and Greeks have used lead sheets to protect their boats, while in the 1800s the advent of iron ships triggered the development of marine antifouling paints. At the beginning they were incorporated with toxic elements such as arsenic and mercury. In the mid-1900s, copper-based paints were predominant until the late 1900s with the development of organotin-based paints (Arai et al., 2009).

Among the organotin biocides, Tributyltin (TBT) was widely used due to its high efficiency against a wide range of colonizing organisms, absence of galvanic corrosion (being suitable for application on aluminum surfaces), and longevity when applied into self-polishing coatings (Bellas, 2007). However, in 2008 the International Maritime Organization (IMO) banned the organotin based paints due to the ecological risk associated with their usage in the marine environment (Martins, Fillmanna, et al., 2018) as this compound was recognized as highly toxic and an endocrine disruptor.

Regulations now demand that antifouling paints must not result in adverse effects on the environment, and a new generation of tin-free antifouling biocides came up to replace the organotin biocides. Most of these biocides were metal based compounds (with Cu2O or CuCHNS) with one or more organic additives (booster biocides) (Dafforn et al., 2011). Among these new antifouling biocides, the following stand out: Diuron, Irgarol, Chlorotalonil, Diclofluanide, Tiram, Triphenylbornane Pyridine, DCOIT, among others (Silva et al., 2019).

The behavior and toxicity of these biocides are not yet fully understood. For instance, Irgarol, one of the new-generation biocides, was banned in 2017 from Europe due to negative effects and persistence in the environment (Fiamma Eugênia Lemos Abreu et al., 2020).

Distinct from other biocides, which were previously used in agriculture as fungicides or herbicides, DCOIT, or 4,5-Dichloro-2-octylisothiazol-3(2H)-one (commercially known as Sea-

Nine 211™) was specifically developed as an antifouling compound. Although DCOIT was once considered to have a low environmental impact (Bellas, 2007), recent studies suggest ecological risks (Figueiredo et al., 2019, 2020a), based on high environmental concentrations (Fiamma Eugênia Lemos Abreu et al., 2021), half-life longer than 8 days in natural coastal environments (Harino & Langston, 2009), and toxicity to non-target organisms (Chen & Lam, 2017a).

Studies on the chronic toxicity of DCOIT, as well as its behavior, mechanisms of action, and persistence in the marine environment are scarce and concentrated in Europe, United States and Japan. Therefore, it becomes necessary not only to quantify this compound in tropical areas, but also to evaluate the water and sediment toxicity to neotropical non-target organisms. The present study aims to:

I. Estimate effects of DCOIT, at sub-individual level, on the tropical oyster *Crassostrea brasiliana*;
II. Evaluate the acute and chronic toxicity of DCOIT in seawater and sediment on neotropical marine invertebrates (bivalve, echinoderm, and crustaceans);
III. Evaluate DCOIT bioaccumulation and trophic transfer on the mussel *Mytilus galloprovincialis*.
IV. Assess the toxicity of SiNC-DCOIT (DCOIT encapsulated in silica nanocapsules) as an environmentally safer alternative for the free DCOIT.

This thesis is structured in the form of scientific articles, in order that each chapter represents at least one manuscript. In general, chapter one provides an overview of the problem of anti-fouling biocides in the marine ecosystem. Chapter 2 focus on the toxicity and bioaccumulation of DCOIT to neotropical organisms. In the third and last chapter, a possible solution for the problem is presented, through the assessment of the toxicity an encapsulated form of DCOIT. The content of each chapter is detailed below.

Chapter one presented the problematic of antifouling biocides in the form of a critical review titled "Occurrence, effects and environmental risk of antifouling biocides (EU PT21): Are marine ecosystems threatened?" Published at *the Critical Reviews in Environmental Science and Technology*. This critical review covers a large gap in the literature on the environmental occurrence and toxicity of the 11 antifouling biocides allowed by the European Union and their environmental risk to global marine ecosystems. In addition to a comprehensive and exhaustive survey on the occurrence and effect of these compounds, this MS has included the hazard and risk assessment of each biocide, carried out for the first time for some antifouling biocides (e.g. tolylfluanid, zineb and Cu2O). Biogeographical and ecological aspects were also critically discussed in a holistic perspective. Finally, it addressed future perspectives and recommendations for regulators.

Chapter two focuses on the chronic and acute toxicity of the DCOIT to neotropical organisms, as well as its behavior on coastal sediments. In this chapter, four manuscripts were presented. The first article is titled "Degradation kinetics of antifouling biocides in sediment during the spiking equilibrium phase" and will be submitted as a short communication to the *Soil and Sediment Contamination*. This article covers a gap in the literature on the degradation of antifouling biocides in coastal sediments, by evaluating the degradation of DCOIT, Irgarol, Diuron, and dichlofluanid during the spiking equilibrium phase of 24 hours in three different time points and concentrations. The findings presented in this article were used to guide the spiking technique applied in the article 3 of this chapter and can also be used as proxy to guide other studies regarding the degradation of DCOIT in sediments and its sediment toxicity.

The second article is titled "A preliminary study on multi-level biomarkers response of the tropical oyster *Crassostrea brasiliana* to exposure to the antifouling biocide DCOIT" and it was published to the "Marine Pollution Bulletin". This manuscript covers a gap in the literature on the sublethal effects of DCOIT on neotropical marine invertebrates. We assessed the effects of the DCOIT on the tropical oyster *Crassostrea brasiliana* after exposing the organisms to environmental relevant concentrations of DCOIT. The effects were evaluated at the biochemical, cellular, and morphological levels. The results were integrated trough statistical and multivariate methods and critically discussed in a holistic perspective. Finally, it addressed the ecological relevance and implication of our findings, suggesting that even in low concentrations DCOIT may cause ecological risk to coastal areas, especially those sites located close to ports and marinas.

The 3rd article presented in the chapter two is titled "Water and sediment toxicity and risk assessment of DCOIT towards neotropical organisms" and will be submitted to the *Environmental Pollution*. This manuscript assessed the acute and chronic toxicity of DCOIT in water for the following species *Perna perna* (bivalve) *Artemia sp* (crustacean), *Echinometra lucunter* (echinoderm), and in sediment for *Nitocra sp* (copepod), *Tiburonella viscana* (anfipod) and *kalliapseudes schubartii* (tanaidae). Furthermore, the obtained data was used to generate endpoints of environmental hazard and risk assessment for water and sediment (for the first time using whole sediment toxicity data) which were compared to the temperate values available in the literature.

The 4th article presented in this chapter was titled:" Bioaccumulation and trophic transfer in mussels after short term exposure to DCOIT and SiNC-DCOIT and will be submitted as a short communication to the *Science of the total environment*. This MS aimed to investigate the bioaccumulation, biomagnification, and trophic transfer of DCOIT and SiNC-DCOIT (a nanoengineered and environmentally friendly alternative of DCOIT) from the marine microalgae *Tetraselmis chuii* to the mussel *Mytilus galloprovincialis*.

In the third chapter, an environmentally safer alternative for DCOIT is presented in the article titled "Chronic and short-term effects of antifouling nanomaterials on early life stages of the oyster *Crassostrea gigas*" and published at the "*Environmental Science and Pollution Research"*. In this manuscript, for the first time, was assessed the short-term and long-term effects on juveniles of *C. gigas* caused by 96 h and 14 d of exposure to novel antifouling nanoadditives (SiNC-DCOIT; SiNC-DCOIT-Ag) and comparing the effects with the counterparts, namely the hollow capsule (SiNC) and both non-encapsulated biocides (DCOIT and Ag).

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Overall Conclusion and final thoughts

The present study found that all the 11 approved antifouling biocides in Europe threaten the marine environment at different magnitudes and indicates that a significant change of paradigm in the coatings industry is needed towards the replacement of coatings containing biocides by other types of antifouling systems. The first chapter also emphasized that for some antifouling biocides such as Chlorothalonil, CuSCN, Medetomidine, Zineb, Cu2O, CuPT, and Tolylfluanid, the toxicity and/or environmental occurrence data is scarce. For other biocides such as DCOIT, more data is needed on different species, from different functional groups. Such data will enable more accurate predictions of risks to the marine and estuarine environments, in order to assure the effective protection of our oceans, seas, and estuaries, thus fulfilling one of the Sustainable Development Goals from the United Nations (SDG 14).

As mentioned in chapter one, considering that the toxicity and environmental fate of DCOIT and other biocides in sediment matrices and tropical species are still unknown, in chapter two we found that DCOIT, Diuron, Irgarol and Dichlofluanid degradation kinetics were biphasic, and an equilibrium phase of at least 6h during sediment spiking procedures is satisfactory. Regarding the toxicity of DCOIT towards neotropical species, chapter 2 also demonstrated that the oyster *Crassostrea brasiliana* is adversely affected when exposed to environmentally relevant concentrations of DCOIT at biochemical, cellular, and histological levels. In gills, there was histopathological damage, but the biochemical biomarkers indicated a lack of effects on the activity of the antioxidant system. In the digestive glands, there was an induction of the antioxidant defense system. DCOIT also caused negative effects on the water on the tropical sea urchin *E. lucunter* at 33 µg/L (EC50), mussel *P. perna* at 8.3 µg/L (EC50), and crustacea *Artemia* sp. at 163 µg/L (LC50). For whole sediment toxicity, DCOIT caused negative effects on the amphipod *T. viscana* at 565 µg/kg (LC50), the copepod *Nitocra sp* at 215 µg/kg (EC50). The water hazard assessment based on toxicity data to tropical species indicates that tropical marine organisms are 1.7 more sensitive to DCOIT compared to non-tropical species. However, hazard assessments based on a large group of species from all climate regions presented similar values compared to the values from tropical organisms. The present study provides the first risk assessment based on whole sediment toxicity data for benthic species, which revealed that as for planktonic and pelagic species DCOIT also pose risk for sediment dweller organisms. Regarding bioaccumulation, neither DCOIT nor SiNC-DCOIT were considered bioaccumulative, however, both biocides presented the capability of being transferred through the trophic chain, with potential to biomagnify under certain circumstances.

Based on the presented findings DCOIT can be considered hazardous and pose risk to the ecosystems located in areas with high traffic or presence of vessels. Yet, DCOIT encapsulation appears to be a promising solution to develop a new version with lower environmental hazard compared to the current commercial DCOIT biocide. The mitigation of DCOIT and other antifouling biocides impacts in the coastal and marine ecosystems can be achieved by rigorous control of the release rates and strict regulation on hotspot areas (e.g. harbors, marinas, dry dock facilities). These actions require regular monitoring of biocides' environmental occurrence and risk, in all worldwide oceans and coastal areas, making data publicly available to better manage and protect local ecosystems. Some countries are aware of these demands, in Europe, REACH (Registration, Evaluation, Authorisation, and Restriction of Chemicals) set up a wide framework for regulating and restricting chemicals to minimize the environmental impacts of such compounds over their whole life cycle. In the last few years, due to the inherent risk, the United Kingdom, Denmark, and Sweden already adopted restrictions on the use of both Irgarol 1051 and Diuronon in vessels larger than 25 m in length (Konstantinou and Albanis, 2004, Thomas et al., 2002).

On the past, when the United Nations Environment Program (UNEP) considered the Persistent Organic Pollutants (POPs) a possible threat to the environment, an international task force emerged to assess the POPs culminating on the Stockholm Convention. As occurred for the POPs, the emerging contaminants, including antifouling biocides, also need this concern and such depth monitoring and studies. The presented findings can be useful in a regulatory context, and to better characterize the risk associated with the use of antifouling paints based on DCOIT