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

2022

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

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).

Essa ficha não pode ser modificada.

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

Summary

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).

References:

Abreu, F. E. L., Martins, S. E., & Fillmann, G. (2021). Ecological risk assessment of booster biocides in sediments of the Brazilian coastal areas. Chemosphere, 276. https://doi.org/10.1016/j.chemosphere.2021.130155

Abreu, F. E. L., Natália, J., Braga, Í., & Fillmann, G. (2020). Are antifouling residues a matter of concern in the largest South American port ? 398(January). https://doi.org/10.1016/j.jhazmat.2020.122937

Bellas, J. (2007). Toxicity of the booster biocide Sea-Nine to the early developmental stages of the sea urchin Paracentrotus lividus. Aquatic Toxicology, 83(1), 52–61. https://doi.org/10.1016/j.aquatox.2007.03.011

Chen, L., & Lam, J. C. W. (2017). SeaNine 211 as antifouling biocide: A coastal pollutant of emerging concern. Journal of Environmental Sciences, 61, 68–79. https://doi.org/10.1016/j.jes.2017.03.040

Dafforn, K. A., Lewis, J. A., & Johnston, E. L. (2011). Antifouling strategies: History and regulation, ecological impacts and mitigation. Marine Pollution Bulletin, 62(3), 453–465. https://doi.org/10.1016/j.marpolbul.2011.01.012

Figueiredo, J., Loureiro, S., & Martins, R. (2020). Hazard of novel anti-fouling nanomaterials and biocides DCOIT and silver to marine organisms. Environmental Science: Nano, 7(6), 1670–1680. https://doi.org/10.1039/d0en00023j

Figueiredo, J., Oliveira, T., Ferreira, V., Sushkova, A., Silva, S., Carneiro, D., Cardoso, D. N., Gonçalves, S. F., Maia, F., Rocha, C., Tedim, J., Loureiro, S., & Martins, R. (2019). Toxicity of innovative anti-fouling nano-based solutions in marine species. Environmental Science Nano, 5. https://doi.org/10.1039/C9EN00011A

Harino, H., & Langston, W. J. (2009). Degradation of alternative biocides in the aquatic environment. In Ecotoxicology of Antifouling Biocides (pp. 397–412). Springer Japan. https://doi.org/10.1007/978-4-431-85709-9_23

Silva, E. R., Ferreira, O., Ramalho, P. A., Azevedo, N. F., Bayón, R., Igartua, A., Bordado, J. C., & Calhorda, M. J. (2019). Eco-friendly non-biocide-release coatings for marine biofouling prevention. Science of the Total Environment, 650, 2499–2511. https://doi.org/10.1016/j.scitotenv.2018.10.010

Chapter 1

Occurrence, effects and environmental risk of antifouling biocides (EU PT21): Are marine ecosystems threatened?

Buno Galvão de Campos[a,b]*, Joana Figueiredo[b], Fernando Perina[a], Denis Moledo de Souza Abessa[a]; Susana Loureiro[c], Roberto Martins[c]

[a]*São Paulo State University (UNESP), Praça Infante Dom Henrique, s/n, Zipcode: 11330-900 São Vicente, São Paulo, Brazil*

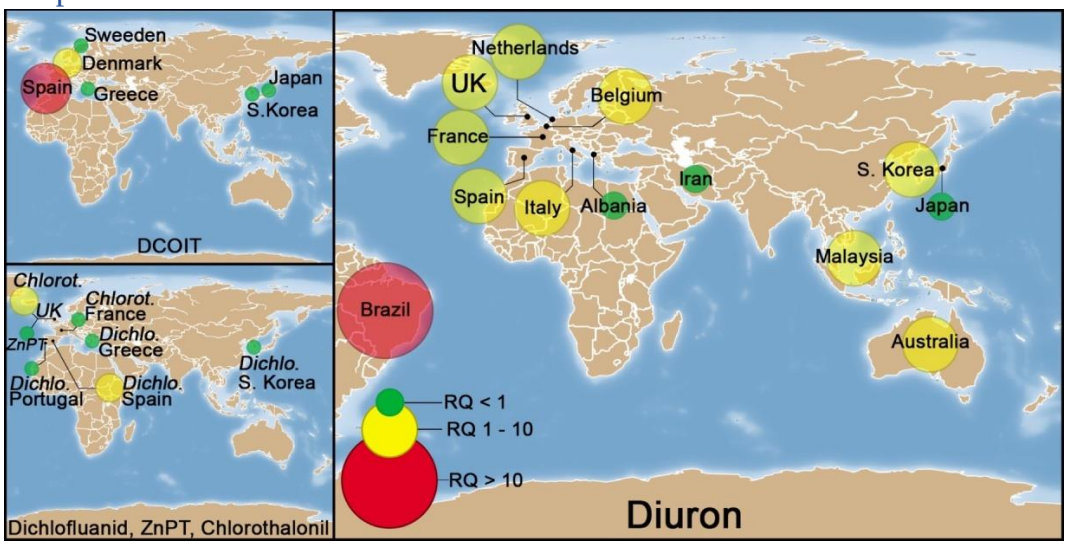
[b]*Department of Biology, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal*

[c]*CESAM - Centre for Environmental and Marine Studies and Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal*

Corresponding Author: *Bruno Galvão de Campos, São Paulo State University (UNESP), Praça Infante Dom Henrique, s/n, Zipcode: 11330-900 São Vicente, São Paulo, Brazil. E-mail: b.campos@unesp.br*

Status*:* Accepted: *DOI: https://doi.org/10.1080/10643389.2021.1910003*

Graphical Abstract



Abstract

This review presents a comprehensive compilation of toxicological and environmental data, further used to assess the ecological risk (ERA) of the 11 EU approved antifouling biocides (PT21). Diuron, chlorothalonil, DCOIT, and pyrithiones are amongst the most toxic biocides towards producer species, while, CuSCN, medetomidine, and zineb showed higher toxicity towards consumers. In addition, medetomidine, CuSNC, zineb, Cu_2O, and chlorothalonil are

up to 400-fold more toxic to non-target organisms than target organisms highlighting their potential threat for marine ecosystems. Biocides hazard, which was assessed based on the marine $PNEC_{probabilistic}$ for biocides having enough and reliable ecotoxicological data, can be summarized in the following order: ZnPT (more hazardous) > CuPT > DCOIT > diuron > chlorothalonil > dichlofluanid > tolylfluanid. For the remaining biocides, the marine hazard was calculated based on the $PNEC_{deterministic}$ as follows: CuSCN (more hazardous) > Cu_2O > medetomidine> zineb. The risk assessment indicates that the following biocides poses risk (quotient >1) for the coastal ecosystems: DCOIT, diuron, dichlofluanid, chlorothalonil, CuSCN, Cu_2O, medetomidine and zineb. In order to protect the services and functions of coastal environments, a systematic and continuous monitoring of these biocides in coastal areas are highly recommended. Current regulatory framework and the replacement of state-of-the-art biocides by safer alternative is also critically discussed.

Keywords: hazard assessment; risk assessment; species sensitivity distribution (SSD); predicted no effect concentration (PNEC); biofouling; regulation.

1. Introduction

Marine biological fouling, also known as biofouling, corresponds to a natural ecological succession characterized by species colonization onto surfaces immersed in seawater (Yebra et al., 2004). There are a wide range of organisms involved in this process from different trophic levels, including bacteria, diatoms, macroalgae, tunicates, barnacles, mussels or tubeworms (Chen & Lam, 2017). The classical model describes the fouling process as a linear succession. First a film composed of organic and inorganic molecules available in seawater adheres to the substrate (Maki & Mitchell, 2003). This thin biochemical layer enables bacterial adhesion and promotes the formation of a biofilm. The following step corresponds to the colonization by diatoms and protozoans, which attachment occurs through polysaccharides or proteins glues to the biofilm (Wahl, 1989). Finally, invertebrates and macroalgae start settling down. However, according to Clare et al. (1992), the actual fouling process is much more complex and better represented by a dynamic nonlinear model that involves physical, chemical, molecular, and behavioral interactions between and within the various fouling categories (e.g. molecular fouling, microfouling, particulate fouling, and macrofouling), thus, according to this model the settlement of fouling organisms are not necessarily in a chronological and linear sequence of succession as often simplified in the literature (Clare et al., 1992; Dobretsov & Rittschof, 2020).

Biofouling of man-made structures causes extensive socioeconomic and environmental impacts (Dafforn et al., 2011). In the shipping industry, this phenomenon causes a considerable

increase in fuel consumption due to the overall reduction of hydrodynamics, mainly through roughness and friction. As consequence, the increase in fuel consumption leads to an increase in greenhouse gas emissions (Figueiredo et al., 2019). Furthermore, biofouling may result in the undesired transport of invasive species through their presence in the hulls of ships (Fernandes et al., 2016). In fixed structures, it causes an important increase in the structure's weight, which may lead to structural instability and failure (e.g. bridges collapse, buoys, or aquaculture cages sink) (Maki & Mitchell, 2003).

Biofouling in human-made structures is commonly tackled through antifouling paints that continually release biocides, forming a protective chemical layer against foulers (Voulvoulis, 2006). Organotin compounds (e.g. TBT) were the most worldwide antifouling biocides used during the 20th century due to their effectiveness against a wide range of fouling species (Omae, 2003). In 2008 they were banned by the International Marine Organization due to its bioaccumulation, toxicity, endocrine disrupting potency, as well as its environmental persistence, with a half-life in sediments of around 1.2 to 4.4 years that can be extended (more than 20 years) if deposited in flakes or in oligotrophic environments (Dafforn et al., 2011; Gittens et al., 2013; International Marine Organization, 2001; Lam et al., 2017). As alternatives, a new generation of antifouling biocides, most of them previously used as fungicides or herbicides (e.g. pyrithiones, diuron, chlorothalonil, Irgarol 1051) and some specially developed as antifouling agents (e.g. DCOIT), came up to replace the previously banned organotin based compounds (Chen & Lam, 2017). According to Lam et al. (2017), after the organotin ban, diuron, Irgarol 1051, and DCOIT become the most frequently used antifouling biocides. However, the lack of a systematic evaluation of the environmental risk of these biocides together with their worldwide use, brought a new threat to the marine environment, as recently recognized for Irgarol 1051 (Lam et al., 2017). Banned in 2017 from the EU PT 21 list, which include all antifouling products approved in the European market, Irgarol 1051 exhibits a relative long half-life in seawater of up to 350 days (Omae, 2003) and high toxicity. Irgarol 1051 is a photosynthesis-inhibitor herbicide, consequently, more toxic to primary producers comparing with TBT (Bao et al., 2011). It has been detected in seawater and sediment in concentrations up to 4.8 µg/L (in Brazil) and 0.23 µg/g (in Korea), respectively (Diniz et al., 2014; S. Lee & Lee, 2017), above the threshold of 0.13 µg/L where ecological risk is expected according to Lam et al. (2017).

The new generation of biocides was intended to be environmentally safer than the former. However several studies reported extensive effects on non-target marine species

(Martins et al., 2018; Viana et al., 2020; Yamada, 2007; Figueiredo et al., 2019, 2020). In addition, the toxicity of the new generation of biocides, mechanism(s) of action, persistence, fate and behavior in the marine environment are not yet fully understood, particularly for some compounds such as copper pyrithione (CuPT), copper thiocyanate (CuSCN), tolyfluanid, dicopper oxide (Cu_2O), medetomidine and zineb. Considering the relevant lack of knowledge, the environmental risk assessment of these molecules is even more challenging and crucial.

This critical review focus on the 11 EU approved compounds (PT21) as follows: *4,5-Dichloro-2-octylisothiazol-3(2H)-one* (DCOIT), *3-(3,4-dichlorophenyl)-1,1-dimethylurea* (diuron), *Zinc Pyrithione* (ZnPT), *Copper Pyrithione* (CuPT), *Copper thiocyanate* (CuSCN), *Dichloro-N-[(dimethylamino)sulphonyl] fluoro-N-(ptolyl)methanesulphenamide* (tolylfluanid), *Dicopper oxide* (Cu_2O), *4-(1-(2,3-Dimethylphenyl)ethyl)-1H-imidazole* (medetomidine), *N-(Dichlorofluoromethylthio)-N',N'-dimethyl-N-phenylsulfamide* (dichlofluanid); *zinc ethylenebis(dithiocarbamate)* (zineb); and *Tetrachloroisophthalonitrile* (chlorothalonil). Their physicochemical properties are summarized in Table 1.

Table 1: Summary of the physico-chemical properties of antifouling biocides. DT_{50} – initial half-life (days); Log K_{ow} – Octanol-Water partitioning coefficient. References: [a] Chen & Lam (2017); [b] Mukherjee et al. (2009); [c] Van Scoy & Tjeerdema (2014); [d] European Chemical Agency [ECHA] (2015a); [e] European Chemical Agency [ECHA] (2016a); [f] European Chemical Agency [ECHA] (2016b); [g] Hilvarsson et al. (2009); [h] European

Biocide	CAS no	Molecular formula	Molecular mass	DT_{50} seawater	Log K_{ow}
DCOIT	64359-81-5	$C_{11}H_{17}Cl_2NOS$	282[a]	0.04[a]	6.4[a]
Diuron	330-54-1	$C_9H_{10}Cl_2N_2O$	233.1[b]	31.4[b]	2.8[b]
Zinc Pyrithione	13463-41-7	$C_{10}H_8N_2O_2S_2Zn$	317.7[b]	0.33[b]	0.93[b]
Dichlofluanid	1085-98-9	$C_9H_{11}Cl_2FN_2O_2S_2$	333.2[b]	0.125[b]	3.5[b]
Clhorotathalonil	1897-45-6	$C_8Cl_4N_2$	265.9[c]	1.8[i]	3[c]
Tolylfluanid	731-27-1	$C_{10}H_{13}Cl_2FN_2O_2S_2$	347.3[b]	0.25[b]	3.9[b]
Copper Pyrithione	154592-20-8	$C_{10}H_{10}CuN_2O_2S_2$	317.9[d]	0.4[d]	2.8[d]
Copper thiocyanate	1111-67-7	$C_2CuN_2S_2$	179[e]	n.a.	n.a.
Dicopper oxide	1317-39-1	Cu_2O	143[f]	n.a.	n.a.
Medetomidine	86347-14-0	$C_{13}H_{16}N_2$	200.2[g]	4[g]	3.13[g]
Zineb	12122-67-7	$C_4H_6N_2S_4Zn$	275.8[h]	9.9[h]	1.30[h]

This review encompasses a comprehensive compilation and discussion of the available environmental and ecotoxicological data for the above-mentioned antifouling biocides (section 3 and 4, respectively), and their environmental hazard and ecological risk in coastal areas, which endpoints were derived and discussed in section 5. The environmental risk posed by each biocide and the threat to coastal areas is critically discussed in the perspective of protection of marine ecosystems (section 5). Gap knowledge, alternative solutions and future perspectives are also discussed in section 6.

2. Methodology

2.1 Graphical representation of the compiled data

The environmental occurrence of AF biocides data (compiled in the supplementary material, Table S1) was used to calculate the percentage of environmental quantification performed in each biogeographical realm.

The short-term toxicity data of AF biocides towards 70 marine species was compiled in the supplementary material (Table S2). The geographic distribution of such species was check through the World Register of Marine Species website (http://www.marinespecies.org). The representativeness of a given marine biogeographic realm (proposed by Costello et al., 2017) in terms of available short-term toxicity data was calculated through the number of recorded species with published toxicity data in proportion to the total number of worldwide records for all biocides, as follows:

$$\%ofspecies \in therealm = \frac{(n^{o}ofspecies \in therealm) \times 100}{70}$$

Species that had a distribution restricted to one realm were classified as endemic, and the percentage of endemic species in each realm was calculated as follows:

$$\%ofendemicspecies = \frac{(endemicspecies \in therealm) \times 100}{n^{o}ofspecies \in agivenrealm}$$

Organisms were also classified according to a major ecological functional trait, as producers, consumers, or decomposers. Both categorical data and numerical variables (e.g. effects or lethal concentrations) were plotted in boxplots were the numerical variables were split into quartiles: the first quartile represents the 25th percentile, the second quartile portraits the 50th percentile and the third quartile shows the 75th percentile; whiskers extend to maximum and minimum values.

The figures presented in this review were generated using the seaborn library and Matplotlib Basemap Toolkit from Python 3 software.

2.2 Determination of the environmental hazard and risk assessment

Predicted no-effect concentration (PNEC) and risk quotient (RQ) values for each antifouling biocide were derived from compiled data presented in the sections 2 and 3. The calculations followed the EU Technical Guidance Document on Risk Assessment (TGD, 2003).

Regarding the hazard assessment, the recommended approaches to derive the PNEC values (TGD, 2003) for the different biocides were followed:

(I) the *deterministic approach*, based on the ratio between the lowest median lethal effect concentration (L/EC_{50}) value available in the literature and an assessment factor (AF) that varies depending on the uncertainty and quality of the database according to the TGD (2003), as follows:

$$PNECdet = \frac{lowest\ L/EC\ 50}{AFdet}$$

A minimum of three toxicity values from representative trophic levels, commonly microalgae (producers), crustaceans (primary consumers), and fish (secondary consumers) is required to obtain the deterministic PNEC (TGD, 2003).

(II) the *probabilistic approach*, based on the species sensitivity distribution (SSD) method, using all available L/EC_{50} values for each biocide (the geometric mean was calculated whenever more than one L/EC_{50} value was available for a given species). The SSD method requires a minimum of 8 taxonomic groups, applied in a probabilistic distribution function, which output is the 5% hazardous concentration (HC_5), a threshold that protects 95% of the ecosystem as a whole (TGD, 2003; Staples et al., 2008). The higher the number of species in the curve, the higher the certainty of the derived value. The HC_5 was calculated using the R software. The probabilistic PNEC was then calculated through the quotient between the HC_5 value and 5, the most conservative AF defined by the TGD taking into consideration the use of short-term toxicity data and the uncertainty regarding the size of the available dataset:

$$PNECprob = \frac{HC5}{5(AF)}$$

For the ecological risk assessment of the approved AF biocides, the risk quotient (RQ) was calculated as follows (if RQ<1, biocide poses no risk):

$$RQ = \frac{MEC(PEC)}{PNEC}$$

where MEC and PEC stands for measured environmental concentration and predicted environmental concentrations, respectively (these values were compiled from literature). The RQ was calculated taking into consideration the deterministic and probabilistic PNEC values for each biocide.

3. Biocides environmental concentrations

Data on biocides concentrations in both seawater and marine sediment are provided as supplementary material (Table S1). Marinas, ship routes, and commercial port areas are hotspots of antifouling biocides contamination (Wezel & Vlaardingen, 2004), and literature regarding environmental concentration outside these hotspots is particularly scarce.

3.1. Degradation, occurrence and persistence of DCOIT, Diuron, chlorothalonil and dichlofluanid

Degradation kinetics of DCOIT in seawater ranges from less than one day (Jacobson & Willingham, 2000) up to 13 days (Sakkas, Konstantinou, & Albanis, 2002) depending on the environmental conditions, such as sunlight, dissolved oxygen or temperature (Chen & Lam, 2017). In a regulatory context, DCOIT is regarded as having low persistence (European Chemical Agency [ECHA], 2014a). As a consequence, in spite of its rapid degradation DCOIT has been detected in Mediterranean, Atlantic and Pacific waters, ranging from 0.003 µg/L in coastal waters of Sweden (Readman, 2006) to 3.7 µg/L in a Spanish marina (Martínez & Barceló, 2001). According to Thomas and Brooks (2010), due to the high Log Koc of DCOIT (4.19), this compound tends to bind and partition to the sediment solid-phase. Chen and Lam (2017) further stated that marine sediments may act as a reservoir for DCOIT. Not surprisingly, its accumulation has already been detected in coastal sediments worldwide, particularly in western Asia (Table 2), where concentration reaches 0.28 µg/g in Korean harbors (M. R. N. Lee et al., 2015).

Diuron has a well-documented persistence in the marine environment (Faÿ et al., 2018) with degradations kinetics ranging from 1 month to 1 year depending on the environmental conditions (Dafforn et al., 2011). It has been detected worldwide in seawater (Table S1, Supplementary material), where concentrations reaches 7.8 µg/L in Brazilian harbors (Table 2) (Diniz et al., 2014).The high Log Koc of 2.6 indicates that Diuron has a high sediment adsorption capacity (Giacomazzi & Cochet, 2004). The highest diuron concentration in coastal sediments was found in the soft-bottom of Korean harbor areas (0.14 µg/g, Lam et al., 2017). Besides, coastal sediments from America, Asia and Europe also present diuron contamination (Table S1, Supplementary material).

Microbial activity is the major process involved in the degradation of chlorothalonil in the marine environment, where its half-life is 1.8 days, presenting a low persistence in water (Sakkas, Lambropoulou, et al., 2002; Kv. Thomas, 2001). Therefore, most studies performed in coastal areas failed to detect chlorothalonil (Albanis et al., 2002; Carbery et al., 2006; Lambropoulou et al., 2000; Sakkas, Konstantinou, Lambropoulou, et al., 2002). However,

concentrations of 0.01 and 1.38 µg chlorothalonil/L were measured in the Atlantic coasts of France and the United Kingdom, respectively (ACE, 2002; Voulvoulis, 2006). Although chlorothalonil has the potential to strongly adsorb to sediment due to its Log Koc = 5 (Armbrust, 2001), the rapid degradation in seawater implies that partitioning to sediment may not be significant. However, sediment contamination was observed in Brazil, Malaysia, Korea and Greece, with concentrations of chlorothalonil at 0.009, 0.006, 0.08 and 0.16 µg/g, respectively (Abreu et al., 2020; Albanis et al., 2002; S. L. Y. Lee, 2017; Mukhtar et al., 2019).

According to Wezel and Vlaardingen (2004), dichlofluanid is highly unstable in water with a half-life of 3 hours. This compound suffers hydrolysis and is rapidly converted to DMSA, a less toxic compound (European Chemical Agency [ECHA], 2006). Despite its low persistence, dichlofluanid has been detected in coastal waters of Greece, Korea, Portugal and Spain (Table S1, Supplementary material). The highest concentrations of dichlofluanid were found in Portugal at 0.02 µg/L (Gonzalez-Rey et al., 2015) and Spain at 3.37 µg/L (Martínez & Barceló, 2001). Dichlofluanid has low solubility in water (< 2 mg/L) and Log Koc of 3.7 (Wezel & Vlaardingen, 2004). Although it has low solubility, accumulation in sediment, is unlikely to occur, due to its rapid degradation in the water. The highest concentration in worldwide coast sediment (Table S1, Supplementary material) ranges from 0.016 µg/g in Brazil (Abreu et al., 2020) to 0.8 µg/g in Malaysia (Mukhtar et al., 2019).

Table 2: Maximum MEC and PEC concentrations of antifouling biocides for marine environments in water (µg/L) and sediment (µg/g). n.a.: data not available

Biocide	Matrix	Country	Site	Year	MEC	PEC	Reference
ZnPT	Water	UK	Mersey Estuary	2002	0.000033	0.52	MacKie et al.(2004); Danish EPA(2012)
Dichlofluanid		Spain	Ports and marinas (catalonia)	2000	3.37	2.6	Martínez et al.(2001); ECHA (2006)
Diuron		Brazil	Port of Itaqui	2011	7.8	8.7	Diniz et al.(2014); Kevin V. Thomas et al.(2001)
DCOIT		Spain	Ports and marinas (catalonia)	1999	3.7	0.52	Martínez and Barceló (2001); Danish EPA(2012)
CuPT		-	Shipping lane	2012	n.a	0.00081	ECHA (2015[a])
CuSCN		-	Marinas	2016	n.a	8.64	ECHA (2016[a])
Tolylfluanid		-	Harbour	2014	n.a	0.17	ECHA (2014)
Cu_2O		-	Marinas	2016	n.a	32.14	ECHA (2016b)
Chlorothalonil		UK	Estuary	1998	1.38	1.4	Voulvoulis et al. (2000); Bellas (2006)
Medetomidine		-	Marinas	2015	n.a	0.0039	ECHA (2015b)
Zineb		-	Marina,harbours	2012	n.a	0.142	New Zealand EPA (2012)
ZnPT	Sediment	-	Pleasure craft harbour	2000	n.a	0.21	Danish EPA(2012)
Dichlofluanid		Malaysia	Sungai Pulai Estuary	n.a.	0.8	0.8	Mukhtar et al.(2019); ECHA (2006)
Diuron		Japan	Otsuchi Bay	2005	0.53	0.22	Harino et al.(2007); Mukherjee et al.(2009)
DCOIT		Korea	Coasts and Harbors	2010-2011	0.28	0.12	Lee et al. (2015); Danish EPA(2012)
CuPT		Japan	Otsuchi Bay	2005	0.022	n.a	Harino et al.(2007)
Copper thiocyanate		-	Marinas	2016	n.a	638.33	ECHA (2016[a])
Dicopper oxide		-	Marinas	2016	n.a	2652.3	ECHA (2016b)
Chlorothalonil		Greece	Ports and marinas	2000	0.16	n.a	Albanis et al. (2002)
Medetomidine		-	Marinas	2015	n.a	0.0004	ECHA (2015b)

3.2. Pyrithiones, CuSCN, Cu2O, Medetomidine, Tolylfluanid and Zineb: Monitoring challenges

Few monitoring studies have been performed focusing on the environmental occurrence of Zn or Cu pyrithiones (PT), CuSCN, Cu_2O, medetomidine, tolylfluanid and zineb (Table 2). The fate of these compounds in the marine environment is poorly studied and understood. The half-life of zineb, medetomidine, and tolylfluanid is 9.9, 4, and 0.25 hours, respectively (cf. Table 1). Their occurrence in coastal environments have not yet been reported, although predicted environmental concentrations (PECs) for seawater are 0.14, 0.003, and 0.17 µg/L, respectively (European Chemical Agency [ECHA], 2014b, 2015b; New Zealand EPA, 2012). For sediments, the PEC was only estimated for medetomidine (0.0004 µg/L; (European Chemical Agency [ECHA], 2015b), and according to Ohlauson et al. (2012), this biocide has a log Koc of 0.35. For zineb and tolylfluanid, the log Koc is 3 and 3.3, respectively, indicating a low to moderate mobility in sediment (European Chemical Agency [ECHA], 2014b; National Center for Biotechnology Information, 2021).

The lack of environmental information regarding medetomidine is basically due to two major factors. First, it is a recent antifouling biocide that is not yet applied on the same scale as those already established in the market, such as diuron and DCOIT (Lennquist et al., 2010), thus, not attracting as much attention to monitoring programs. The second factor is due to analytical inconsistencies. The current method to quantify medetomidine is based on a combined gas chromatography-mass spectrometry (GC-MS) in chemical ionization, negative ions mode (NCI) (Bellas et al., 2005). However, several authors have reported difficulties in analyzing this compound, especially at low concentrations (up to 84.1 µg/L) (Bellas et al., 2006; Hilvarsson et al., 2007; Lennquist et al., 2010).

Unlike medetomidine, studies involving tolylfluanid and zineb do not present analytical inconsistencies in the literature. The methods used to detect these compounds are based on liquid chromatography (Reemtsma et al., 2013), as well as a novel sensing colorimetric method proposed by Mohamadjafari and Rastegarzadeh (2017). However, the absence of environmental data on tolylfluanid and zineb is attributed to their novelty and recent regulation as an antifouling compound (Regulation (EU) No 2015/419; Regulation (EU) No 92/2014), indicating that it has been used and focused in monitoring programs on a smaller scale compared to other biocides.

Regarding the pyrithiones, both ZnPT and CuPT are particularly challenging to quantify, primarily due to their short photolytic half-life (cf. Table 1). Moreover, these

pyrithiones chelate and transchelate with other metallic ions, severely influencing their stability. ZnPT tends to transchelate into the more stable form of CuPT when copper ions are available (Katja & Dahllo, 2005). However, since 1999 (Kevin V Thomas, 1999), analytical methods have been developed to overcome these constraints. Bones et al. (2006) developed a technique that simultaneously measures ZnPT and CuPT by adding Cu ions to the samples, thus, comparing the ZnPT transchelation with a control. Yamaguchi et al. (2006) proposed another technique that prevents the transchelation of ZnPT by adding ammonium acetate in order to stabilize copper ions. Despite the existence of techniques for quantifying pyrithiones in the marine environment, the data presented in this review show that these techniques are not widely applied yet. In addition, a factor that continues to be limiting for the quantification of pyrithione is its rapid degradation (which occurs even during the analytical procedure) (Soon et al., 2019). As far as we are concerned, only one study reported ZnPT in the water column, more precisely in the Mersey Estuary at 3.3×10^{-5} µg/L (MacKie et al., 2004), and there is no ZnPT data on sediments. However, the Danish EPA (2012) determined a PEC in sediments of 0.21 µg/g. For CuPT, there is one report to sediment concentration of 0.02 µg/g measured in Japan (Harino et al., 2007). According to Yamada (2007) the log Koc for both ZnPT and CuPT is 0.7 indicating a low mobility in the sediment. The available environmental data towards ZnPT or CuPT does not allow a clear understanding of the environmental fate of pyrithiones as well as its distribution in coastal areas. New analytical advances are needed to accurately detect both pyrithiones, preferably techniques with higher sensitivity and capacity to handle with the unstable nature of the pyrithiones in seawater. The most recent study about the development of an analytical method for pyrithiones detection in the environment is from 2015 (Kim et al., 2015). As a comparison, the limit of detection (LOD) value based on the technique of liquid chromatography for ZnPT (0.018 µg/L; Bones et al., 2006) is 600 times higher than for DCOIT (0.003 µg/L; Harino et al., 2005) demonstrating relevant analytical discrepancies between biocides.

CuSCN and Cu_2O have copper as an antifouling agent, the most commonly used metallic biocide in antifouling paints since the mid of mid-nineteenth century (Yebra et al., 2004). However, the challenge of quantifying such compounds in water and sediment samples relies on their environmental fate. According to Thomas and Brooks (2010), leachates from antifouling paints containing inorganic Cu-based biocides enter into the water mostly as Cu^{+} rich solutions, and then it is oxidized to Cu^{2+}, which may form complexes with inorganic and organic ligands present in the water. Not surprisingly, due to the diffuse and multiple sources

of Cu contamination, especially in harbors and marinas, there is a lack of studies that specifically relate to the Cu contamination with Cu-based antifouling biocides, such as CuSCN and Cu_2O.

In environmental studies, organometallic antifouling compounds have been identified indirectly by the detection of their respective free ion forms; then, through the application of mathematical models, the environmental concentration of the parental biocide is predicted (PECs are presented in Table 2). A possible alternative, not yet applied for antifouling biocides, would be the comparison between the isotopic signatures of Cu found in the environment and the various antifouling formulations available in the market. This method was already used by Lepak et al. (2015) to determine mercury sources in the environment.

4. Adverse effects to marine organisms

In this section, data available on the toxicity of biocides to marine species is reported and discussed. For each biocide, marine taxonomic groups were ranked according to the sensitivity, based on EC_{50} (median effect concentration) and LC_{50} (median lethal concentration). The endpoints of representative species are presented as supplementary material (Table S2, Supplementary material). Generally, data refers to short term exposures, the majority of the studies are based on lethal endpoints (for invertebrates) and growth inhibition for producers, while whole sediment toxicity data is globally scarce or absent for all biocides.

4.1. DCOIT

The toxicity of DCOIT to marine organisms is fairly well studied. The sensitivity of the different taxonomic groups towards DCOIT can be summarized as follows: microalgae > cyanobacteria > macroalgae > bacteria > diatoms > crustaceans > fish > bivalves > echinoderms > ascidians > polychaetes (Table S2). Among the reported EC_{50} values, the microalgae *Emiliania huxleyi* presented the highest sensitivity with 72h-EC_{50} of 0.4 µg/L (Devilla et al., 2005); and the highest value was estimated for the ascidian *Ciona intestinalis*, which 24h-EC_{50} of 43 µg/L (Bellas, 2006). Regarding lethal effects, the reported LC50 values range from 5 µg/L (96 h-LC50) for the mysid *Americamysis bahia* (Shade et al., 1993) to 150 µg/L (24 h-LC50) for the rotifer *Brachionus plicatilis* (Figueiredo et al., 2019).

4.2. Diuron

The toxicity of diuron to marine species is relatively well documented. The sensitivity of the major taxonomic groups towards diuron is ranked as: microalgae > cyanobacteria > diatoms > cnidarians > coralline algae > crustaceans > fish > bivalves > echinoderms > macroalgae > polychaetes > dinoflagellates (Table S2). EC_{50} values found in the literature range from 0.4 µg/L (96 h-EC_{50}) for microalgae *Tetraselmis suecica* (Dupraz et al., 2018) to 19,000

µg/L (24 h-EC_{50}) for the dinoflagellate *Pyrocystis lunula* (Stauber et al., 2008). The diuron LC_{50} values reported for marine species range from 5 µg/L (10 d-LC_{50}) for the cnidarian *Aurelia aurita* (Pinteus et al., 2020) to 16,000 µg/L (48 h-LC_{50}) for the polychaeta *Hydroides elegans* (Bao et al., 2011).

4.3. Chlorothalonil

The most sensitive groups towards chlorothalonil are the rotifers > diatoms > echinoderms > microalgae > annelids > crustaceans > bivalves > fish > tunicates > dinoflagellates (Table S2). The EC50 values described in the literature range from 0.9 µg/L (72 h-EC_{50}) for the diatom *Skeletonema costatum*, (Onduka et al., 2012) to 66 µg/L (24 h-EC_{50}) for the dinoflagellate *Pyrocystis lunula* (Bao et al., 2011). The LC_{50} values range from 0.5 µg/L (24 h-LC_{50}) for the rotifer *Philodina acuticornis* (Hagen et al., 2010) to 5,940 µg/L (96 h-LC_{50}) for the bivalve *Mytilus edulis* (Ernst et al., 1991).

4.4. Dichlofluanid

Comparatively to the previous biocides, dichlofluanid toxicity data is scarce. There are data just for six taxonomic groups (Table S2), which sensitivity can be organized as follows: fish > bivalves > echinoderms > microalgae > diatoms. Dichlofluanid EC_{50} values range from 15 µg/L (96 h-EC_{50}) for the fish species *Dicentrarchus labrax* (Carteau et al., 2014) to 377 µg/L for the diatom *Nitzschia pungens* (Jung et al., 2017). The 48h-LC_{50} value of dichlofluanid for the microcrustacean Artemia sp. Is set on 154,000 µg/L (Jung et al., 2017).

4.5. Zinc pyrithione

Unlike the scarcity of environmental data for ZnPT, the toxicity of this compound on marine species is well documented in the literature (Table S2). The most sensitive organisms to ZnPT are ranked as: dinoflagellates > diatoms > echinoderms > bivalves > crustaceans > polychaetes > bryozoans > cyanobacteria > fish > macroalgae > microalgae > cnidarians. EC_{50} values range from 0.4 µg/L (24 h-EC_{50}) for the dinoflagellate species *Pyrocystis lunul*, (Bao et al., 2011) to 280 µg/L (96 h-EC_{50}) for the *microalgae Tetraselmis chuii* (Avelelas et al., 2017). The primary LC_{50} values were estimated at 5.0 µg/L (96 h-LC_{50}) for the crustacean *Americamysis bahia* (Environmental Protection Agency [EPA], 1992); 3,200 µg/L (72 h-LC_{50}) for the bivalve *Mytilus galloprovincialis* (Gutner-Hoch et al., 2018); and 98,000 µg/L (96 h-LC_{50}) for the fish species *Pagrus major* (Mochida et al. (2006).

4.6. Copper pyrithione

Despite the lack of environmental data, the toxicity for CuPT is known for a total of 11 taxonomic groups (Table S2). The sensitivity to CuPT can be summarized as follows: diatoms > microalgae > polychaetes > fish > crustaceans > echinoderms > cyanobacteria >

dinoflagellates > corals > bryozoans > bivalves. EC_{50} values documented in the literature range from 0.7 µg/L (96 h-EC_{50}) for the diatom *Thalassiosira pseudonana* (Bao et al., 2011) to 3,200 µg/L (72 h-EC_{50}) for the bivalve *Mytilus galloprovincialis* (Gutner-Hoch et al., 2018). Regarding lethal effects, LC_{50} reported range from 6.0 µg/L for the polychaeta *Hydroides elegans* (Bao et al., 2011) to 3,800 µg/L for the *bivalve Mytilus galloprovincialis* (Gutner-Hoch et al. 2018).

4.7. CuSCN and Cu2O

The toxicity of the inorganic antifouling biocides CuSCN and Cu2O towards marine species is, as far as we are concerned, poorly studied (cf. Table S2). Regarding CuSCN, the 96 h-L_{C50} value of 9.6 µg/L was calculated for the fish *Pleuronectes platessa* (Environmental Protection Agency [EPA], 2000) and 72h-EC_{50} of 306 µg/L for the diatom *Fragilaria pinnata* (Silkina et al., 2012).

4.8. Medetomidine

The sensitivity of marine species towards medetomidine can be ordered in this way: bivalves > crustaceans > fish > algae. For this biocide, the estimated EC_{50} values span 5 orders of magnitude ranging from 1 µg/L (24 h-EC_{50}) for the bivalve *Abra nitida* to 20,020 µg/L for the macroalgae *Ulva lactuca* (cf. Table S2). The only information on the lethality of medetomidine was carried out with the copepod *Acartia tonsa* with 48h-LC50 calculated at 48.3 µg/L (Wendt et al., 2016).

4.9. Tolylfluanid

Regarding tolylfluanid, the most sensitive group of organisms are bacteria > macroalgae > echinoderm > diatom > bivalves > ascidian. EC_{50} values of this biocide range between 22 µg/L, i.e., the 72 h-EC_{50} value that inhibits settlement and growth of natural marine biofilms (Arrhenius et al., 2014), and 217 µg/L (72 h-EC_{50}) for the larval development of the ascidian *Ciona intestinalis* (Bellas, 2006) (cf. Table S2).

4.10. Zineb

The toxicity of the fungicide zineb is relatively well documented for freshwater species contrasting with the marine organisms. Thus, taking into consideration the available data, fish are the most sensitive group, followed by crustaceans, diatoms, and macroalgae (less sensitive) (cf. Table S2). However, the endpoints determined for fish and crustaceans are lethality, while for diatoms and macroalgae are related to a growth inhibition effect. Therefore, considering short-term chronic toxicity data, the endpoints values of zineb reported were 96 h-EC_{50} = 232 µg/L for the diatom *Nitzschia pungens*. (Jung et al., 2017), and 48 h-EC_{50} = 870 µg/L for the macroalgae Hormosira banksia (Myers et al., 2006). Regarding lethality data of zineb, the 96

h-LC_{50} estimated for the fish species *Pagrus major* is 29 µg/L (Hano et al., 2017); and the 48 h-LC_{50} calculated for *Artemia sp*. is 41 µg/L (Jung et al., 2017).

4.11. Biogeographic representativeness of data

An environmental risk assessment is influenced by the existence of an extensive, representative and reliable database on both environmental occurrence and ecotoxicological data.

The present review demonstrates that most of the published biocides environmental monitoring studies have been carried out in Europe and Asia, concentrated in only 4 out of 30 marine biogeographic realms (described by Costello et al., 2017), namely, Northeastern Atlantic, Mediterranean, Tropical Indo-pacific and offshore Western Pacific. Some ecologically relevant biogeographic realms (number of endemic species greater than 50% of the total known biodiversity) have not been monitored yet, such as, the Black, Baltic, Red or Tasmania seas, Chilean coast, Gulf of Guinea or the South-east Pacific (Costello et al., 2017).

On the other side, the distribution of the species tested for anti-fouling biocides is also very restricted. According to information available on the Word Register of Marine Species portal the majority of species used in the biocides' exposure testing are mainly distributed in the Mediterranean, Northeastern Atlantic and Tropical Western Atlantic, as shown in Figure 1 (numbers 5, 3 and 11, respectively). The number of endemic species in each realm is also low and restricted to some Atlantic and Pacific realms. The Offshore Southern Atlantic realm concentrate the highest percentage of tested species which are endemic of this region (21%), followed by the Offshore Western Pacific (14%) and the Tropical Western Atlantic (12%) (Figure 1A). More than half of the species with antifouling biocides ecotoxicological data exhibits a cosmopolitan distribution, being distributed in at least two biogeographic realms of the temperate and tropical regions (Figure 2). None of the tested species were strictly distributed in the polar regions (latitude $\geq 60°$N/S), so no information for the Artic or Antarctic Oceans can be retrieved for any biocide. The biocides Tolylfluanid, CuSNC, Dichlofluanid, Medetomidine, Cu2O, Zineb and DCOIT have no information exclusively inhabiting the Indian Ocean while Medetomidine, Cu2O and Diuron have no representation of endemic species of the Pacific Ocean (Figure 2).

An important effort to equilibrate this unbalance in terms of biogeographic distribution is therefore highly recommended for a future reassessment of the antifouling biocides environmental risk.

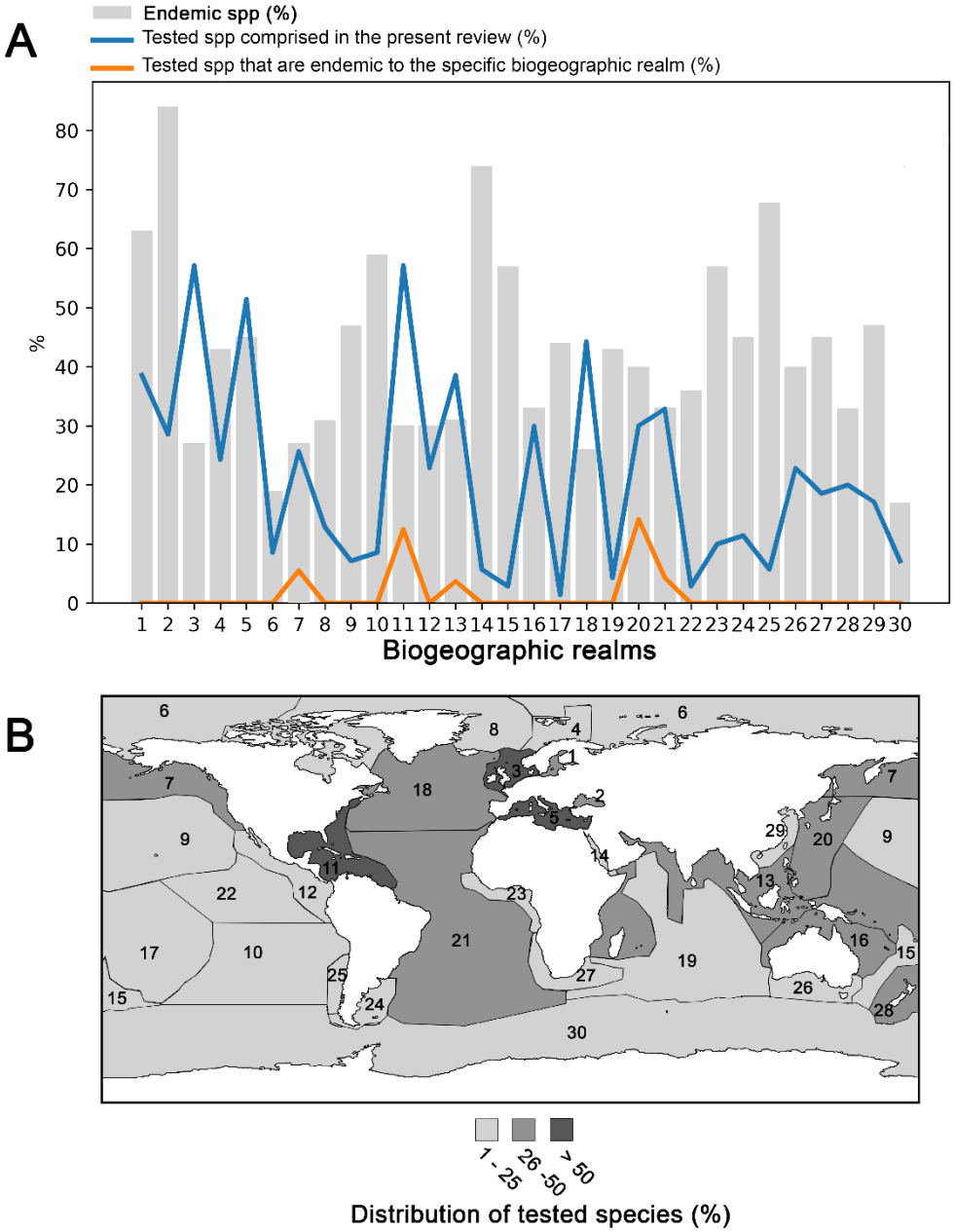


Figure 1: A: Marine biogeographic realms numbered 1-30. Gray bars indicate the percentage of reported endemism in each realm. The blue line indicates the representativity of each realm within the overall set of species comprised in the present review. Orange line indicates the percentage of the species under the review that are endemic to each realm. B: Spatial representation of the biogeographic realms representativity in the current review dataset (e.g. 50% indicates that half of the tested species are distributed in that realm, among eventually others). Biogeographic realms legend: 1: Inner Baltic Sea; 2: Black Sea; 3: NE Atlantic; 4: Arctic Europe; 5: Mediterranean; 6: Arctic; 7: North Pacific, 8: N Atlantic boreal & sub-Arctic; 9: Mid-tropical North Pacific Ocean; 10: South-east Pacific; 11: Tropical W Atlantic; 12: Tropical E Pacific; 13: Tropical Indo-Pacific (East Indies) & coastal Indian Ocean; 14: Red Sea, 15: Tasman Sea to SW Pacific; 16: Tropical Australia & Coral Sea; 17: Mid South Tropical Pacific; 18: Offshore & NW North Atlantic; 19: Offshore Idian Ocean; 20: Offshore W Pacific; 21: Offshore S Atlantic; 22: Offshore mid-E Pacific; 23: Tropical E Atlhantic; 24: Argentina; 25: Chile; 26: S Australia; 27: S Africa; 28:New Zeland: 29: North West Pacific; 30:Southern Ocean

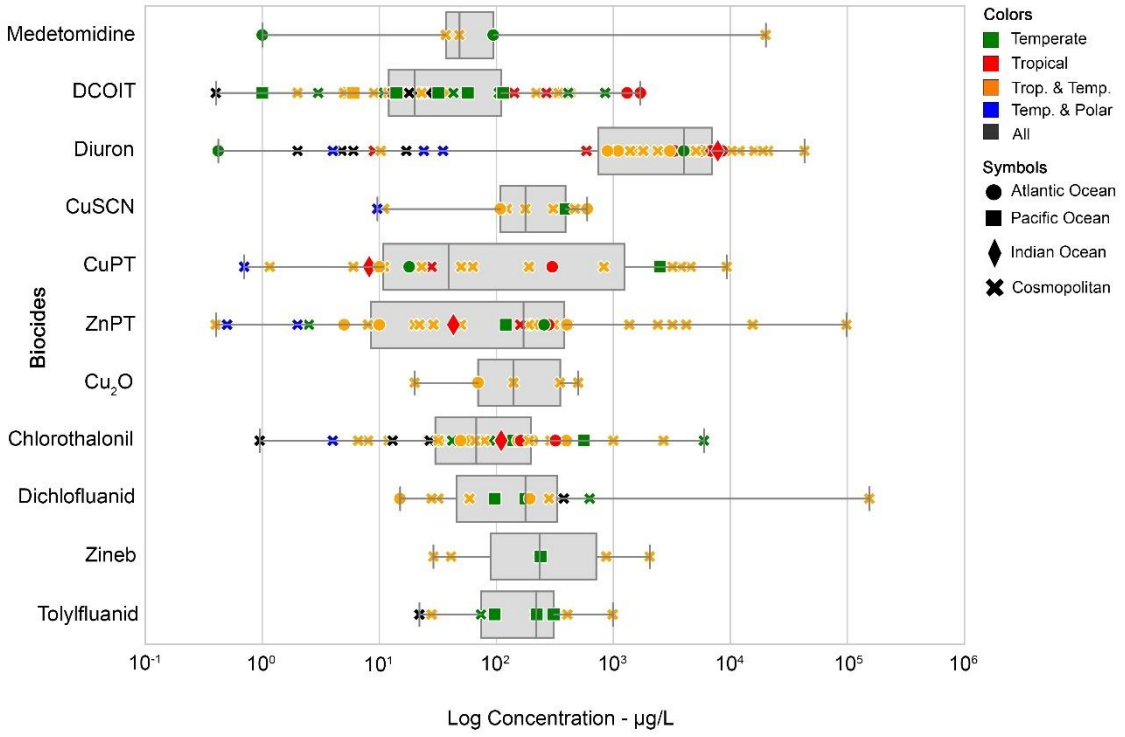


Figure 2: Toxicity gradient of antifouling biocides (EU PT21) according to the distribution of the marine species by the major climate regions: temperate, tropical, temperate & tropical, temperate & polar, and every region. Oceanographic distribution of tested organisms is also represented by symbols. The box shows the quartiles of the toxicity dataset while the whiskers extend to maximum and minimum EC_{50} or LC_{50} values. Each marker represents an endpoint value.

4.12. Insights on ecological traits and biocides mode of action

In order to compare the toxicity and species sensitivity towards the approved biocides, organisms were classified in terms of a major ecological trait (producers, consumers, and decomposers) and whether or not they are targeted by antifouling biocides (full data available as supplementary material, table S2). The overall toxicity and species sensitivity are presented in Figure 3.

Because primary producers are the basis of the marine food chain, effects caused by the exposure to biocides can impair the entire ecosystem. Toxicity data on this particular group is, therefore, key to quantify and to protect against the risks imposed by antifouling biocides to the marine ecosystems. The present review highlights that diuron, chlorothalonil, DCOIT, and pyrithiones are amongst the most toxic biocides towards primary producer species. Furthermore, ZnPT, CuPT, and chlorothalonil have higher average toxicity towards target primary producers, while DCOIT and diuron are more toxic to non-target primary producers (cf. Figure 3). Besides the limited toxicity data towards organisms of this trophic level, dichlofluanid presented high toxicity for target macroalgae. According to the literature, diuron and dichlofluanid are photosynthesis inhibitors, firstly used commercially as herbicides, then used as antifoulants preventing algae growth (Dafforn et al., 2011). Although DCOIT toxicity mechanism is not yet well understood, it is known that DCOIT causes oxidative stress followed by oxidative damage, especially in membrane lipids (Chen & Lam, 2017). In their turn, chlorothalonil's mode of action occurs by electron transport inhibition, while, both zinc and copper pyrithiones can impair several metabolic processes such as the downregulation of phosphoenolpyruvate carboxykinase (PEPCK) causing changes in energy production.(Dafforn et al., 2011)

Consumers play an essential role in the function and structure of the ecosystem and the release of antifouling biocides into seawater can impair top-down and bottom-up processes in marine ecosystems. For instance, a decline in consumers can lead to an uncontrolled increase of producers, followed by a long-term depletion of nutrients and consequent reduction in their population (Verity & Smetacek, 1996). Despite the limited toxicity data, CuSCN, medetomidine, and zineb indicate extreme toxicity towards non-target consumers impairing the organisms even at low concentrations (few µg/L). On aquatic invertebrates, the fungicide zineb causes neurotoxicity through the impairment of monoaminergic neurotransmitters. Besides, it causes oxidative stress through the downregulation of important antioxidant enzymes, such as glutathione (GSH) (Grosicka-Maciag et al., 2013; Laisi et al., 1985). Medetomidine promotes the activation of octopamine receptors inhibiting the settlement of invertebrates larvae (Lind et

al., 2010). CuSCN reduces the mitosis rates by the breakage of the cellular antioxidant defense system through glutathione reduction (Reading, 2005).

According to the Biocidal Products Regulation (BPR) (Regulation 528/2012) of the European Union, antifouling biocides must degrade fast to reduce the environmental persistence, present low potential for bioaccumulation and low toxicity to non-target marine organisms. Medetomidine, CuSNC, zineb, Cu2O, and chlorothalonil do not fulfil completely these parameters as they present average toxicity 3 to 400 times higher for non-target organisms than the target ones. DCOIT, CuPT, and diuron presented similar toxicity for target and non-target organisms (an average difference of ≤ 1.5). These findings set an alert for those antifouling biocides. On the contrary, dichlofluanid, ZnPT, and tolylfluanid presented average toxicity 3 to 80 times higher for foulers comparing with non-target organisms.

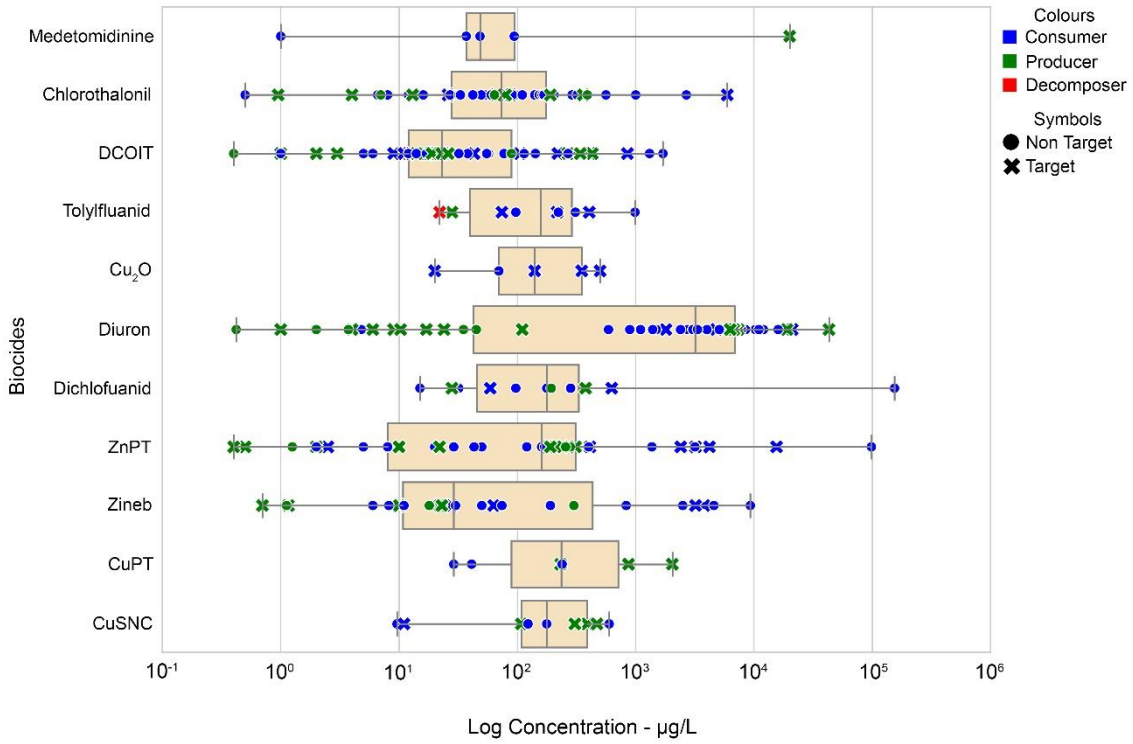


Figure 3: Toxicity gradient of antifouling biocides (EU PT21) towards marine producers, consumers or decomposers. Target/fouling and non-target/fouling organisms are also represented as circles and crosses, respectively. The box shows the quartiles of the dataset while the whiskers extend to maximum and minimum EC_{50} or LC_{50} values. Each marker represents an endpoint value.

5. Environmental hazard and ecological risk assessment

The marine hazard (deterministic PNEC, probabilistic PNEC, HC_5) and risk endpoints RQ) for the 11 approved biocides are compiled in Table S3 as supplementary material (the assessment factors used to derive the hazard endpoints are also provided).

5.1.Hazard assessment

According to the EU Technical Guidance Document on Risk Assessment (TGD, 2003), the predicted non-effect concentration (PNEC) is an important reference safety value used to protect the organisms of a given ecosystem. It is mandatory for the ecological risk assessment of a given chemical, and therefore an important tool for managing toxic chemicals.

As far as we are concerned, this is the first study to determine the marine environmental hazard for tolylfluanid, zineb and Cu_2O. Biocides hazard based on $PNEC_{probabilistic}$ can be summarized in the following order: ZnPT (0.05 µg/L, more hazardous) > CuPT > DCOIT > diuron > chlorothalonil > dichlofluanid > tolylfluanid (5.52 µg/L, less hazardous; cf. Table S3). CuSCN, Cu_2O, medetomidine and zineb were excluded due the absence of sufficient toxicity data for the SSD method. The hazard assessment based on the SSD method is regarded as more accurate and ecologically relevant according to Sorgog and Kamo (2019) and Figueiredo et al. (2020) and, thus, highly recommended in the framework of an ecological risk assessment (Gredelj et al., 2018; Guo et al., 2020; Mensah et al., 2013). Nevertheless, by applying the deterministic approach, biocides hazard based on $PNEC_{deterministic}$ tend to increase substantially being summarized as follows: Dichlofluanid (2.0×10^{-5} µg/L, more hazardous) > medetomidine > DCOIT > ZnPT > diuron > chlorothalonil > CuPT > CuSCN > Cu_2O > tolylfluanid > zineb (0.03 µg/L, less hazardous; cf. Table S3). As observed in Table S3 (for those biocides which did not present enough data for the calculation of the probabilistic PNEC), the deterministic PNECs range from 0.0001 (medetomidine) to 0.03 µg/L (zineb), as a result of the uncertainty of some of the datasets which implied the application of high assessment factors to derive the PNEC values. Further studies regarding the ecotoxicological effects of such compounds are crucial to increase the reliability of each PNEC value, to reduce the uncertainty, and better reflect the real threat of such compounds to the marine ecosystems.

5.2. Ecological risk assessment

According to the estimation of the risk quotients, it is demonstrated that the following biocides pose risk (RQ_{prob} > 1) for the coastal ecosystems (countries in brackets): *DCOIT* (Denmark and Spain), *diuron* (Netherlands, United Kingdom, France, Spain, Belgium, Italy,

Brazil, South Korea, Malaysia and Australia), *dichlofluanid* (Spain) and *chlorothalonil* (United Kingdom). The RQ of CuPT, CuSCN, tolylfluanid, Cu_2O, medetomidine and zineb was based on PEC values, and only CuPT and tolylfluanid do not pose risk to the environment (RQ<1).

Globally, it was demonstrated that marine organisms are sensitive to all antifouling biocides. Diuron, DCOIT, CuSCN, Cu_2O are the biocides that most threaten the environment, and the replacement by eco-friendly alternatives is highly recommended. The ECHA (2014a) indicates that DCOIT only poses risk within commercial ports/marinas, not in the surrounding areas. However, according to Chen and Lam (2017), most marine organisms are endangered by DCOIT. Thus, a systematic environmental risk assessment was recommended to prevent irreversible impacts on marine ecosystems worldwide. Onduka et al. (2013) also confirmed that DCOIT pose ecological risk to some coastal areas in Japan. Regarding diuron, the potential environmental risk was confirmed along the Brazilian, South Korean and Spanish coasts by Lam et al. (2017), Mijangos et al. (2018) and Viana et al. (2020). According to the present findings, ZnPT, CuPT, medetomidine and zineb present lower marine environmental risk, however further environmental quantification data is required to confirm this assumption and validate them as safer alternatives for the marine environment. This aspect needed to take into consideration, especially if we consider the high toxicity of these biocides to non-target organisms.

The present review focus on the effects and the environmental occurrence of all antifouling biocides approved in Europe, and it provides a systematic environmental risk assessment of these chemicals also for all areas worldwide where they were reported. The major source of uncertainty on risk assessments is the lack, dispersion and reliability of information on the environmental occurrence and ecotoxicity. This lack of data makes a site-specific risk assessment unattainable, and increase the uncertainty reducing the efficacy and accuracy of the RQ values (Sorgog & Kamo, 2019). As a lesson from the banned organotin compounds and the recently banned Irgarol 1051 in Europe, there is an urgency to comprehensively evaluate the environmental fate and toxicity of such compounds at local and regional levels, especially in Africa, Middle East, South Asia, Oceania and America where data is particularly scarce or absent at all. This effort will be critical to regulators, policy makers, and industry towards the implementation of solutions that can mitigate the threats posed by commercially available biocides. However, we recognize that protecting non-target organisms and, simultaneously, tackling fouling biota, is often a difficult balance to achieve.

6. Current advances and future perspectives

Research advances have been recently given to find environmentally friendly but simultaneously efficient antifoulants alternatives to current state-of-the-art antifouling biocides. Marine organisms are a vast and natural source of compounds with promising biocidal activity against fouling organisms (Omae, 2006) widely explored in a context of blue growth. Several molecules (e.g. terpenes, phenols, steroids, nitrogen compounds, peptoids) were identified in sessile organisms. Such substances have been artificially reproduced, synthesized and tested in terms of antifouling activity (Yebra et al., 2004). According to Wang et al. (2020), indole derivatives, a class of nitrogen-containing compound isolated from bryozoan and ascidians, have a potent antifouling performance. These substances reduced up to 90% the colonization density of barnacles in marine field tests, inhibited bacterial growth (inhibition ratio > 90% for *Escherichia coli* and *Staphylococcus aureus*) (Feng & Li, 2019) and inhibited the growth of the diatoms *Nitzschia losterium f.* and *Navicula climacospheniae* (Yang & Xia, 2015). Additionally, Feng et al. (2019) demonstrated that these derivatives are more efficient in terms of antibacterial activity and algal inhibition comparing with state-of-the-art antifouling biocide chlorothalonil while Yang et al. (2015) demonstrated that the antifouling activity of the indole derivative 2, 5, 6-tribromo-1-methyl-gramine (TBG) was up to six times higher than tributyltin oxide or $CuSO_4$. Lysozymes have also been proposed as eco-friendly alternatives able to hydrolyze the bacterial cell wall, thus, interrupting the first phase of the fouling process, the biofilm formation, on coated surfaces (Caro et al., 2010). These are only two major types of natural molecules that have been explored as potential substitutes of current antifoulants.

The difficulty of producing natural antifoulants relies on the feasibility of the scale up, which usually fails due to technical and economic constraints (Gittens et al., 2013). Furthermore, these new molecules also face the same regulatory process as any other antifouling biocide. As a result, the timeline for the development and approval is approximately ten years (Rittschof, 2000), which may compromise the entrance in the market of new molecules during this decade.

Meanwhile, the encapsulation of current biocides in nanostructured materials has been widely explored as a promising eco-friendly alternative. This procedure aims to control the biocide release over time, reduce the environmental impact, and solve compatibility problems with the coating ingredients (Andersson et al., 2015; Avelelas et al., 2017; Figueiredo et al., 2019, 2020; Gutner-Hoch et al., 2018, 2019; Maia et al., 2015). According to Figueiredo et al. (2019, 2020), the encapsulation of DCOIT in engineered silica mesoporous nanocapsules reduced its toxicity towards several non-target marine organisms. Additionally, these studies

demonstrated that DCOIT encapsulation did not affect its antifouling efficacy and reduced its marine environmental hazard. Avelelas et al. (2017) and Gutner-Hoch et al. (2018) tested the antifouling efficacy of ZnPT or CuPT encapsulated in silica mesoporous nanocapsules or immobilized in layered double hydroxides. As a result, these authors demonstrated that the antifouling efficacy towards mussels and bryozoans was similar or even better than the respective free forms, and that these biocides were less toxic towards microalgae, crustaceans or echinoderms, in some cases 3 orders of magnitude, when immobilized in such nanomaterials or in polyurea capsules. In addition, Ruggiero et al. (2018) successfully encapsulated zosteric sodium salt, a natural non-commercial and environmentally friendly antifouling biocide, into silica nanocapsules. As a result, they obtained an innovative filler with controlled-release properties for antifouling coatings. These promising findings indicate that the biocides encapsulation may help coating producers to save antifouling agents and to improve compatibility issues on formulations. Also, nanoengineered encapsuled biocides may contribute to reduce the ecological footprint of the maritime industry at short and mid-terms.

However, the new alternative seems to be the use of biocides-free antifouling coatings. Although biocides are used worldwide in the large majority of antifouling coatings, non-biocidal-based paints are already a reality in the market. Ultra-hydrophobic or ultra-hydrophilic non-stick coatings can prevent the adhesion of fouling organisms (Amara et al., 2018; Dafforn et al., 2011). These systems providing a low-friction and ultra-smooth surface, for instance, based on silicon technology (as marketed by one of the big players worldwide). Another alternative was proposed by Azemaret al. (2015) through a hybrid antifouling paint using, as binders, Poly(ε-caprolactone) (PCL) and poly(dimethylsiloxane) (PDMS) homopolymers. This product presented efficacy comparable to an existing commercial paint, but with a reduced environmental impact. Other alternative includes chemical-free coatings based on ultraviolet light-emitting diodes (UV-LED) to prevent foulers settlement, a technology under development by companies from the Netherlands. It is important to note that such alternatives may present other ingredients, such as organotin catalysts, that can cause toxicity towards the marine biota (Chen et al., 2014; Hakan Alyuruk et al., 2010; Manzo et al., 2014). Thus, it is recommended a carefully assessment of the chemical composition and ecotoxicity of their leachates to the seawater over time.

7 .Conclusions

Despite the strict environmental regulation in Europe, it is demonstrated that the 11 approved antifouling biocides still pose risk for the marine environment with different magnitude scales. A significant change of paradigm in the coatings industry is needed towards

the replacement of biocides-containing paints by other types of antifouling systems. However, coatings industry seems to be very conservative, mostly due to economic and technical constraints (e.g. absence of very efficient and long-lasting antifouling non-biocidal coatings). Only a more ambitious legislative framework may lead to a desired green technological revolution to protect immersed human-made structures from biofouling.

Meanwhile, the mitigation of the 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 a 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.

Bioassays and their use in ecotoxicological approaches are crucial to derive hazard for biocides, and more data is needed on different species, from different functional groups. This will enable a more accurate prediction of risk to the marine environment and to protect our oceans, seas and estuaries, one of the Sustainable Development Goals from the United Nations (SDG 14).

8. Acknowledgments

Thanks are due to the financial support to São Paulo Research Foundation (FAPESP) under Grant FAPESP: 2019/19898-3 and FAPESP: 2017/10211-0 and to FCT/MCTES for the financial support to CESAM (UIDP/50017/2020+UIDB/50017/2020), through national funds. Dr. Roberto Martins is funded by national funds (OE), through FCT – Fundação para a Ciência e a Tecnologia, I.P., in the scope of the framework contract foreseen in the numbers 4, 5 and 6 of the article 23, of the Decree-Law 57/2016, of August 29, changed by Law 57/2017, of July 19 (CEECIND/01329/2017). This project was also carried out in the framework of the bilateral project “Exposure and bioaccumulation assessment of anti-fouling nanomaterials in marine organisms from temperate and tropical waters” funded by FCT and CAPES (ref. 4265 DRI/FCT) and the project NANOGREEN (CIRCNA/BRB/0291/2019) funded by national funds (OE), through FCT.

9. References

Abreu, F. E. L., Natália, J., Braga, Í., & Fillmann, G. (2020). *Are antifouling residues a matter of concern in the largest South American port ? 398*(January). https://doi.org/10.1016/j.jhazmat.2020.122937

ACE. (2002). *Assessment of antifouling agents in coastal environments (ACE), Final Scientific and Technical Report. Mas3-Ct98-0178.*

Albanis, T. A., Lambropoulou, D. A., Sakkas, V. A., & Konstantinou, I. K. (2002). Antifouling paint booster biocide contamination in greek marine sediments. *Chemosphere*, *48*(5), 475–485. https://doi.org/10.1016/S0045-6535(02)00134-0

Amara, I., Miled, W., Ben, R., & Ladhari, N. (2018). Antifouling processes and toxicity effects of antifouling

paints on marine environment . A review. *Environmental Toxicology and Pharmacology*, *57*(2018), 115–130. https://doi.org/10.1016/j.etap.2017.12.001

Andersson, M., Nordstierna, L., Bergek, J., Blanck, H., Holmberg, K., & Nydén, M. (2015). Use of microcapsules as controlled release devices for coatings. *Advances in Colloid and Interface Science*, *222*, 18–43. https://doi.org/10.1016/j.cis.2014.06.003

Armbrust, K. L. (2001). Chlorothalonil and chlorpyrifos degradation products in golf course leachate. *Pest Management Science*, *57*(9), 797–802. https://doi.org/10.1002/ps.361

Avelelas, F., Martins, R., Oliveira, T., Maia, F., Malheiro, E., Soares, A. M. V. M., Loureiro, S., & Tedim, J. (2017). Efficacy and Ecotoxicity of Novel Anti-Fouling Nanomaterials in Target and Non-Target Marine Species. *Mar Biotechnol*, *19*(2), 164–174. https://doi.org/10.1007/s10126-017-9740-1

Azemar, F., Fay, F., Réhel, K., & Linossier, I. (2015). *Progress in Organic Coatings Development of hybrid antifouling paints*. *87*, 10–19. https://doi.org/10.1016/j.porgcoat.2015.04.007

Bao, V. W. W., Leung, K. M. Y., Qiu, J.-W., & Lam, M. H. W. (2011). Acute toxicities of five commonly used antifouling booster biocides to selected subtropical and cosmopolitan marine species. *Marine Pollution Bulletin*, *62*(5), 1147–1151. https://doi.org/10.1016/J.MARPOLBUL.2011.02.041

Bellas, J. (2006). Comparative toxicity of alternative antifouling biocides on embryos and larvae of marine invertebrates. *Science of the Total Environment*, *367*, 573–585. https://doi.org/10.1016/j.scitotenv.2006.01.028

Bellas, J., Hilvarsson, A., Birgersson, G., & Granmo, Å. (2006). Effects of medetomidine, a novel antifouling agent, on the burrowing bivalve Abra nitida (Müller). *Chemosphere*, *65*(4), 575–582. https://doi.org/10.1016/j.chemosphere.2006.02.045

Bellas, J., Hilvarsson, A., & Granmo, Å. (2005). Sublethal effects of a new antifouling candidate on lumpfish (Cyclopterus lumpus L.) and Atlantic cod (Gadus morhua L.) larvae. *Biofouling*, *21*(3–4), 207–216. https://doi.org/10.1080/08927010500285236

Bones, J., Thomas, K. V., & Paull, B. (2006). Improved method for the determination of zinc pyrithione in environmental water samples incorporating on-line extraction and preconcentration coupled with liquid chromatography atmospheric pressure chemical ionisation mass spectrometry. *Journal of Chromatography A*, *1132*(1–2), 157–164. https://doi.org/10.1016/j.chroma.2006.07.068

Carbery, K., Owen, R., Frickers, T., Otero, E., & Readman, J. (2006). Contamination of Caribbean coastal waters by the antifouling herbicide Irgarol 1051. *Marine Pollution Bulletin*, *52*(6), 635–644. https://doi.org/10.1016/j.marpolbul.2005.10.013

Caro, A., Humblot, V., Méthivier, C., Minier, M., Barbes, L., Li, J., Salmain, M., & Pradier, C. M. (2010). Bioengineering of stainless steel surface by covalent immobilization of enzymes. Physical characterization and interfacial enzymatic activity. *Journal of Colloid and Interface Science*, *349*(1), 13–18. https://doi.org/10.1016/j.jcis.2009.12.001

Carteau, D., Vallée-Réhel, K., Linossier, I., Quiniou, F., Davy, R., Compère, C., Delbury, M., & Faÿ, F. (2014). Development of environmentally friendly antifouling paints using biodegradable polymer and lower toxic substances. *Progress in Organic Coatings*, *77*(2), 485–493. https://doi.org/10.1016/j.porgcoat.2013.11.012

Chen, L., & Lam, J. C. W. (2017). SeaNine 211 as antifouling biocide: A coastal pollutant of emerging concern. *Journal of Environmental Sciences*, *61*, 68–79. https://doi.org/10.1016/j.jes.2017.03.040

Chen, L., Zhang, H., Sun, J., Wong, Y. H., Han, Z., Au, D. W. T., Bajic, V. B., & Qian, P. Y. (2014). Proteomic changes in brain tissues of marine medaka (Oryzias melastigma) after chronic exposure to two antifouling compounds: Butenolide and 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT). *Aquatic Toxicology*, *157*, 47–56. https://doi.org/10.1016/j.aquatox.2014.09.010

Clare, A. S., Rittschof, D., Gerhart, D. J., & Maki, J. S. (1992). Molecular approaches to nontoxic antifouling. *Invertebrate Reproduction and Development*, *22*(1–3), 67–76. https://doi.org/10.1080/07924259.1992.9672258

Costello, M. J., Tsai, P., Wong, P. S., Cheung, A. K. L., Basher, Z., & Chaudhary, C. (2017). Marine biogeographic realms and species endemicity. *Nature Communications*, *8*(1), 1–9.

https://doi.org/10.1038/s41467-017-01121-2

Dafforn, K. A., Lewis, J. A., & Johnston, E. L. (2011). Antifouling strategies: History and regulation, ecological impacts and mitigation. *Marine Pollution Bulletin*, *62*(3), 453–465. https://doi.org/10.1016/j.marpolbul.2011.01.012

Danish EPA. (2012). *Environmental Project no. 531 2000 Ecotoxicological Assessment of Antifouling Biocides and Nonbiocidal Antifouling Paints*.

Devilla, R., Brown, M., Donkin, M., Tarran, G., Aiken, J., & Readman, J. (2005). Impact of antifouling booster biocides on single microalgal species and on a natural marine phytoplankton community. *Marine Ecology Progress Series*, *286*, 1–12. https://doi.org/10.3354/meps286001

Diniz, L. G. R., Jesus, M. S., Dominguez, L. A. E., Fillmann, G., Vieira, E. M., & Franco, T. C. R. S. (2014). First Appraisal of Water Contamination by Antifouling Booster Biocide of 3rdGeneration at Itaqui Harbor (São Luiz - Maranhão - Brazil). *Journal of the Brazilian Chemical Society*, *25*(2).

Dobretsov, S., & Rittschof, D. (2020). Love at first taste: Induction of larval settlement by marine microbes. *International Journal of Molecular Sciences*, *21*(3). https://doi.org/10.3390/ijms21030731

Dupraz, V., Stachowski-Haberkorn, S., Ménard, D., Limon, G., Akcha, F., Budzinski, H., & Cedergreen, N. (2018). Combined effects of antifouling biocides on the growth of three marine microalgal species. *Chemosphere*, *209*, 801–814. https://doi.org/10.1016/j.chemosphere.2018.06.139

ECB. (2003). *European Commission Bureau.Technical guidance document on risk assessment, part II. European Commission – Joint Research Centre, Institute for Health and Consumer protection, European Chemical Bureau;* (p. 328).

Environmental Protection Agency [EPA]. (1992). *Pesticide Ecotoxicity Database*. https://cfpub.epa.gov/ecotox/search.cf

Environmental Protection Agency [EPA]. (2000). *Pesticide Ecotoxicity Database*. https://cfpub.epa.gov/ecotox/search.cf

Ernst, W., Doe, K., Jonah, P., Young, J., Julien, G., & Hennigar, P. (1991). The toxicity of chlorothalonil to aquatic fauna and the impact of its operational use on a pond ecosystem. *Archives of Environmental Contamination and Toxicology*, *21*(1), 1–9. https://doi.org/10.1007/BF01055550

European Chemical Agency [ECHA]. (2006). *Assessment report, Dichlofluanid, Regulation (EU) n°528/2012 concerning the making available on the market and use of biocidal products, evaluation of active substances*.

European Chemical Agency [ECHA]. (2013). *Assessment report, Zineb, Regulation (EU) n°528/2012 concerning the making available on the market and use of biocidal products, evaluation of active substances.*

European Chemical Agency [ECHA]. (2014a). *Assessment report, 4,5-Dichloro-2-octyl-2H-isothiazol-3-one (DCOIT), Regulation (EU) n°528/2012 concerning the making available on the market and use of biocidal products, evaluation of active substances*.

European Chemical Agency [ECHA]. (2014b). *Assessment report, Tolylfluanid, Regulation (EU) n°528/2012 concerning the making available on the market and use of biocidal products, evaluation of active substances.*

European Chemical Agency [ECHA]. (2015a). *Assessment report, Copper Pyrithione, Regulation (EU) n°528/2012 concerning the making available on the market and use of biocidal products, evaluation of active substances.*

European Chemical Agency [ECHA]. (2015b). *Assessment report, Medetomidine, Regulation (EU) n°528/2012 concerning the making available on the market and use of biocidal products, evaluation of active substances.*

European Chemical Agency [ECHA]. (2016a). *Assessment report, Copper thiocyanate, Regulation (EU) n°528/2012 concerning the making available on the market and use of biocidal products, evaluation of active substances.*

European Chemical Agency [ECHA]. (2016b). *Assessment report, Dicopper oxide, Regulation (EU) n°528/2012 concerning the making available on the market and use of biocidal products, evaluation of active substances.*

Faÿ, F., Horel, G., Linossier, I., & Vallée-réhel, K. (2018). *Effect of biocidal coatings on microfouling: In vitro and in situ results*. *114*(2018), 162–172. https://doi.org/10.1016/j.porgcoat.2017.10.017

Feng, K., Ni, C., Yu, L., Zhou, W., & Li, X. (2019). Synthesis and evaluation of acrylate resins suspending indole derivative structure in the side chain for marine antifouling. *Colloids and Surfaces B: Biointerfaces*, *184*, 110518. https://doi.org/10.1016/j.colsurfb.2019.110518

Fernandes, J. A., Santos, L., Vance, T., Fileman, T., Smith, D., Bishop, J. D. D., Viard, F., Queirós, A. M., Merino, G., Buisman, E., & Austen, M. C. (2016). Costs and benefits to European shipping of ballast-water and hull-fouling treatment: Impacts of native and non-indigenous species. *Marine Policy*, *64*, 148–155. https://doi.org/10.1016/j.marpol.2015.11.015

Figueiredo, J., Loureiro, S., & Martins, R. (2020). Hazard of novel anti-fouling nanomaterials and the biocides DCOIT and silver to marine organisms. *Environmental Science: Nano*. https://doi.org/10.1039/d0en00023j

Figueiredo, J., Oliveira, T., Ferreira, V., Sushkova, A., Silva, S., Carneiro, D., Cardoso, D. N., Gonçalves, S. F., Maia, F., Rocha, C., Tedim, J., Loureiro, S., & Martins, R. (2019). Toxicity of innovative anti-fouling nano-based solutions in marine species. *Environmental Science Nano*, *5*. https://doi.org/10.1039/C9EN00011A

Giacomazzi, S., & Cochet, N. (2004). Environmental impact of diuron transformation: A review. In *Chemosphere* (Vol. 56, Issue 11, pp. 1021–1032). Elsevier Ltd. https://doi.org/10.1016/j.chemosphere.2004.04.061

Gittens, J. E., Smith, T. J., Suleiman, R., & Akid, R. (2013). Current and emerging environmentally-friendly systems for fouling control in the marine environment. *Biotechnology Advances*, *31*(8), 1738–1753. https://doi.org/10.1016/j.biotechadv.2013.09.002

Gonzalez-Rey, M., Tapie, N., Le Menach, K., Dévier, M. H., Budzinski, H., & Bebianno, M. J. (2015). Occurrence of pharmaceutical compounds and pesticides in aquatic systems. *Marine Pollution Bulletin*, *96*(1–2), 384–400. https://doi.org/10.1016/j.marpolbul.2015.04.029

Gredelj, A., Barausse, A., Grechi, L., & Palmeri, L. (2018). Deriving predicted no-effect concentrations (PNECs) for emerging contaminants in the river Po, Italy, using three approaches: Assessment factor, species sensitivity distribution and AQUATOX ecosystem modelling. *Environment International*, *119*, 66–78. https://doi.org/10.1016/j.envint.2018.06.017

Grosicka-Maciag, E., Szumiło, M., Czeczot, H., Kurpios-Piec, D., Skrzycki, M., & Rahden-Staroń, I. (2013). Modulation of antioxidant defense system by the dithiocarbamate fungicides Maneb and Zineb in Chinese hamster V79 cells and the role of N-acetyl-L-cysteine. *Food and Chemical Toxicology*, *60*, 130–134. https://doi.org/10.1016/j.fct.2013.07.034

Guo, Q., Wei, D., Zhao, H., & Du, Y. (2020). Predicted no-effect concentrations determination and ecological risk assessment for benzophenone-type UV filters in aquatic environment. *Environmental Pollution*, *256*, 113460. https://doi.org/10.1016/j.envpol.2019.113460

Gutner-Hoch, E., Id, R. M., Oliveira, T., Maia, F., Soares, A. M. V. M., Id, S. L., Piller, C., Preiss, I., Weis, M., Larroze, S. B., Teixeira, T., & Benayahu, Y. (2018). *Antimacrofouling Efficacy of Innovative Inorganic Nanomaterials Loaded with Booster Biocides*. 1–12. https://doi.org/10.3390/jmse6010006

Gutner-Hoch, E., Martins, R., Maia, F., Oliveira, T., Shpigel, M., Weis, M., Tedim, J., & Benayahu, Y. (2019). Toxicity of engineered micro- and nanomaterials with antifouling properties to the brine shrimp Artemia salina and embryonic stages of the sea urchin Paracentrotus lividus. *Environmental Pollution*, *251*, 530–537. https://doi.org/10.1016/j.envpol.2019.05.031

Hagen, T., Allinson, G., Wightwick, A., Salzman, S. A., & Nugegoda, D. (2010). Utilization of a new bdelloid rotifer (Philodina acuticornis odiosa) assay to evaluate the effect of salinity on the toxicity of chlorothalonil. *Toxicological & Environmental Chemistry*, *92*(4), 743–748. https://doi.org/10.1080/02772240903074619

Hakan Alyuruk, Elmas Doner, Zeynelabidin Karabay, & Levent Cavas. (2010). Antifouling Performances and Acute Toxicities of Eco-Friendly Biocides. *HACETTEPE JOURNAL OF BIOLOGY AND CHEMISTRY*, *38*(4), 277–286.

Harino, H., Mori, Y., Yamaguchi, Y., Shibata, K., & Senda, T. (2005). Monitoring of antifouling booster biocides in water and sediment from the port of Osaka, Japan. *Archives of Environmental Contamination and Toxicology*, *48*(3), 303–310. https://doi.org/10.1007/s00244-004-0084-2

Harino, H., Yamamoto, Y., Eguchi, S., Kawai, S., Kurokawa, Y., Arai, T., Ohji, M., Okamura, H., & Miyazaki, N. (2007). Concentrations of antifouling biocides in sediment and mussel samples collected from Otsuchi Bay, Japan. *Archives of Environmental Contamination and Toxicology*, *52*(2), 179–188. https://doi.org/10.1007/s00244-006-0087-2

Hilvarsson, A., Halldórsson, H. P., & Granmo, Å. (2007). Medetomidine as a candidate antifoulant: Sublethal effects on juvenile turbot (Psetta maxima L.). *Aquatic Toxicology*, *83*(3), 238–246. https://doi.org/10.1016/j.aquatox.2007.04.008

Hilvarsson, A., Ohlauson, C., Blanck, H., & Granmo, Å. (2009). Bioaccumulation of the new antifoulant medetomidine in marine organisms. *Marine Environmental Research*, *68*(1), 19–24. https://doi.org/10.1016/j.marenvres.2009.03.007

International Marine Organization. (2001). *International Convention on the Control of Harmful Antifouling Systems on Ships*.

Jacobson, A. H., & Willingham, G. L. (2000). Sea-nine antifoulant: An environmentally acceptable alternative to organotin antifoulants. *Science of the Total Environment*, *258*(1–2), 103–110. https://doi.org/10.1016/S0048-9697(00)00511-8

Jung, S. M., Bae, J. S., Kang, S. G., Son, J. S., Jeon, J. H., Lee, H. J., Jeon, J. Y., Sidharthan, M., Ryu, S. ., & Shin, H. W. (2017). Acute toxicity of organic antifouling biocides to phytoplankton Nitzschia pungens and zooplankton Artemia larvae. *Marine Pollution Bulletin*. https://doi.org/10.1016/j.marpolbul.2016.11.047

Katja, G., & Dahllo, I. (2005). ENVIRONMENTAL FATE OF THE ANTIFOULING COMPOUND ZINC PYRITHIONE IN SEAWATER. *Environmental Toxicology and Chemistry*, *24*(12), 3001–3006.

Kim, N. S., Hong, S. H., An, J. G., Shin, K. H., & Shim, W. J. (2015). Distribution of butyltins and alternative antifouling biocides in sediments from shipping and shipbuilding areas in South Korea. *Marine Pollution Bulletin*, *95*(1), 484–490. https://doi.org/10.1016/j.marpolbul.2015.03.010

Lagerström, M., Yngsell, D., Eklund, B., & Ytreberg, E. (2018). *Identification of commercial and recreational vessels coated with banned organotin paint through screening of tin by portable XRF*. https://doi.org/10.1016/j.jhazmat.2018.09.038

Laisi, A., Tuominen, R., Männistö, P., Savolainen, K., & Mattila, J. (1985). The effect of maneb, zineb, and ethylenethiourea on the humoral activity of the pituitary-thyroid axis in rat. *Archives of Toxicology. Supplement. Archiv Fur Toxikologie. Supplement*, *8*, 253–258. https://doi.org/10.1007/978-3-642-69928-3_37

Lam, N. H., Jeong, H. ho, Kang, S. dong, Kim, D. J., Ju, M. J., Horiguchi, T., & Cho, H. S. (2017). Organotins and new antifouling biocides in water and sediments from three Korean Special Management Sea Areas following ten years of tributyltin regulation: Contamination profiles and risk assessment. *Marine Pollution Bulletin*, *121*(1–2), 302–312. https://doi.org/10.1016/j.marpolbul.2017.06.026

Lambropoulou, D. A., Konstantinou, I. K., & Albanis, T. A. (2000). Determination of fungicides in natural waters using solid-phase microextraction and gas chromatography coupled with electron-capture and mass spectrometric detection. *Journal of Chromatography A*, *893*(1), 143–156. https://doi.org/10.1016/S0021-9673(00)00750-0

Lee, M. R. N., Kim, U. J., Lee, I. S., Choi, M., & Oh, J. E. (2015). Assessment of organotin and tin-free antifouling paints contamination in the Korean coastal area. *Marine Pollution Bulletin*, *99*(1–2), 157–165. https://doi.org/10.1016/j.marpolbul.2015.07.038

Lee, S. L. Y. (2017). Determination of five alternative antifouling agents in Korean marine sediments. *Environmental Earth Sciences*, *76*(17), 1–9. https://doi.org/10.1007/s12665-017-6954-5

Lee, S., & Lee, Y. W. (2017). Determination of five alternative antifouling agents in Korean marine sediments. *Environmental Earth Sciences*, *76*(17), 615. https://doi.org/10.1007/s12665-017-6954-5

Lennquist, A., Mårtensson Lindblad, L. G. E., Hedberg, D., Kristiansson, E., & Förlin, L. (2010). Colour and melanophore function in rainbow trout after long term exposure to the new antifoulant medetomidine. *Chemosphere*, *80*(9), 1050–1055. https://doi.org/10.1016/j.chemosphere.2010.05.014

Lepak, R. F., Yin, R., Krabbenhoft, D. P., Ogorek, J. M., Dewild, J. F., Holsen, T. M., & Hurley, J. P. (2015). Use of Stable Isotope Signatures to Determine Mercury Sources in the Great Lakes. *Environmental Science and Technology Letters*, *2*(12), 335–341. https://doi.org/10.1021/acs.estlett.5b00277

Lind, U., Rosenblad, M. A., Frank, L. H., Falkbring, S., Brive, L., Laurila, J. M., Pohjanoksa, K., Vuorenpää, A., Kukkonen, J. P., Gunnarsson, L., Scheinin, M., Mårtensson Lindblad, L. G. E., & Blomberg, A. (2010). Octopamine receptors from the barnacle Balanus improvisus are activated by the α2-adrenoceptor agonist medetomidine. *Molecular Pharmacology*, *78*(2), 237–248. https://doi.org/10.1124/mol.110.063594

MacKie, D. S., Van Den Berg, C. M. G., & Readman, J. W. (2004). Determination of pyrithione in natural waters by cathodic stripping voltammetry. *Analytica Chimica Acta*, *511*(1), 47–53. https://doi.org/10.1016/j.aca.2004.01.033

Maia, F., Silva, A. P., Fernandes, S., Cunha, A., Almeida, A., Tedim, J., Zheludkevich, M. L., & Ferreira, M. G. S. (2015). Incorporation of biocides in nanocapsules for protective coatings used in maritime applications. *CHEMICAL ENGINEERING JOURNAL*, *270*, 150–157. https://doi.org/10.1016/j.cej.2015.01.076

Maki, J. S., & Mitchell, R. (2003). Biofouling in the Marine Environment. *Encyclopedia of Environmental Microbiology*. https://doi.org/doi:10.1002/0471263397.env275

Manzo, S., Ansanelli, G., Parrella, L., Di Landa, G., Massanisso, P., Schiavo, S., Minopoli, C., Lanza, B., Boggia, R., Aleksi, P., & Tabaku, A. (2014). First evaluation of the threat posed by antifouling biocides in the Southern Adriatic Sea. *Environmental Sciences: Processes and Impacts*, *16*(8), 1981–1993. https://doi.org/10.1039/c3em00724c

Martínez, K., & Barceló, D. (2001). Determination of antifouling pesticides and their degradation products in marine sediments by means of ultrasonic extraction and HPLC-APCI-MS. *Fresenius' Journal of Analytical Chemistry*, *370*(7), 940–945. https://doi.org/10.1007/s002160100904

Martínez, K., Ferrer, I., Hernando, M. D., Fernández-Alba, A. R., Marcé, R. M., Borrull, F., & Barceló, D. (2001). Occurrence of antifouling biocides in the spanish mediterranean marine environment. *Environmental Technology (United Kingdom)*, *22*(5), 543–552. https://doi.org/10.1080/09593332208618258

Martins, S. E., Fillmanna, G., Lillicrapb, A., & Thomasb, K. V. (2018). Review: ecotoxicity of organic and organo-metallic antifouling co-biocides and implications for environmental hazard and risk assessments in aquatic ecosystems. *Biofouling*, *7014*, 1–19. https://doi.org/10.1080/08927014.2017.1404036

Mensah, P. K., Palmer, C. G., & Muller, W. J. (2013). Derivation of south african water quality guidelines for roundup® using species sensitivity distribution. *Ecotoxicology and Environmental Safety*, *96*, 24–31. https://doi.org/10.1016/j.ecoenv.2013.06.009

Mijangos, L., Ziarrusta, H., Ros, O., Kortazar, L., Fernández, L. A., Olivares, M., Zuloaga, O., Prieto, A., & Etxebarria, N. (2018). Occurrence of emerging pollutants in estuaries of the Basque Country: Analysis of sources and distribution, and assessment of the environmental risk. *Water Research*, *147*, 152–163. https://doi.org/10.1016/j.watres.2018.09.033

Mohamadjafari, S., & Rastegarzadeh, S. (2017). A sensing colorimetric method based on in situ formation of gold nanoparticles after dispersive liquid-liquid microextraction for determination of zineb. *Microchemical Journal*, *132*, 154–160. https://doi.org/10.1016/j.microc.2017.01.021

Mukherjee, A., Mohan Rao, K. V., & Ramesh, U. S. (2009). Predicted concentrations of biocides from antifouling paints in Visakhapatnam Harbour. *Journal of Environmental Management*, *90*(SUPPL. 1), S51–S59. https://doi.org/10.1016/j.jenvman.2008.07.018

Mukhtar, A., Mohamat-Yusuff, F., Zulkifli, S., Harino, H., Ismail, A., & Inoue, K. (2019). Concentration of Organotin and Booster Biocides in Sediments of Seagrass Area from Sungai Pulai Estuary, South of Johor,

Malaysia. *Environments*, *6*(2), 26. https://doi.org/10.3390/environments6020026

Myers, J. H., Gunthorpe, L., Allinson, G., & Duda, S. (2006). Effects of antifouling biocides to the germination and growth of the marine macroalga, Hormosira banksii (Turner) Desicaine. *Marine Pollution Bulletin*, *52*(9), 1048–1055. https://doi.org/10.1016/j.marpolbul.2006.01.010

National Center for Biotechnology Information. (2021). *PubChem Annotation Record for Zineb, Source: Hazardous Substances Data Bank (HSDB). Retrieved February 25, 2021*.

New Zealand EPA. (2012). *Antifouling paints reassessment. Preliminary Risk Assessment. New Zealand Government.*

Ohlauson, C., Eriksson, K. M., & Blanck, H. (2012). Short-term effects of medetomidine on photosynthesis and protein synthesis in periphyton, epipsammon and plankton communities in relation to predicted environmental concentrations. *Biofouling*, *28*(5), 491–499. https://doi.org/10.1080/08927014.2012.687048

Omae, I. (2003). Organotin antifouling paints and their alternatives. *Applied Organometallic Chemistry*, *17*(2), 81–105. https://doi.org/10.1002/aoc.396

Omae, I. (2006). General Aspects of Natural Products Antifoulants in the Environment. *Antifouling Paint Biocides*, 227–262. https://doi.org/10.1007/698_5_057

Onduka, T., Kakuno, A., Kono, K., Ito, K., Mochida, K., & Fujii, K. (2012). Toxicity of chlorothalonil to marine organisms. *Fisheries Science*, *78*(6), 1301–1308. https://doi.org/10.1007/s12562-012-0562-9

Onduka, T., Ojima, D., Ito, M., Ito, K., Mochida, K., & Fujii, K. (2013). Toxicity of the antifouling biocide Sea-Nine 211 to marine algae, crustacea, and a polychaete. *Fisheries Science*, *79*(6), 999–1006. https://doi.org/10.1007/s12562-013-0678-6

Pinteus, S., Lemos, M. F. L., Freitas, R., Duarte, I. M., Alves, C., Silva, J., Marques, S. C., & Pedrosa, R. (2020). Medusa polyps adherence inhibition: A novel experimental model for antifouling assays. *Science of the Total Environment*, *715*, 136796. https://doi.org/10.1016/j.scitotenv.2020.136796

Reading, F. (2005). *Antagonistic and Synergistic Effects of Antifouling Chemicals in Mixture*. 194–203.

Readman, J. W. (2006). Development, Occurrence and Regulationof Antifouling Paint Biocides: Historical Review and Future Trends. *Antifouling Paint Biocides*, 1–15. https://doi.org/10.1007/698_5_047

Reemtsma, T., Alder, L., & Banasiak, U. (2013). Emerging pesticide metabolites in groundwater and surface water as determined by the application of a multimethod for 150 pesticide metabolites. *Water Research*, *47*(15), 5535–5545. https://doi.org/10.1016/j.watres.2013.06.031

Regulation (EU) No 2015/419. (n.d.). *Regulation EU No 2015/419 of 12 March 2015 approving tolylfluanid as an active substance for use in biocidal products for product-type 21 Text with EEA relevance. Council Directive (L68, 13.03.2015, p. 39).*

Regulation (EU) No 92/2014. (n.d.). *Regulation (EU) No 92/2014 of 31 January 2014 approving zineb as an existing active substance for use in biocidal products for product-type 21*.

Rittschof, D. (2000). Natural product antifoulants: One perspective on the challenges related to coatings development. *Biofouling*, *15*(1–3), 119–127. https://doi.org/10.1080/08927010009386303

Ruggiero, L., Crociani, L., Zendri, E., El Habra, N., & Guerriero, P. (2018). Incorporation of the zosteric sodium salt in silica nanocapsules: synthesis and characterization of new fillers for antifouling coatings. *Applied Surface Science*, *439*, 705–711. https://doi.org/10.1016/j.apsusc.2017.12.228

Sakkas, V. A., Konstantinou, I. K., & Albanis, T. A. (2002). Aquatic phototransformation study of the antifouling agent Sea-Nine 211: Identification of byproducts and the reaction pathway by gas chromatography-mass spectroscopy. *Journal of Chromatography A*, *959*(1–2), 215–227. https://doi.org/10.1016/S0021-9673(02)00430-2

Sakkas, V. A., Konstantinou, I. K., Lambropoulou, D. A., & Albanis, T. A. (2002). Survey for the occurrence of antifouling paint booster biocides in the aquatic environment of Greece. *Environmental Science and Pollution Research International*, *9*(5), 327–332. https://doi.org/10.1007/BF02987576

Sakkas, V. A., Lambropoulou, D. A., & Albanis, T. A. (2002). Study of chlorothalonil photodegradation in

natural waters and in the presence of humic substances. *Chemosphere*, *48*(9), 939–945. https://doi.org/10.1016/S0045-6535(02)00121-2

Silkina, A., Bazes, A., Mouget, J. L., & Bourgougnon, N. (2012). Comparative efficiency of macroalgal extracts and booster biocides as antifouling agents to control growth of three diatom species. *Marine Pollution Bulletin*, *64*(10), 2039–2046. https://doi.org/10.1016/j.marpolbul.2012.06.028

Soon, Z. Y., Jung, J., Jang, M., Kang, J., Jang, M., Lee, J., & Kim, M. (2019). Zinc Pyrithione (ZnPT) as an Antifouling Biocide in the Marine Environment — a Literature Review of Its Toxicity , Environmental Fates , and Analytical Methods. *Water Air Soil Pollution*, *230*(12), 1–18. https://doi.org/10.1007/s11270-019-4361-0

Sorgog, K., & Kamo, M. (2019). Quantifying the precision of ecological risk: Conventional assessment factor method vs. species sensitivity distribution method. *Ecotoxicology and Environmental Safety*, *183*(May), 109494. https://doi.org/10.1016/j.ecoenv.2019.109494

Staples, C. A., Woodburn, K. B., Klecka, G. M., Mihaich, E. M., Hall, A. T., Ortego, L., Caspers, N., & Hentges, S. G. (2008). Comparison of four species sensitivity distribution methods to calculate predicted no effect concentrations for bisphenol A. *Human and Ecological Risk Assessment*, *14*(3), 455–478. https://doi.org/10.1080/10807030802074170

Stauber, J. L., Binet, M. T., Bao, V. W. W., Boge, J., Zhang, A. Q., Leung, K. M. Y., & Adams, M. S. (2008). Comparison of the Qwiklite™ algal bioluminescence test with marine algal growth rate inhibition bioassays. *Environmental Toxicology*, *23*(5), 617–625. https://doi.org/10.1002/tox.20400

TGD. (2003). *Technical Guidance Document on Risk Assessment in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances, Commission Regulation (EC) No 1488/94*.

Thomas, Kv. (2001). The environmental fate and behaviour of antifouling paint booster biocides: A review. *Biofouling*, *17*(1), 73–86. https://doi.org/10.1080/08927010109378466

Thomas, K. V., & Brooks, S. (2010). The environmental fate and effects of antifouling paint biocides. *Biofouling*, *26*(1), 73–88. https://doi.org/10.1080/08927010903216564

Thomas, Kevin V., Fileman, T. W., Readman, J. W., & Waldock, M. J. (2001). Antifouling paint booster biocides in the UK coastal environment and potential risks of biological effects. *Marine Pollution Bulletin*, *42*(8), 677–688. https://doi.org/10.1016/S0025-326X(00)00216-2

Thomas, Kevin V. (1999). Determination of the antifouling agent zinc pyrithione in water samples by copper chelate formation and high-performance liquid chromatography-atmospheric pressure chemical ionisation mass spectrometry. *Journal of Chromatography A*, *833*(1), 105–109. https://doi.org/10.1016/S0021-9673(98)01009-7

Van Scoy, A. R., & Tjeerdema, R. S. (2014). Environmental fate and toxicology of chlorothalonil. *Reviews of Environmental Contamination and Toxicology*, *232*, 89–105. https://doi.org/10.1007/978-3-319-06746-9_4

Verity, P., & Smetacek, V. (1996). Organism life cycles, predation, and the structure of marine pelagic ecosystems. *Marine Ecology Progress Series*, *130*(1–3), 277–293. https://doi.org/10.3354/meps130277

Viana, J. L. M., dos Santos Diniz, M., dos Santos, S. R. V., Verbinnen, R. T., Almeida, M. A. P., & dos Santos Franco, T. C. R. (2020). Antifouling substances as a continuous threat to the aquatic environment: Sources, temporal trends and ecological risk assessment in an impacted region of Brazil. *Science of The Total Environment*, *730*, 139026. https://doi.org/10.1016/j.scitotenv.2020.139026

Voulvoulis, N. (2006). Antifouling Paint Booster Biocides: Occurrence and Partitioning in Waterand Sediments. *Antifouling Paint Biocides*, 155–170. https://doi.org/10.1007/698_5_053

Voulvoulis, N., Scrimshaw, M. D., & Lester, J. N. (2000). Occurrence of four biocides utilized in antifouling paints, as alternatives to organotin compounds, in waters and sediments of a commercial estuary in the UK. *Marine Pollution Bulletin*, *40*(11), 938–946. https://doi.org/10.1016/S0025-326X(00)00034-5

Wahl, M. (1989). Marine epibiosis. I. Fouling and antifouling: some basic aspects Martin. *Marine Ecology Progress Series*, *58*, 175–189. https://doi.org/10.4028/www.scientific.net/AMR.178.344

Wang, X., Wang, X., Dong, M., Li, Z., Liu, Z., Lu, J., Lin, Q., & Yang, J. (2020). Synthesis and biological

activities of 1H-indole-1-carboxylic acid aryl esters as a marine antifouling coating. *Journal of Coatings Technology and Research*, *17*(2), 553–561. https://doi.org/10.1007/s11998-019-00305-3

Wendt, I., Backhaus, T., Blanck, H., & Arrhenius, Å. (2016). The toxicity of the three antifouling biocides DCOIT, TPBP and medetomidine to the marine pelagic copepod Acartia tonsa. *Ecotoxicology*, *25*(5), 871–879. https://doi.org/10.1007/s10646-016-1644-8

Wezel, A. P. Van, & Vlaardingen, P. Van. (2004). *Environmental risk limits for antifouling substances*. *66*, 427–444. https://doi.org/10.1016/j.aquatox.2003.11.003

Yamada, H. (2007). *Behaviour, Occurrence, and Aquatic Toxicity of New Antifouling Biocides and Preliminary Assessment of Risk to Aquatic Ecosystems*. Bulletin of Fisheries Research Agency. https://pdfs.semanticscholar.org/6f07/7705084e8240b83a8c7a4e147b07c4bef380.pdf?_ga=2.25401982.217765747.1591648473-948558388.1591648475

Yamaguchi, Y., Kumakura, A., Sugasawa, S., Harino, H., Yamada, Y., Shibata, K., & Senda, T. (2006). Direct analysis of zinc pyrithione using LC-MS. *International Journal of Environmental Analytical Chemistry*, *86*(1–2), 83–89. https://doi.org/10.1080/03067310500249930

Yang, C., Sun, W., Liu, S., & Xia, C. (2015). Comparative effects of indole derivatives as antifouling agents on the growth of two marine diatom species. *Chemistry and Ecology*, *31*(4), 299–307. https://doi.org/10.1080/02757540.2015.1022536

Yebra, D. M., Kiil, S., & Dam-Johansen, K. (2004). Antifouling technology—past, present and future steps towards efficient and environmentally friendly antifouling coatings. *Progress in Organic Coatings*, *50*(2), 75–104. https://doi.org/10.1016/J.PORGCOAT.2003.06.001

Supplementary Material

Table S1: MECs concentrations of antifouling biocides for marine environments in water and sediment.

Biocide	Matrix	Country	Site	Date	Concentration	Reference	DOI
DCOIT	Water	Korea	Jinhae Bay	2009-2010	6 ng/L	Kim et al. (2014)	https://doi.org/10.1016/j.marpolbul.2013.10.043
	Sed.	Korea	Coasts and Harbors	2010-2011	nd - 281 ng/g	Lee et al. (2015)	https://doi.org/10.1016/j.marpolbul.2015.07.038
	Water	Japan	Osaka Port	2003	<0.03 - 0.55 ng/L	Harino et al. (2005)	http://dx.doi.org/10.1017/S0025315405010799h
	Sed.	Japan	Osaka Port	2004	<0.04 -110 ng/g	Harino et al. (2010)	https://link.springer.com/article/10.1007%2Fs00244-006-0087-2
	Water	Japan	Hiroshima Bay	2010	0.1 - 11 ng/L	mochida et al. (2015)	https://doi.org/10.1016/j.envpol.2015.05.012
	Sed.	Japan	Hiroshima Bay	2010	<1 - 55 ng/g	mochida et al. (2015)	https://doi.org/10.1016/j.envpol.2015.05.012
	Sed.	Japan	Otsuchi Bay	205	<0.04 - 150ng/g	Harino et al. (2006)	https://doi.org/10.1007/s00244-006-0087-2
	Water	Spain	Ports and marinas (catalonia)	1999	2700-3300 ng/L	Martínez et al. (2000)	https://doi.org/10.1016/S0021-9673(00)00307-1
	Water	Spain	Ports and marinas (catalonia)	1999	2600-3700 ng/L	Martínez et al. (2001)	https://doi.org/10.1080/09593332208618258
	Sed.	Spain	Ports and marinas (catalonia)	2000	<0.4 - 4 ng/g	Martínez & Barceló (2001)	https://doi.org/10.1007/s002160100904
	Water	Sweeden	n.a.	2005	<1-3 ng/L	Readman (2005)	https://doi.org/10.1007/698_5_047
	Water	Greece	Patras marina	2000	6.3 - 49 ng/L	Sakkas et al. (2002)	https://doi.org/10.1007/BF02987576
	Water	United Kingdown	Southampton	2000	<1 ng/L	Thomas et al. (2002)	https://doi.org/10.1016/S0048-9697(01)01153-6
	Water	Denmark	Harbors	1998	<5 - 283 ng/L	Steen et al. (2004)	https://doi.org/10.1016/j.chemosphere.2004.06.043
	Sed.	Denmark	Harbors	1998	<20 ng/g	Steen et al. (2004)	https://doi.org/10.1016/j.chemosphere.2004.06.043
	Sed.	Brazil	Santos Bay	2020	63.7 ng/g	Abreu et al. (2020)	https://doi.org/10.1016/j.jhazmat.2020.122937
	Sed.	Malaysia	Coast	2006	<0.04 -4.2ng/g	Harino et al. (2009)	https://doi.org/10.1007/s00244-008-9252-0
	Sed.	Thailand	Coast	2004	<0.04 - 0.09ng/g	Harino et al. (2006)	https://doi.org/10.1007/s00244-005-0246-x
	Sed.	Indonesia	Coast	2004	<0.4 - 150ng/g	Harino et al. (2012)	https://doi.org/10.1007/s00244-011-9747-y
	Sed.	Viatnam	Coast	2002	0.09 - 1.3 ng/g	Harino et al. (2006)	https://doi.org/10.1017/S0025315406014147
	Water	Korea	Coast	2017	<0.16 - 2.44 ng/L	Lam et al. (2017)	https://doi.org/10.1016/j.marpolbul.2017.06.026
	Sed.	Korea	Bays and harbors	2017	<0.06 - 117 ng/g	Lam et al. (2017)	https://doi.org/10.1016/j.marpolbul.2017.06.026
	Sed.	Panama	Coast	2016	<0.38 - 123.4 ng/g	Batista-Andrade et al. (2018)	https://doi.org/10.1016/j.envpol.2017.11.063
	Sed.	Korea	Bays and harbors	2009-2010	nd - 5.5 ng/g	kim et al. (2015)	https://doi.org/10.1016/j.marpolbul.2015.03.010
	Sed.	Malaysia	Sungai Pulai Estuary	n.a.	9.1 - 170 ng/g	Mukhtar et al. (2019)	https://doi.org/10.3390/environments6020026
	Sed.	Korea	Coast	2013	nd - 271.4 ng/g	Lee & Lee (2017)	https://doi.org/10.1007/s12665-017-6954-5
Diuron	Water	Korea	Jinhae Bay	2009-2010	35 - 1360 ng/L	Kim et al. (2014)	https://doi.org/10.1016/j.marpolbul.2013.10.043

Biocide	Matrix	Country	Site	Date	Concentration	Reference	DOI
	Sed.	Brazil	São Marcos Bay	2019	<5.0–55.2 ng/g	Viana et al. (2019)	https://doi.org/10.1016/j.envpol.2019.112988
	water	Korea	Harbors and costal areas	2017	<0.31 - 41.9 ng/L	Lam et al. (2017)	https://doi.org/10.1016/j.marpolbul.2017.06.026
	Sed.	Korea	Harbors and costal areas	2017	<0.06 - 144 ng/g	Lam et al. (2017)	https://doi.org/10.1016/j.marpolbul.2017.06.026
	Sed.	Panama	Harbors and costal areas	2016	<0.75 - 15.4 ng/g	Batista-Andrade et al. (2018)	https://doi.org/10.1016/j.envpol.2017.11.063
	Water	Japan	Harbors and costal areas	2012-2014	31 ± 13 (54) ng/L	Kaonga et al. (2016)	https://doi.org/10.1016/j.chemosphere.2015.11.100
	Sed.	Japan	Harbors and costal areas	2012-2014	897 ± 215 (1280) ng/L	Kaonga et al. (2016)	https://doi.org/10.1016/j.chemosphere.2015.11.100
	Sed.	Korea	Bays and harbors	2009-2010	9 - 62.3 ng/g	kim et al. (2015)	https://doi.org/10.1016/j.marpolbul.2015.03.010
	Sed.	USA	Marinas, Southern California	2008	<0.3–4.2 ng/g	Sapozhnikova et al. (2013)	https://doi.org/10.1016/j.marpolbul.2013.01.039
	Water	Malaysia	Peninsular Malaysia (harbors and coastal areas)	2012	6.26 - 97.8 ng/L	Ali et al. (2014)	https://doi.org/10.1016/j.marpolbul.2014.05.049
	Water	Spain	Ports and marinas (catalonia)	2000	2 - 2190 ng/L	Martinez et al. (2001)	https://doi.org/10.1080/09593332208618258
	Water	UK	Ports and marinas	1999	10.5 - 305.2 ng/L	Boxall et al. (2000)	https://doi.org/10.1016/S0025-326X(00)00021-7
	Sed.	Japan	Otsuchi Bay	2005	0.08 - 530 ng/g	Harino et al. (2007)	https://doi.org/10.1007/s00244-006-0087-2
	Sed.	Brazil	Santos Bay	2020	9.9 ng/g	Abreu et al. (2020)	https://doi.org/10.1016/j.jhazmat.2020.122937
	Water	Brazil	Port of Itaqui	2011	<6 - 7800ng/L	Diniz et al. (2014)	http://dx.doi.org/10.5935/0103-5053.20130289
	Sed.	Malaysia	Sungai Pulai Estuary	n.a.	<0.1 - 22.9 ng/g	Mukhtar et al. (2019)	https://doi.org/10.3390/environments6020026
	Water	Italy	Gulf of Napoli	2004	6.2 - 475 ng/L	Landa et al. (2006)	https://doi.org/10.1016/j.marpolbul.2006.05.027
	Water	Italy	Gulf of Napoli	2005	1.6 - 34.8 ng/L	Ansanelli et al. (2017)	https://doi.org/10.1016/j.rsma.2017.09.011
	Water	Italy	Gulf of La Spezia	2005	0.2 - 9.7 ng/L	Ansanelli et al. (2017)	https://doi.org/10.1016/j.rsma.2017.09.011
	Water	Korea	South-East Korea	2014-2015	<0.31 - 96.2 ng/L	Lam et al. (2017)	https://doi.org/10.1016/j.marpolbul.2017.06.026
	Water	Irã	Bushehr, Northern Persian Gulf	2013	<4.8-29.1 ± 3 ng/L	Saleh et al. (2014)	https://doi.org/10.1016/j.chroma.2014.06.057
	Water	Australia	Perth coastal waters	2008	20–2160 ng/L	Reitsema (2008)	https://www.water.wa.gov.au/__data/assets/pdf_file/0005/5477/84012.pdf
	Water	Australia	Sydney estuary water	2013	15.1 - 96.7 ng/L	Birch et al. (2015)	https://doi.org/10.1016/j.marpolbul.2015.06.038
	Water	Netherlands	n.a.	2000	90-1130 ng/L	Lamoree et al. (2002)	https://doi.org/10.1016/s0021-9673(02)00878-6
	Water	UK	Shoreham Harbour, Brighton Marina,	2003-2004	<7-366 ng/L	Gatidou et al. (2007)	https://doi.org/10.1016/j.envint.2006.07.002
	Water	France	NW Atlantic French Coast	2006-2007	<50 - 268 ng;/L	Caquet et al. (2013)	https://doi.org/10.1007/s11356-012-1171-y
	Water	Belgium	n.a.	2010	up to 263 ng/L	Wille et al. (2011)	https://doi.org/10.1016/j.chroma.2011.10.039
	Water	Albania	Southern Adriatic Sea	2012	1.9–93.9	Manzo et al. (2014)	https://doi.org/10.1039/C3EM00724C
	Water	Italy	Tyrrhenian coast	2010	<1.0 - 34.8 ng/L	Ansanelli et al. (2017)	https://doi.org/10.1039/C3EM00724C
	Sed.	Spain	Canary Islands, Spain	2008-2009	2.3 - 2003 ng/L	Sanchez-Rodrıguez et al. (2011)	https://doi.org/10.1016/j.chemosphere.2010.09.064

Biocide	Matrix	Country	Site	Date	Concentration	Reference	DOI
ZnPT	Water	UK	Southern England - Marinas	1998	<20 ng/l	Thomas (1999)	https://doi.org/10.1016/S0021-9673(98)01009-7
ZnPT	Water	UK	Mersey Estuary	2002	0.0334± 0.001ng/l	Mackie et al. (2004)	https://doi.org/10.1016/j.aca.2004.01.033
CuSCN	Water	Germany	n.a.	n.a.	<10 ng/l	Daehne et al.(2017)	https://doi.org/10.1002/ieam.1896
Tolylfluanid	Water	Germany	n.a.	n.a.	<1000 ng/L	Daehne et al.(2017)	https://doi.org/10.1002/ieam.1896
Dichlofluanid	Water	Spain	Ports and marinas (catalonia)	2000	2600 - 3370	Martinez et al. (2001)	https://doi.org/10.1080/09593332208618258
	Sed.	Greece	Ports and marinas	2000	nd - 195	Albanis et al (2002)	https://doi.org/10.1016/S0045-6535(02)00134-0
	Sed.	Japan	Otsuchi Bay	2005	<0.4 - 14 ng/g	Harino et al. (2007)	https://doi.org/10.1007/s00244-006-0087-2
	Sed.	Malaysia	Sungai Pulai Estuary	n.a.	48.7 - 800 ng/g	Mukhtar et al. (2019)	https://doi.org/10.3390/environments6020026
	Sed.	Spain	Canary Islands, Spain	2008-2009	nd - 16.6 ng/g	Sanchez-Rodrıguez et al. (2011)	https://doi.org/10.1016/j.chemosphere.2010.09.064
	Sed.	Korea	Harbors and costal areas	2013	nd - 6.6 ng/g	Lee & Lee (2017)	https://doi.org/10.1007/s12665-017-6954-5
	Water	Korea	Harbors and costal areas	2006-2009	<1.7 - 21.77 ng/L	Lee et al. (2011)	https://doi.org/10.1016/j.jhazmat.2010.10.048
	Water	Spain	Ports and marinas (catalonia)	1998-1999	300 -600 ng/L	Martinez et al. (2000)	https://doi.org/10.1016/S0021-9673(00)00307-1
	Water	Portugal	n.a.	n.a.	7-22 ng/L	Gonzalez-Rey et. al (2015)	https://doi.org/10.1016/j.marpolbul.2015.04.029
	Water	Italy	Tyrrhenian coast	2010	<1ng/L	Ansanelli et al. (2017)	https://doi.org/10.1016/j.rsma.2017.09.011
	Sed.	Brazil	Santos Bay	2020	16 ng/g	Abreu et al. (2020)	https://doi.org/10.1016/j.jhazmat.2020.122937
	Water	Greece	marinass	2003	<3 - 36 ng/L	Hamwijk et al. (2005)	https://doi.org/10.1016/j.chemosphere.2005.01.072
	Water	Panama	Harbors and costal areas	2016	<2.7 ng/L	Batista-Andrade et al. (2016)	https://doi.org/10.1016/j.marpolbul.2016.07.045
	Sed.	Korea	Ports and marinas	2010	<50 ng/L	Kim et al. (2014)	https://doi.org/10.1016/j.marpolbul.2013.10.043
	Water	Ausralia	Perth coastal waters, Australia	2003	<100 ng/l	Reitsema (2008)	https://www.water.wa.gov.au/__data/assets/pdf_file/0005/5477/84012.pdf
Chlorothalonil	Sed.	Greece	Ports and marinas	2000	<2.5 - 165 ng/g	Albanis et al (2002)	https://doi.org/10.1016/S0045-6535(02)00134-0
	Sed.	Malaysia	Sungai Pulai Estuary	n.a.	<0.1 - 6.2 ng/g	Mukhtar et al. (2019)	https://doi.org/10.3390/environments6020026
	Sed.	Korea	Ports and marinas	2013	<2.9 - 82.7 ng/g	Lee & Lee (2017)	https://doi.org/10.1007/s12665-017-6954-5
	Water	France	Atlantic coast of France and UK	1998-2002	<0.8 - 10.9 ng/L	ACE (2002)	https://www.bodc.ac.uk/resources/inventories/edmed/report/725/
	Sed.	Brazil	Santos Bay	2020	9.2 ng/g	Abreu et al. (2020)	https://doi.org/10.1016/j.jhazmat.2020.122937
	Water	UK	Estuary	1998	360-1380 ng/L	Voulvoulis et al. (2000)	https://doi.org/10.1016/S0025-326X(00)00034-5
	Sed.	Korea	Ports and marinas	2013	<2.9 - 82.7 ng/g	Lee et al. (2015)	https://doi.org/10.1016/j.marpolbul.2015.07.038

Biocide	Organism	Species	Endpoint	Parameter	Value (mg/L)	CI 95% (mg/L)	Target	Reference	DOI
	Rotifers	*Philodina acuticornis odiosa*	24h LC_{50}	Mortality	0.0005	0.0004 - 0.0007	NT	Hagen et al. (2010)	https://doi.org/10.1080/02772240903074619
	Crustaceans	*Cancer magister*	48h LC_{50}	Larval mortality	0.56	n.a.	NT	Armstrong et al. (1976)	https://doi.org/10.1016/0022-2011(76)90007-0
	Crustaceans	*Cancer magister*	96h LC_{50}	Larval mortality	0.14	n.a.	NT	Armstrong et al. (1976)	https://doi.org/10.1016/0022-2011(76)90007-1
	Crustaceans	*Cancer magister*	48h EC_{50}	Behavior (movement)	0.17	n.a.	NT	Armstrong et al. (1976)	https://doi.org/10.1016/0022-2011(76)90007-2
	Annelida	*Hydroides elegans*	48h LC_{50}	Larval mortality	0.012	0.01 - 0.014	NT	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Crustaceans	*Tigriopus japonicus*	24h LC_{50}	Mortality	0.168	0.160 - 0.176	NT	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Crustaceans	*Tigriopus japonicus*	24h LC_{50}	Mortality	0.098	0.090 - 0.106	NT	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Crustaceans	*Tigriopus japonicus*	96h LC_{50}	Mortality	0.096	0.091 - 0.103	NT	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
Chlorothalonil	Crustaceans	*Tigriopus japonicus*	96h LC_{50}	Mortality	0.091	0.086 - 0.096	NT	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Crustaceans	*Tigriopus japonicus*	96h LC_{50}	Mortality	0.06	0.044 - 0.081	T	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Crustaceans	*Elasmopus rapax*	96h LC_{50}	Mortality	0.067	0.053 - 0.084	NT	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Diatoms	*Skeletonema costatum*	96h IC_{50}	Growth inhibition	0.013	0.007 - 0.023	T	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Diatoms	*Thalassiosira pseudonana*	96h IC_{50}	Growth inhibition	0.004	0.002 - 0.1	T	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Dinoflagellates	*Pyrocystis lunula*	24h EC_{50}	Bioluminescence	0.066	0.055 - 0.080	T	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Dinoflagellates	*Pyrocystis lunula*	24h EC_{50}	Bioluminescence	0.19	0.124 - 0.289	T	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Dinoflagellates	*Pyrocystis lunula*	24h EC_{50}	Bioluminescence	0.0796	0.071 - 0.089	T	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Fish	*Oryzias melastigma*	96h LC_{50}	Larval mortality	0.11	0.1 - 0.11	NT	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041

Biocide	Organism	Species	Endpoint	Parameter	Value (mg/L)	CI 95% (mg/L)	Target	Reference	DOI
	Microalgae	*Synechococcus* sp.	96h IC_{51}	Growth inhibition	0.39	0.34 - 0.46	NT	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Crustaceans	*Amphiascus tenuiremis*	96h LC_{50}	Mortality	0.027	n.a.	T	Bejarano et al. (2005)	https://doi.org/10.1016/j.jembe.2005.01.003
	Crustaceans	*Amphiascus tenuiremis*	96h LC_{50}	Mortality	0.053	n.a.	T	Bejarano et al. (2005)	https://doi.org/10.1016/j.jembe.2005.01.004
	Crustaceans	*Amphiascus tenuiremis*	96h LC_{50}	Mortality	0.028	± 0.0005	T	Bejarano et al. (2005)	https://doi.org/10.1016/j.jembe.2005.01.005
	Bivalves	*Crassostrea virginica*	96h EC_{50}	Morphology (shell deposition)	0.026	n.a.	T	Belas (2006)	https://doi.org/10.1016/j.scitotenv.2006.01.028
	Bivalves	*Mytilus edulis*	48h EC_{50}	Embryotoxicity	0.088	n.a.	T	Belas (2006)	https://doi.org/10.1016/j.scitotenv.2006.01.029
	Echinoderms	*Paracentrotus lividus*	48h EC_{50}	Embryonic development	0.0066	n.a.	NT	Belas (2006)	https://doi.org/10.1016/j.scitotenv.2006.01.030
	Tunicates	*Ciona intestinalis*	48h EC_{50}	Embryonic development	0.033	n.a.	NT	Belas (2006)	https://doi.org/10.1016/j.scitotenv.2006.01.031
	Tunicates	*Ciona intestinalis*	48h EC_{50}	Larval settlement	0.042	n.a.	NT	Belas (2006)	https://doi.org/10.1016/j.scitotenv.2006.01.032
	Echinoderms	*Paracentrotus lividus*	48h EC_{50}	Growth (Length)	0.008	0.007 - 0.0084	NT	Bellas et al. (2008)	https://doi.org/10.1016/j.aquatox.2008.05.011
	Microalgae	*Dunaliella tertiolecta*	96h IC_{50}	Growth inhibition	0.064	n.a.	NT	DeLorenzo et al. (2003)	https://doi.org/10.1081/PFC-120023511
	Crustaceans	*Penaeus duorarum*	96h LC_{50}	Mortality	0.165	0.1 - 0.27	NT	EPA (1992)	Consulted at EPA Ecotox Database (https://cfpub.epa.gov/ecotox/search.cf)
	Fish	*Cyprinodon variegatus*	96h LC_{50}	Larval mortality	0.032	0.03 - 0.036	NT	EPA (1992)	Consulted at EPA Ecotox Database (https://cfpub.epa.gov/ecotox/search.cf)
	Bivalves	*Mytilus edulis*	96h LC_{50}	Mortality	5.94	n.a.	T	Ernst et al. (1991)	https://doi.org/10.1007/BF01055550

Biocide	Organism	Species	Endpoint	Parameter	Value (mg/L)	CI 95% (mg/L)	Target	Reference	DOI
	Fish	*Gasterosteus aculeatus*	96h LC_{50}	Mortality	0.027	0.0125 - 0.05	NT	Ernst et al. (1993)	Consulted at: http://ceqg-rcqe.ccme.ca/download/en/163
	Crustaceans	*Artemia salina*	48h LC_{50}	Mortality	2.68	n.a.	NT	Jung et al. (2017)	https://doi.org/10.1016/j.marpolbul.2016.11.047
	Diatoms	*Nitzschia pungens*	96h IC_{50}	Growth inhibition	0.361	n.a.	T	Jung et al. (2017)	https://doi.org/10.1016/j.marpolbul.2016.11.048
	Crustaceans	*Palaemonetes pugio*	96h LC_{50}	Embryo mortality	0.396	n.a.	NT	Key et al. (2003)	https://doi.org/10.1081/PFC-120023512
	Crustaceans	*Palaemonetes pugio*	96h LC_{50}	Larvae mortality	0.0495	n.a.	NT	Key et al. (2003)	https://doi.org/10.1081/PFC-120023513
	Crustaceans	*Palaemonetes pugio*	96h LC_{50}	Mortality	0.153	0.120 - 0.195	NT	Key et al. (2003)	https://doi.org/10.1081/PFC-120023514
	Crustaceans	*Palaemonetes pugio*	48h LC_{50}	Mortality	0.204	0.164 - 0.254	NT	Key et al. (2003)	https://doi.org/10.1081/PFC-120023515
	Crustaceans	*Artemia salina*	24h LC_{50}	Mortality	1	0.8 - 1.2	NT	Koutsaftis and Aoyama (2007)	https://doi.org/10.1016/j.scitotenv.2007.07.023
	Crustaceans	*Penaeus duorarum*	48h LC_{50}	Mortality	0.32	n.a.	NT	Mayer (1987)	Consulted at: https://doi.org/10.1007/978-1-4615-4911-6 (Tucker, J. (1998) Marine Fish Culture. Springer)
	Crustaceans	*Penaeus duorarum*	48h EC_{50}	Intoxication/Immobilization	0.32	n.a.	NT	Mayer (1987)	Consulted at: https://doi.org/10.1007/978-1-4615-4911-6 (Tucker, J. (1998) Marine Fish Culture. Springer)
	Fish	*Leiostomus xanthurus*	48h LC_{50}	Juvenile mortality	0.032	n.a.	NT	Mayer (1987)	Consulted at: https://doi.org/10.1007/978-1-4615-4911-6 (Tucker, J. (1998) Marine Fish Culture. Springer)
	Crustaceans	*Penaeus duorarum*	96h LC_{50}	Mortality	0.162	n.a.	NT	Montforts (1999)	Consulted at: EPA Ecotox Database (https://cfpub.epa.gov/ecotox/search.cf

Biocide	Organism	Species	Endpoint	Parameter	Value (mg/L)	CI 95% (mg/L)	Target	Reference	DOI
									m)
	Crustaceans	*Tigriopus japonicus*	24h EC_{50}	Larval Intoxication/Immobilization	0.016	0.014 - 0.018	NT	Onduka et al. (2012)	https://doi.org/10.1007/s12562-012-0562-9
	Crustaceans	*Marsupenaeus japonicus*	96h LC_{50}	Mortality	0.29	0.26 - 0.33	NT	Onduka et al. (2012)	https://doi.org/10.1007/s12562-012-0562-10
	Diatoms	*Skeletonema costatum*	72h IC_{50}	Growth inhibition	0.00095	0.00087 - 0.001	T	Onduka et al. (2012)	https://doi.org/10.1007/s12562-012-0562-11
	Fish	*Fundulus heteroclitus*	96h LC_{50}	Mortality	0.06	0.056 - 0.066	NT	Onduka et al. (2012)	https://doi.org/10.1007/s12562-012-0562-12
CuSCN	Crustacean	*Crangon crangon*	96h LC_{50}	Mortality	> 1.02	n.a.	NT	EPA (2000)	Consulted at EPA Ecotox Database (https://cfpub.epa.gov/ecotox/search.cf
	Fish	*Pleuronectes platessa*	96h LC_{50}	Mortality	0.0096	0.006 - 0.024	NT	EPA (2000)	Consulted at EPA Ecotox Database (https://cfpub.epa.gov/ecotox/search.cf)
	Diatoms	*Phaedactylum tricornutum*	72h EC_{50}	growth inhibition	108	n.a.	T	Carteau et al. 2014	10.1016/j.porgcoat.2013.11.012
	Crustacean	*Acartia tonsa*	72h EC_{50}	Egg production	123	n.a.	NT	Carteau et al. 2014	10.1016/j.porgcoat.2013.11.012
	Bivalves	*Crassostrea gigas*	48h EC_{50}	Embryonic development	11	n.a.	T	Carteau et al. 2014	10.1016/j.porgcoat.2013.11.012
	Echinoderms	*Paracentrotus lividus*	48h EC_{50}	Embryonic development	177	n.a.	NT	Carteau et al. 2014	10.1016/j.porgcoat.2013.11.012
	Fish	*Dicentrarchus labrax*	EC_{50}	n.a.	597	n.a.	NT	Carteau et al. 2014	10.1016/j.porgcoat.2013.11.012
	Diatoms	*Sargassum muticum*	72h EC_{50}	growth inhibition	0.47	± 0.002	T	Silkina et al. (2012)	https://doi.org/10.1016/j.marpolbul.2012.06.028
	Diatoms	*Ceramium botryocarpum*	72h EC_{50}	growth inhibition	0.39	± 0.001	T	Silkina et al. (2012)	https://doi.org/10.1016/j.marpolbul.2012.06.029
	Diatoms	*Fragilaria pinnata*	72h EC_{50}	growth inhibition	0.306	± 0.001	T	Silkina et al. (2012)	https://doi.org/10.1016/j.marpolbul.2012.06.030
Cu_2O	Crustaceans	*Americamysis bahia*	96h LC_{50}	Mortality	0.0697	0.057-0.092	NT	EPA (2000)	Consulted at EPA Ecotox Database

Biocide	Organism	Species	Endpoint	Parameter	Value (mg/L)	CI 95% (mg/L)	Target	Reference	DOI
									(https://cfpub.epa.gov/ecotox/search.cf)
	Fish	*Cyprinodon variegatus*	96h LC_{50}	Mortality	> 0.173	n.a.	NT	EPA (2000)	Consulted at EPA Ecotox Database (https://cfpub.epa.gov/ecotox/search.cf)
	Bivalves	*Balanus improvisus*nauplii	96h LC_{50}	Mortality	0.02	n.a.	T	Koryakova (1993)	Consulted at: EPA Ecotox Database (https://cfpub.epa.gov/ecotox/search.cfm)
	Bivalves	*Balanus improvisus*nauplii	72h LC_{50}	Mortality	0.14	n.a.	T	Koryakova (1993)	Consulted at: EPA Ecotox Database (https://cfpub.epa.gov/ecotox/search.cfm)
	Bivalves	*Balanus improvisus*nauplii	48h LC_{50}	Mortality	0.35	n.a.	T	Koryakova (1993)	Consulted at: EPA Ecotox Database (https://cfpub.epa.gov/ecotox/search.cfm)
	Bivalves	*Balanus improvisus*nauplii	24h LC_{50}	Mortality	0.5	n.a.	T	Koryakova (1993)	Consulted at: EPA Ecotox Database (https://cfpub.epa.gov/ecotox/search.cfm)
	Diatoms	*Phaeodactylum tricornutum*	96h EC_{50}	Growth inhibition	0.01	0.010 - 0.020	T	Avelelas et al. (2017)	https://doi.org/10.1007/s10126-017-9740-1
	Microalgae	*Tetraselmis chuii*	96h IC_{50}	Growth inhibition	0.3	0.170 - 0.530	NT	Avelelas et al. (2017)	https://doi.org/10.1007/s10126-017-9740-1
	Crustaceans	*Tigriopus japonicus*	96h LC_{50}	Mortality	0.074	0.069 - 0.079	NT	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
CuPT	Coral	*Acropora valida*	24h LC_{50}	Mortality	0.028	0.001 - 0.630	T	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Crustaceans	*Tigriopus japonicus*	96h LC_{50}	Mortality	0.03	0.026 - 0.036	NT	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Crustaceans	*Elasmopus rapax*	96h LC_{50}	Mortality	0.011	0.009 - 0.013	NT	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.201

Biocide	Organism	Species	Endpoint	Parameter	Value (mg/L)	CI 95% (mg/L)	Target	Reference	DOI
									1.02.041
	Crustaceans	*Amphibalanus amphitrite*	24h LC_{50}	Mortality	0.063	0.059 - 0.067	T	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Cyanobacteria	*Synechococcus* sp.	96h EC_{50}	Growth inhibition	0.022	0.014 - 0.033	T	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Diatoms	*Thalassiosira pseudonana*	96h EC_{50}	Growth inhibition	0.0007	0.0005 - 0.001	T	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Dinoflagellate	*Pyrocystis lunula*	24h EC_{50}	Bioluminescence	0.023	0.012 - 0.042	T	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Fish	*Oryzias melastigma*	96h LC_{50}	Mortality	0.0082	0.008 - 0.0084	NT	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Polychaetes	*Hydroides elegans*	48h LC_{50}	Mortality	0.006	0.005 - 0.006	NT	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Microalgae	*Tisochrysis lutea*	96h EC_{50}	Growth inhibition	0.00112	± 0.00004	NT	Dupraz et al. (2018)	https://doi.org/10.1016/j.chemosphere.2018.06.139
	Microalgae	*Skeletonema marinoi*	96h EC_{51}	Growth inhibition	0.00116	± 0.00002	NT	Dupraz et al. (2018)	https://doi.org/10.1016/j.chemosphere.2018.06.140
	Microalgae	*Tetraselmis suecica*	96h EC_{52}	Growth inhibition	0.018	± 0.00146	NT	Dupraz et al. (2018)	https://doi.org/10.1016/j.chemosphere.2018.06.141
	Bivalves	*Mytilus galloprovincialis*	72h EC_{50}	Settlement inhibition (Atl Sea)	3.2	1.60 - 6.56	T	Gutner-Hoch et al. (2018)	https://doi.org/10.3390/jmse6010006
	Bivalves	*Mytilus galloprovincialis*	72h EC_{50}	Settlement inhibition (Red Sea)	> 100	n.a.	T	Gutner-Hoch et al. (2018)	https://doi.org/10.3390/jmse6010006
	Bivalves	*Mytilus galloprovincialis*	72h LC_{50}	Mortality (Atl Sea)	3.8	3.18 - 4.67	T	Gutner-Hoch et al. (2018)	https://doi.org/10.3390/jmse6010006
	Bivalves	*Mytilus galloprovincialis*	72h LC_{50}	Mortality (Red Sea)	> 100	n.a.	T	Gutner-Hoch et al. (2018)	https://doi.org/10.3390/jmse6010006
	Bryozoans	*Bugula neritina*	72h EC_{50}	Settlement inhibition (Red Sea)	0.05	0.04 - 0.06	NT	Gutner-Hoch et al. (2018)	https://doi.org/10.3390/jmse6010006
	Bryozoans	*Bugula neritina*	72h EC_{50}	Settlement inhibition (Med Sea)	0.19	0.07 - 0.4	NT	Gutner-Hoch et al. (2018)	https://doi.org/10.3390/jmse6010006
	Crustaceans	*Artemia salina*	24h LC_{50}	Mortality	4.58	0.82 - 25.64	NT	Gutner-Hoch et al. (2019)	https://doi.org/10.1016/j.envpol.2019.0

Biocide	Organism	Species	Endpoint	Parameter	Value (mg/L)	CI 95% (mg/L)	Target	Reference	DOI
									5.031
	Echinoderms	*Paracentrotrus lividus*	48h EC_{50}	Larval development	0.011	0.007 - 0.015	NT	Gutner-Hoch et al. (2019)	https://doi.org/10.1016/j.envpol.2019.05.031
	Crustaceans	*Artemia salina*	24h LC_{50}	Mortality	0.83	0.580 - 1.07	NT	Koutsaftis and Aoyama (2007)	https://doi.org/10.1016/j.scitotenv.2007.07.023
	Crustaceans	*Heptacarpus futilirostris*	96h LC_{50}	Mortality	2.5	1.00 - 6.60	NT	Mochida et al. (2006)	https://doi.org/10.1897/05-688R.1
	Fish	*Pagrus major*	96h LC_{50}	Mortality	9.3	8.10 - 10.7	NT	Mochida et al. (2006)	https://doi.org/10.1897/05-688R.1
DCOIT	Microalgae	*Chlorella fusca var. vacuolata*	24h EC_{50}	Growth inhibition	0.089	0.081 – 0.097	NT	Arrhenius et al. (2006)	https://doi.org/10.1016/j.marpolbul.2014.07.011
	Microbial	*Periphyton community*	72h EC_{50}	Photosynthesis	0.026	n.a.	T	Arrhenius et al. (2006)	https://doi.org/10.1016/j.marpolbul.2014.07.011
	Ascidiacea	*Ciona intestinalis*	24h EC_{50}	Embryonic development	0.105	0.094 - 0.119	T	Belas (2006)	https://doi.org/10.1016/j.scitotenv.2006.01.028
	Ascidiacea	*Ciona intestinalis larval*	24h EC_{50}	Larval settlement	0.043	0.042 - 0.046	T	Belas (2006)	https://doi.org/10.1016/j.scitotenv.2006.01.028
	Bivalves	*Mytilus edulis embryo*	48h EC_{50}	Intoxication (Immobilization)	0.011	0.009 - 0.013	T	Belas (2006)	https://doi.org/10.1016/j.scitotenv.2006.01.028
	Echinoderms	*Paracentrotus lividus 4-arm larvae*	48h EC_{50}	Larval development	0.012	0.011 - 0.013	NT	Belas (2006)	https://doi.org/10.1016/j.scitotenv.2006.01.028
	Echinoderms	*Paracentrotus lividus*	48h EC_{50}	Larval growth (Length)	0.025	0.020 - 0.032	NT	Belas (2006)	https://doi.org/10.1016/j.scitotenv.2006.01.028
	Echinoderms	*Paracentrotus lividus 4-arm larvae*	48h EC_{50}	Larval development	0.019	0.019 – 0.021	NT	Bellas (2007)	https://doi.org/10.1016/j.aquatox.2007.03.011
	Echinoderms	*Paracentrotus lividus*	48h EC_{50}	Larval growth (Length)	0.02	0.019 – 0.022	NT	Bellas (2007)	https://doi.org/10.1016/j.aquatox.2007.03.011
	Macroalgae	*Fucus serratus zygotes*	24h EC_{50}	Germination percentage	0.019	n.a.	T	Braithwaite & Fletcher (2005)	https://doi.org/10.1016/j.jembe.2005.01.015
	Bivalves	*Mytilus edulis*	48h EC_{50}	Intoxication	0.411	n.a.	T	DCOIT assessment	Consulted at:

Biocide	Organism	Species	Endpoint	Parameter	Value (mg/L)	CI 95% (mg/L)	Target	Reference	DOI
		embryo		(Immobilization)				report (2014)	https://circabc.europa.eu/sd/a/5d2b12c8-7690-4636-a962-ab277f4b183d/DCOIT%20-%20PT%2021%20(assessment%20report%20as%20finalised%20on%2013.03.2014).pdf
	Bivalves	*Crassostrea virginica embryo*	48h EC_{50}	Intoxication (Immobilization)	0.012	n.a.	T	DCOIT assessment report (2014)	Consulted at: https://circabc.europa.eu/sd/a/5d2b12c8-7690-4636-a962-ab277f4b183d/DCOIT%20-%20PT%2021%20(assessment%20report%20as%20finalised%20on%2013.03.2014).pdf
	Fish	*Takifugu rubripes*	96h LC_{50}	Mortality	0.006	n.a.	NT	DCOIT assessment report (2014)	Consulted at: https://circabc.europa.eu/sd/a/5d2b12c8-7690-4636-a962-ab277f4b183d/DCOIT%20-%20PT%2021%20(assessment%20report%20as%20finalised%20on%2013.03.2014).pdf
	Cyanobacteria	*Synechococcus* sp.	72h EC_{50}	Growth inhibition	0.001	n.a.	T	Devilla et al. (2005)	https://doi.org/10.3354/meps286001
	Microalgae	*Emiliania huxleyi*	72h EC_{50}	Growth inhibition	0.0004	n.a.	NT	Devilla et al. (2005)	https://doi.org/10.3354/meps286001
	Polychaete	*Perinereis aibuhitensis*	24h LC_{50}	Mortality	0.268	0.192 -0.296	T	Eom et al. (2019)	https://doi.org/10.1016/j.cbpc.2019.05.001
	Polychaete	*Perinereis aibuhitensis*	96h LC_{50}	Mortality	0.142	0.089 - 0.182	T	Eom et al. (2019)	https://doi.org/10.1016/j.cbpc.2019.05.001
	Polychaete	*Perinereis*	14d LC_{50}	Mortality	0.055	0.028 - 0.086	T	Eom et al. (2019)	https://doi.org/10.1

Biocide	Organism	Species	Endpoint	Parameter	Value (mg/L)	CI 95% (mg/L)	Target	Reference	DOI
		aibuhitensis							016/j.cbpc.2019.05.001
	Bivalves	*Mytilus edulis adult*	48h EC_{50}	Intoxication (Immobilization)	0.851	0.13 - 1	T	EPA (1992)	Consulted at EPA Ecotox Database (https://cfpub.epa.gov/ecotox/search.cf)
	Bivalves	*Mytilus edulis embryo*	48h EC_{50}	Intoxication (Immobilization)	0.003	0.0025 - 0.003	T	EPA (1992)	Consulted at EPA Ecotox Database (https://cfpub.epa.gov/ecotox/search.cf)
	Bivalves	*Crassostrea virginica embryo*	48h EC_{50}	Intoxication (Immobilization)	0.009	0.0087 - 0.01	T	EPA (1992)	Consulted at EPA Ecotox Database (https://cfpub.epa.gov/ecotox/search.cf)
	Crustaceans	*Penaeus aztecus*	96h LC_{50}	Mortality	0.027	0.018 - 0.053	NT	EPA (1992)	Consulted at EPA Ecotox Database (https://cfpub.epa.gov/ecotox/search.cf)
	Crustaceans	*Uca pugilator*	96h LC_{50}	Mortality	1.7	1 - 3.2	NT	EPA (1992)	Consulted at EPA Ecotox Database (https://cfpub.epa.gov/ecotox/search.cf)
	Diatoms	*Skeletonema costatum*	96h EC_{50}	Growth inhibition	0.018	0.009 - 0.038	T	EPA (1992)	Consulted at EPA Ecotox Database (https://cfpub.epa.gov/ecotox/search.cf)
	Fish	*Cyprinodon variegatus*	96h LC_{50}	Mortality	0.023	0.018 - 0.029	NT	EPA (1992)	Consulted at EPA Ecotox Database (https://cfpub.epa.gov/ecotox/search.cf)
	Bacteria	*Vibrio fischeri*	30 min EC_{50}	Luminescence inhibition	0.003	± 0.0003	T	Fernández-Alba et al. (2002)	https://doi.org/10.1100/tsw.2002.221
	Crustaceans	*Penaeus aztecus*	96h LC_{50}	Mortality	0.016	n.a.	NT	Heitmuller (1977)	Consulted at: EPA Ecotox Database (https://cfpub.epa.gov/ecotox/search.cfm)
	Crustaceans	*Acartia tonsa*	72h EC_{50}	Egg production	0.038	±0.008	NT	Hjorth et al. (2006)	https://doi.org/10.1016/j.marenvres.2005.11.003
	Crustacea	*Balanus amphitrite larvae*	24h LC_{50}	Mortality	0.34	n.a.	T	Jacobson & Willingham (2000)	https://doi.org/10.1016/S0048-9697(00)00511-8

Biocide	Organism	Species	Endpoint	Parameter	Value (mg/L)	CI 95% (mg/L)	Target	Reference	DOI
	Diatoms	*Amphora coffeaeformis*	LC_{50}	Growth inhibition	0.003	n.a.	T	Jacobson & Willingham (2000)	https://doi.org/10.1016/S0048-9697(00)00511-8
	Macroalgae	*Ulva intestinalis*	120h EC_{50}	Population (Chlorophyll A concentration)	0.002	n.a.	T	Jacobson & Willingham (2000)	https://doi.org/10.1016/S0048-9697(00)00511-8
	Crustaceans	*Artemia salina*	48h LC_{50}	Mortality	0.318	n.a.	NT	Jung et al. (2017)	https://doi.org/10.1016/j.marpolbul.2016.11.04
	Diatoms	*Nitzschia pungens*	96h EC_{50}	Growth inhibition	0.267	n.a.	T	Jung et al. (2017)	https://doi.org/10.1016/j.marpolbul.2016.11.04
	Fish	*Pagrus major*	96h LC_{50}	Mortality	0.005	n.a.	NT	Kawashima (1997)	Consulted at: EPA Ecotox Database (https://cfpub.epa.gov/ecotox/search.cfm)
	Fish	*Fundulus heteroclitus*	96h LC_{50}	Mortality	0.005	n.a.	NT	Mochida et al. (2010)	https://doi.org/10.11403/jset.13.105
	Macroalgae	*Hormosira banksii*	48h EC_{50}	Germination	0.34	0.28 - 0.44	T	Myers et al. (2006)	https://doi.org/10.1016/j.marpolbul.2006.01.010
	Macroalgae	*Hormosira banksii*	48h EC_{50}	Rhizoid growth	0.43	0.29 - 0.65	T	Myers et al. (2006)	https://doi.org/10.1016/j.marpolbul.2006.01.010
	Crustaceans	*Americamysis bahia*	96h LC_{50}	Mortality	0.005	0.003 – 0.006	NT	Shade et al. (1993)	https://doi.org/10.1520/STP13169S
	Crustaceans	*Penaeus aztecus*	96h LC_{50}	Mortality	0.012	0.006 – 0.023	NT	Shade et al. (1993)	https://doi.org/10.1520/STP13169S
	Crustaceans	*Uca pugilator*	96h LC_{50}	Mortality	1.31	0.695 – 2.47	NT	Shade et al. (1993)	https://doi.org/10.1520/STP13169S
	Diatoms	*Skeletonema costatum*	96h EC_{50}	Growth inhibition	0.014	0.007 – 0.029	T	Shade et al. (1993)	https://doi.org/10.1520/STP13169S
	Fish	*Cyprinodon variegatus*	96h LC_{50}	Mortality	0.017	0.012 – 0.025	NT	Shade et al. (1993)	https://doi.org/10.1520/STP13169S
	Bivalves	*Crassostrea gigas eggs*	24h LC_{50}	Mortality	0.017	0.014 - 0.021	T	Tsunemasa & Okamura (2011)	https://doi.org/10.1007/s00244-010-9598-y
	Echinoderms	*Strongylocentrotus intermedius*	50h EC_{50}	Embryonesis success	0.014	n.a.	NT	Wang et al. (2011)	https://doi.org/10.1002/etc.440
	Echinoderms	*Strongylocentrotus intermedius*	50h EC_{50}	Embryonesis success	0.032	n.a.	NT	Wang et al. (2011)	https://doi.org/10.1002/etc.440

Biocide	Organism	Species	Endpoint	Parameter	Value (mg/L)	CI 95% (mg/L)	Target	Reference	DOI
	Echinoderms	*Strongylocentrotus intermedius*	50h EC_{50}	Embryonesis success	0.057	n.a.	NT	Wang et al. (2011)	https://doi.org/10.1002/etc.440
	Echinoderms	*Strongylocentrotus intermedius*	50h EC_{50}	Embryonesis success	0.114	n.a.	NT	Wang et al. (2011)	https://doi.org/10.1002/etc.440
	Diatoms	*Skeletonema costatum*	96h EC_{50}	Growth inhibition	0.026	n.a.	T	Wendt (2013)	http://hdl.handle.net/2077/33888
	Macroalgae	*Ulva lactuca*	72h EC_{50}	Population (Chlorophyll A concentration)	0.023	0.021 - 0.026	T	Wendt et al. (2013)	https://doi.org/10.1007/s00128-013-1057-9
	Crustaceans	*Acartia tonsa*	48h LC_{50}	Mortality	0.016	0.013 – 0.018	NT	Wendt et al. (2016)	https://doi.org/10.1007/s10646-016-1644-8
	Crustacea	*Balanus amphitrite*	24h EC_{50}	Settlement inhibition	0.22	n.a.	T	Willemsen et al. (1998)	https://doi.org/10.1007/s10646-016-1644-8
	Bivalves	*Crassostrea virginica embryo*	48h EC_{50}	Intoxication (Immobilization)	0.024	n.a.	T	Willingham & Jacobson (1996)	https://doi.org/10.1021/bk-1996-0640.ch011
	Diatoms	*Skeletonema costatum*	96h EC_{50}	Growth inhibition	0.02	n.a.	T	Willingham & Jacobson (1996)	https://doi.org/10.1021/bk-1996-0640.ch012
	Echinoderms	*Glyptocidaris crenularis 4-arm larvae*	53h EC_{50}	Larval development	0.001	0.0001 - 0.003	NT	Xu et al. (2010)	https://doi.org/10.1177/0960327110385958
	Crustaceans	*Tigriopus japonicus*	24h EC_{50}	n.a	0.03	n.a.	NT	Yamada (2007)	Available at: https://pdfs.semanticscholar.org/6f07/7705084e8240b83a8c7a4e147b07c4bef380.pdf?_ga=2.254019822.217765747.1591648473-948558388.1591648473
	Bacteria	*Vibrio fischeri*	15 min EC_{50}	Bioluminescence inhibition	0.299	0.19-0.46	T	Figueiredo et al. (2019)	https://doi.org/10.1039/C9EN00011A
	Microalgae	*Phaeodactylum tricornutum*	72h EC_{50}	Growth inhibition	0.04	0.003 - 0.005	NT	Figueiredo et al. (2019)	https://doi.org/10.1039/C9EN00011A
	Bivalves	*Mytilus galloprovincialis*	72h LC_{50}	Mortality	1.27	0.865 - 1.87	T	Figueiredo et al. (2019)	https://doi.org/10.1039/C9EN00011A
	Microalgae	*Isochrysis galbana*	72h EC_{50}	Growth inhibition	0.032	0.023 - 0.046	NT	Figueiredo et al. (2019)	https://doi.org/10.1039/C9EN00011A

Biocide	Organism	Species	Endpoint	Parameter	Value (mg/L)	CI 95% (mg/L)	Target	Reference	DOI
	Microalgae	*Nannochloropsis gaditana*	72h EC_{51}	Growth inhibition	0.035	0.009 - 0.130	NT	Figueiredo et al. (2019)	https://doi.org/10.1039/C9EN00011A
	Rotifers	*Brachionus plicatilis*	24h LC_{50}	Mortality	0.15	0.66 - 0.342	NT	Figueiredo et al. (2019)	https://doi.org/10.1039/C9EN00011A
	Bivalves	*Cerastoderma edule*	96h LC_{50}	Mortality	0.325	0.085 - 1.24	NT	Figueiredo et al. (2019)	https://doi.org/10.1039/C9EN00011A
	Polychaetes	*Hediste diversicolor*	96h LC_{50}	Mortality	3.43	0.397 - 0.673	NT	Figueiredo et al. (2019)	https://doi.org/10.1039/C9EN00011A
	Crustaceans	*Artemia salina*	48h LC_{50}	Mortality	0.351	0.308 - 0.398	NT	Figueiredo et al. (2019)	https://doi.org/10.1039/C9EN00011A
	Crustaceans	*Palaemon varians*	96h LC_{50}	Mortality	1.31	0.539 - 3.19	NT	Figueiredo et al. (2019)	https://doi.org/10.1039/C9EN00011A
	Echinoderm	*Paracentrotus lividus*	48h LC_{50}	Mortality	0.025	0.023 - 0.927	NT	Figueiredo et al. (2019)	https://doi.org/10.1039/C9EN00011A
	Crustaceans	*Tigriopus japonicus*	24h LC_{50}	Mortality	0.077	n.a.	NT	Yamada (2007)	https://pdfs.semanticscholar.org/6f07/7705084e8240b83a8c7a4e147b07c4bef380.pdf?_ga=2.254019822.217765747.1591648473-948558388.1591648474
	Crustaceans	*Mysidopsis juniae*	96h LC_{50}	Mortality	0.008	n.a.	NT	De Jesus et al. (2020)	https://doi.org/10.1016/j.marpolbul.2021.111970
	Crustaceans	*Penaeus japonicus*	96h LC_{50}	Mortality	0.013	n.a.	NT	Yamada (2007)	https://pdfs.semanticscholar.org/6f07/7705084e8240b83a8c7a4e147b07c4bef380.pdf?_ga=2.254019822.217765747.1591648473-948558388.1591648475
Dichlofluanid	Bivalves	*Mytilus edulis*	48h EC_{50}	Larval development	0.627	0.574 - 0.785	T	Belas (2006)	https://doi.org/10.1016/j.scitotenv.2006.01.028
	Echinoderm	*Paracentrotus lividus*	48h EC_{50}	Larval development	0.282	0.26 - 0.298	NT	Belas (2006)	https://doi.org/10.1016/j.scitotenv.2006.01.029
	Crustaceans	*Acartia tonsa*	48h LC_{10}	Mortality	0.017	n.a.	NT	Carteau et al. 2014	https://doi.org/10.1

Biocide	Organism	Species	Endpoint	Parameter	Value (mg/L)	CI 95% (mg/L)	Target	Reference	DOI
									016/j.porgcoat.2013.11.012
	Fish	*Dicentrarchus labrax*	96h EC_{50}	n.a	0.015	n.a.	NT	Carteau et al. 2014	https://doi.org/10.1016/j.porgcoat.2013.11.012
	Microalgae	*Phaedactylum tricornutum*	72h EC_{50}	Growth inhibition	0.193	n.a.	NT	Carteau et al. 2014	https://doi.org/10.1016/j.porgcoat.2013.11.012
	Bivalves	*Crassostrea virginica* juvenile	96h EC_{50}	Growth	0.059	n.a.	T	consulted at: EPA toxicity database	Consulted at EPA Ecotox Database (https://cfpub.epa.gov/ecotox/search.cf)
	Fish	*Leiostomus xanthurus*	24h LC_{50}	Mortality	0.032	n.a.	NT	consulted at: EPA toxicity database	Consulted at EPA Ecotox Database (https://cfpub.epa.gov/ecotox/search.cf)
	Microalgae	*Saccharina latissima*	3h NOEC	Growth inhibition	0.00001	n.a.	NT	Johansson et al. 2012	https://doi.org/10.1007/s00244-012-9778-z
	Crustaceans	*Artemia* sp.	48h LC_{50}	Mortality	154	n.a.	NT	Jung et al. (2017)	https://doi.org/10.1016/j.marpolbul.2016.11.047
	Diatoms	*Nitzschia pungens*	96h EC_{50}	Growth inhibition	0.377	n.a.	T	Jung et al. (2017)	https://doi.org/10.1016/j.marpolbul.2016.11.048
	Macroalgae	*Ulva lactuca*	72h EC_{50}	Population (Chrolophyll A concentration)	0.028	0.018 - 0.035	T	Wendt et al. (2013)	https://doi.org/10.1007/s00128-013-1057-9
	Echinoderm	*Glyptocidaris crenularis*	53h EC_{50}	Larval development	0.097	0.038 - 0.211	NT	Xu et al. (2010)	https://doi.org/10.1177/0960327110385958
	Echinoderm	*Glyptocidaris crenularis*	53h EC_{50}	Larval development	0.177	0.060 - 0.487	NT	Xu et al. (2010)	https://doi.org/10.1177/0960327110385958
Diuron	Coral	*Acropora valida*	24h LC_{50}	Mortality	4.8	0.670 - 4.00	T	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Crustaceans	*Trigiopus japonicus*	96h LC_{50}	Mortality	11	10.5 - 11.4	NT	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Crustaceans	*Amphibalanus amphitrite*	24h LC_{50}	Mortality	21	21.0 - 22.0	T	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.201

Biocide	Organism	Species	Endpoint	Parameter	Value (mg/L)	CI 95% (mg/L)	Target	Reference	DOI
									1.02.041
	Cyanobacteria	*Synechococcus* sp.	96h EC_{50}	Growth inhibition	0.11	0.089 - 0.114	T	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Diatoms	*Skeletonema costatum*	96h EC_{50}	Growth inhibition	0.006	0.005 - 0.007	T	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Diatoms	*Thalassiosira pseudonana*	96h EC_{50}	Growth inhibition	0.004	0.004 - 0.005	T	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Dinoflagellate	*Pyrocystis lunula*	24h EC_{50}	Bioluminescence	43	38.0 - 49.0	T	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Fish	*Oryzias melastigma*	96h LC_{50}	Mortality	7.8	7.60 - 7.90	NT	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Polychaetes	*Hydroides elegans*	48h LC_{50}	Mortality	16	15.0 - 17.0	NT	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Crustaceans	*Palaemon serratus*	24h LC_{50}	Mortality	3.04	2.09 - 3.23	NT	Bellas et al. (2005)	https://doi.org/10.1007/s10646-004-6370-y
	Echinoderms	*Paracentrotus lividus*	48h EC_{50}	Larval development	5.6	5.40 - 5.70	NT	Bellas et al. (2005)	https://doi.org/10.1007/s10646-004-6370-y
	Cyanobacteria	*Synechococcus* sp.	72h EC_{50}	Growth inhibition	0.001	n.a.	T	Devilla et al. (2005)	https://doi.org/10.3354/meps286001
	Microalgae	*Emiliania huxleyi*	72h EC_{50}	Growth inhibition	0.002	n.a.	NT	Devilla et al. (2005)	https://doi.org/10.3354/meps286002
	Microalgae	*Tisochrysis lutea*	96h EC_{50}	Growth inhibition	0.00373	± 0.00035	NT	Dupraz et al. (2018)	https://doi.org/10.1016/j.chemosphere.2018.06.139
	Microalgae	*Skeletonema marinoi*	96h EC_{51}	Growth inhibition	0.0103	± 0.00080	NT	Dupraz et al. (2018)	https://doi.org/10.1016/j.chemosphere.2018.06.140
	Microalgae	*Tetraselmis suecica*	96h EC_{52}	Growth inhibition	0.00042	± 0.00012	NT	Dupraz et al. (2018)	https://doi.org/10.1016/j.chemosphere.2018.06.141
	Bivalves	*Crassostrea virginica*	96h EC_{50}	n.a	4.8	4.49 - 5.18	T	EPA (1992)	Consulted at EPA Ecotox Database (https://cfpub.epa.gov/ecotox/search.cf)

Biocide	Organism	Species	Endpoint	Parameter	Value (mg/L)	CI 95% (mg/L)	Target	Reference	DOI
	Crustaceans	*Americamysis bahia*	96h LC_{50}	Mortality	1.1	0.970 - 1.30	NT	EPA (1992)	Consulted at EPA Ecotox Database (https://cfpub.epa.gov/ecotox/search.cf)
	Fish	*Cyprinodon variegatus*	96h LC_{50}	Mortality	0.89	0.490 - 1.30	NT	EPA (1992)	Consulted at EPA Ecotox Database (https://cfpub.epa.gov/ecotox/search.cf)
	Crustaceans	*Nitocra spinipes*	96h LC_{50}	Mortality	4	3.3 - 5.2	NT	Karlsson et al. (2006)	https://doi.org/10.1016/j.marpolbul.2006.06.007
	Crustaceans	*Artemia salina*	24h LC_{50}	Mortality	12	11.4 - 12.6	NT	Koutsaftis and Aoyama (2007)	https://doi.org/10.1016/j.scitotenv.2007.07.023
	Crustaceans	*Artemia salina*	24h LC_{50}	Mortality	10.3	6.81 - 17.25	NT	Lee et al. (2017)	https://doi.org/10.1007/s13530-017-0326-0
	Crustaceans	*Artemia salina*	48h LC_{50}	Mortality	6.14	3.15 - 9.58	NT	Lee et al. (2017)	https://doi.org/10.1007/s13530-017-0326-0
	Crustaceans	*Artemia salina*	72h LC_{50}	Mortality	2.76	0.921 - 4.48	NT	Lee et al. (2017)	https://doi.org/10.1007/s13530-017-0326-0
	Echinoderms	*Paracentrotus lividus*	48h EC_{50}	Larval development	2.39	± 0.210	NT	Manzo et al. (2006)	https://doi.org/10.1007/s00244-004-0167-0
	Echinoderms	*Paracentrotus lividus*	48h EC_{50}	Sperms growth	5.09	± 0.450	NT	Manzo et al. (2006)	https://doi.org/10.1007/s00244-004-0167-1
	Bivalves	*Crassostrea virginica*	96h EC_{50}	Growth inhibition	1.8	n.a.	T	Mayer (1987)	https://doi.org/10.1007/978-1-4615-4911-6
	Microalgae	*Dunaliella tertiolecta*	24h EC_{50}	Growth inhibition	0.035	n.a.	NT	McFeters et al. (1983)	https://doi.org/10.1016/0043-1354(83)90197-5
	Microalgae	*Raphidocelis subcapitata*	72h EC_{50}	Growth inhibition	0.045	n.a.	NT	Mezcua et al. (2002)	https://doi.org/10.1007/BF02493212
	Macroalgae	*Hormosira banksii*	48h EC_{50}	Germination	6.29	5.93 - 7.59	T	Myers et al. (2006)	https://doi.org/10.1016/j.marpolbul.2006.01.010
	Macroalgae	*Hormosira banksii*	72h EC_{50}	Germination	6.82	n.a.	T	Myers et al. (2006)	https://doi.org/10.1016/j.marpolbul.200

Biocide	Organism	Species	Endpoint	Parameter	Value (mg/L)	CI 95% (mg/L)	Target	Reference	DOI
									6.01.010
	Macroalgae	*Hormosira banksii*	48h EC_{50}	Growth inhibition	6.75	n.a.	T	Myers et al. (2006)	https://doi.org/10.1016/j.marpolbul.2006.01.010
	Macroalgae	*Hormosira banksii*	72h EC_{50}	Growth inhibition	7.33	n.a.	T	Myers et al. (2006)	https://doi.org/10.1016/j.marpolbul.2006.01.010
	Coralline algae	*Neogoniolithon fosliei*	24h IC_{50}	n.a.	0.009	n.a.	T	Negri et al. (2011)	https://doi.org/10.4319/lo.2011.56.2.0471
	Crustaceans	*Acartia tonsa*	48h LC_{50}	Mortality	1.08	0.402 - 2.88	NT	Perina (2009)	Thesis available at: https://sistemas.furg.br/sistemas/sab/arquivos/bdtd/00000011352.pdf
	Crustaceans	*Acartia tonsa*	48h LC_{50}	Mortality	1.49	0.890 - 2.48	NT	Perina (2009)	Thesis available at: https://sistemas.furg.br/sistemas/sab/arquivos/bdtd/00000011352.pdf
	Crustaceans	*Acartia tonsa*	48h LC_{50}	Mortality	1.39	1.17 - 1.64	NT	Perina (2009)	Thesis available at: https://sistemas.furg.br/sistemas/sab/arquivos/bdtd/00000011352.pdf
	Crustaceans	*Mysidopsis juniae*	96h LC_{50}	Mortality	0.589	0.442 - 0.784	NT	Perina (2009)	Thesis available at: https://sistemas.furg.br/sistemas/sab/arquivos/bdtd/00000011352.pdf
	Crustaceans	*Kalliapseudes schubartii*	96h LC_{50}	Mortality	8.48	6.25 - 8.34	NT	Perina (2009)	Thesis available at: https://sistemas.furg.br/sistemas/sab/arquivos/bdtd/00000011352.pdf
	Crustaceans	*Kalliapseudes schubartii*	96h LC_{50}	Mortality	7.56	6.57 - 8.71	NT	Perina (2009)	Thesis available at: https://sistemas.furg.br/sistemas/sab/arquivos/bdtd/00000011352.pdf
	Crustaceans	*Kalliapseudes schubartii*	96h LC_{50}	Mortality	7.06	4.83 - 8.57	NT	Perina (2009)	Thesis available at: https://sistemas.fur

Biocide	Organism	Species	Endpoint	Parameter	Value (mg/L)	CI 95% (mg/L)	Target	Reference	DOI
									g.br/sistemas/sab/arquivos/bdtd/00000011352.pdf
	Echinoderms	*Lytechinus variegatus*	24h EC_{50}	Larval development	6.82	5.95 - 7.07	NT	Perina (2009)	Thesis available at: https://sistemas.furg.br/sistemas/sab/arquivos/bdtd/00000011352.pdf
	Echinoderms	*Lytechinus variegatus*	24h EC_{50}	Larval development	5.74	5.65 - 5.83	NT	Perina (2009)	Thesis available at: https://sistemas.furg.br/sistemas/sab/arquivos/bdtd/00000011352.pdf
	Echinoderms	*Lytechinus variegatus*	24h EC_{50}	Larval development	3.33	3.17 - 3.41	NT	Perina (2009)	Thesis available at: https://sistemas.furg.br/sistemas/sab/arquivos/bdtd/00000011352.pdf
	Cnidaria	*Aurelia aurita*	10d LC_{50}	polyps Mortality	0.00478	0.00342 - 0.0067	NT	Pinteus et al. (2020)	https://doi.org/10.1016/j.scitotenv.2020.136796
	Diatoms	*Cylindrotheca closterium*	72h IC_{50}	Growth inhibition	0.017	n.a.	T	Stauber et al. (2008)	https://doi.org/10.1002/tox.20400
	Diatoms	*Entomoneis punctulata*	72h IC_{50}	Growth inhibition	0.024	n.a.	T	Stauber et al. (2008)	https://doi.org/10.1002/tox.20401
	Dinoflagellate	*Pyrocystis lunula*	24h EC_{50}	Bioluminescence	19	n.a.	T	Stauber et al. (2008)	https://doi.org/10.1002/tox.20402
	Bivalves	*Abra nitida*	24h EC_{50}	Behavior (Burrowing behavior, burrowing length)	0.094	n.a.	T	Bellas et al. (2006)	https://doi.org/10.1016/j.chemosphere.2006.02.045
	Bivalves	*Abra nitida*	24h EC_{50}	Behavior (Feeding)	0.001	n.a.	T	Bellas et al. (2006)	https://doi.org/10.1016/j.chemosphere.2006.02.046
Medetomidine	Fish	*Psetta maxima*	96h LOEC	respiration frequency	4.21E-07	n.a.	NT	Hilvarsson et al. (2017)	https://doi.org/10.1016/j.aquatox.2007.04.008
	Fish	*Psetta maxima*	96h LOEC	oxygen consumed	0.084	n.a.	NT	Hilvarsson et al. (2017)	https://doi.org/10.1016/j.aquatox.2007.04.008
	Algae	*Ulva lactuca*	72h EC_{50}	inhibition of settling and growth	20.02	n.a.	T	Wendt 2013	http://hdl.handle.net/2077/33888
	Crustaceans	*Acartia tonsa*	48h LC_{50}	Mortality	0.0483	0.018 - 0.217	NT	Wendt et al. (2016)	https://doi.org/10.1

Biocide	Organism	Species	Endpoint	Parameter	Value (mg/L)	CI 95% (mg/L)	Target	Reference	DOI
									007/s10646-016-1644-8
	Crustaceans	*Acartia tonsa*	48h EC_{50}	Egg production	0.037	0.009 - 0.168	NT	Wendt et al. (2016)	https://doi.org/10.1007/s10646-016-1644-9
Tolylfluanid	Diatoms	*Cylindrotheca closterium*	4h EC_{50}	Population growth	0.022	n.a.	T	Arrhenius et al. (2014)	https://doi.org/10.1016/j.marpolbul.2014.07.011
	Photoautotrophic biofilm	Periphyton	72h EC_{50}	Settling and growth	0.022	0.153 - 0.037	T	Arrhenius et al. (2014)	https://doi.org/10.1016/j.marpolbul.2014.07.011
	Ascidian	*Ciona intestinalis*	48h EC_{50}	Larval development	0.217	0.134 - 0.254	T	Belas (2006)	https://doi.org/10.1016/j.scitotenv.2006.01.028
	Bivalves	*Mytilus edulis*	48h EC_{50}	Larval development	0.074	0.061 - 0.092	T	Belas (2006)	https://doi.org/10.1016/j.scitotenv.2006.01.028
	Bivalves	*Paracentrotus lividu*	48h EC_{50}	Larval development	0.405	0.169 - 0.521	T	Belas (2006)	https://doi.org/10.1016/j.scitotenv.2006.01.028
	Echinoderm	*Paracentrotus lividu*	48h EC_{50}	Larval development	0.986	0.868 - 1.171	NT	Belas (2006)	https://doi.org/10.1016/j.scitotenv.2006.01.028
	Echinoderm	*Strongylocentrotus intermedius*	4h EC_{50}	Fecundation	0.309	0.219 - 0.410	NT	Wang et al. (2010)	http://doi.wiley.com/10.1002/etc.440
	Echinoderm	*Strongylocentrotus intermedius*	50h EC_{50}	Larval development	0.222	0.054 - 0.357	NT	Wang et al. (2010)	http://doi.wiley.com/10.1002/etc.440
	Macroalgae	*Ulva lactuca*	72h EC_{50}	Population (Chlorophyll A concentration)	0.028	0.018 - 0.035	T	Wendt et al. (2013)	https://doi.org/10.1007/s00128-013-1057-9
	Echinoderm	*Glyptocidaris crenularis*	53h EC_{50}	Larval development	0.097	0.038 - 0.211	NT	Xu et al. (2010)	https://doi.org/10.1177/0960327110385958
Zineb	Bivalves	*Crassostrea virginica* spat	48h EC_{50}	Intoxication (Immobilization)	> 1	n.a.	T	EPA (2000)	Consulted at EPA Ecotox Database (https://cfpub.epa.gov/ecotox/search.cf)
	Fish	*Pagrus major*	96h LC_{50}	Mortality	0.029	0.011 - 0.047	NT	Hano et al. (2017)	https://doi.org/10.1016/j.ecoenv.2016.12.019
	Fish	*Spotted halibut*	96h LC_{50}	Mortality	0.239	0.121 - 0.356	NT	Hano et al. (2017)	https://doi.org/10.1016/j.ecoenv.2016.1

Biocide	Organism	Species	Endpoint	Parameter	Value (mg/L)	CI 95% (mg/L)	Target	Reference	DOI
									2.019
	Crustaceans	*Artemia* sp.	48h LC_{50}	Mortality	0.041	n.a.	NT	Jung et al. (2017)	https://doi.org/10.1016/j.marpolbul.2016.11.047
	Diatoms	*Nitzschia pungens*	96h EC_{50}	Growth inhibition	0.232	n.a.	T	Jung et al. (2017)	https://doi.org/10.1016/j.marpolbul.2016.11.048
	Macroalgae	*Hormosira banksii*	48h EC_{50}	Germination	0.87	0.66 - 1.19	T	Myers et al. (2006)	https://doi.org/10.1016/j.marpolbul.2006.01.012
	Macroalgae	*Hormosira banksii*	48h EC_{50}	Rhizoid growth	2.04	0.79 - 3.53	T	Myers et al. (2006)	https://doi.org/10.1016/j.marpolbul.2006.01.012
ZnPT	Diatoms	*Phaeodactylum tricornutum*	96h EC_{50}	Growth inhibition	0.01	0.010 - 0.020	T	Avelelas et al. (2017)	https://doi.org/10.1007/s10126-017-9740-1
	Microalgae	*Tetraselmis chuii*	96h IC_{50}	Growth inhibition	0.28	0.140 - 0.580	NT	Avelelas et al. (2017)	https://doi.org/10.1007/s10126-017-9740-2
	Crustaceans	*Elasmopus rapax*	96h LC_{50}	Mortality	0.029	0.019 - 0.046	NT	Bao et al. (2008)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Diatoms	*Thalassiosira pseudonana*	96h EC_{50}	Growth inhibition	0.002	0.0016 - 0.0023	T	Bao et al. (2008)	https://doi.org/10.1016/j.marpolbul.2011.02.042
	Cnidarians	*Aiptasia* sp.	96h LC_{50}	Mortality	0.41	0.350 - 0.480	T	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Coral	*Acropora valida*	24h LC_{50}	Mortality	0.18	n.a.	T	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Crustaceans	*Tigriopus japonicus*	96h LC_{50}	Mortality	0.17	0.150 - 0.190	NT	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Crustaceans	*Amphibalanus amphitrite*	24h LC_{50}	Mortality	0.21	0.200 - 0.230	T	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Cyanobacteria	*Synechococcus* sp.	96h EC_{50}	Growth inhibition	0.022	0.009 - 0.050	T	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Diatoms	*Thalassiosira pseudonana*	96h EC_{50}	Growth inhibition	0.0005	0.0005 - 0.0006	T	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.201

Biocide	Organism	Species	Endpoint	Parameter	Value (mg/L)	CI 95% (mg/L)	Target	Reference	DOI
									1.02.041
	Diatoms	*Skeletonema costatum*	96h EC_{50}	Growth inhibition	0.002	0.0015 - 0.0019	T	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Dinoflagellate	*Pyrocystis lunula*	24h EC_{50}	Bioluminescence	0.0004	0.0003 - 0.001	T	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Fish	*Oryzias melastigma*	96h LC_{50}	Mortality	0.043	0.032 - 0.057	NT	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Polychaetes	*Hydroides elegans*	48h LC_{50}	Mortality	0.008	0.007 - 0.009	NT	Bao et al. (2011)	https://doi.org/10.1016/j.marpolbul.2011.02.041
	Bivalves	*Mytilus edulis*	48h EC_{50}	Embryonic development	0.0025	n.a.	T	Bellas et al. (2005)	http://doi.org/10.1016/j.marpolbul.2005.06.010
	Microalgae	*Tisochrysis lutea*	96h EC_{50}	Growth inhibition	0.00125	± 0.00007	NT	Dupraz et al. (2018)	https://doi.org/10.1016/j.chemosphere.2018.06.139
	Microalgae	*Skeletonema marinoi*	96h EC_{51}	Growth inhibition	0.00198	± 0.00005	NT	Dupraz et al. (2018)	https://doi.org/10.1016/j.chemosphere.2018.06.139
	Microalgae	*Tetraselmis suecica*	96h EC_{52}	Growth inhibition	0.256	± 0.0181	NT	Dupraz et al. (2018)	https://doi.org/10.1016/j.chemosphere.2018.06.139
	Bivalves	*Crassostrea virginica*	96h EC_{50}	n.a.	0.022	n.a.	T	EPA (1992)	Consulted at EPA Ecotox Database (https://cfpub.epa.gov/ecotox/search.cf)
	Crustaceans	*Americamysis bahia*	96h LC_{50}	Mortality	0.005	n.a.	NT	EPA (1992)	Consulted at EPA Ecotox Database (https://cfpub.epa.gov/ecotox/search.cf)
	Fish	*Cyprinodon variegatus*	96h LC_{50}	Mortality	0.4	n.a.	NT	EPA (1992)	Consulted at EPA Ecotox Database (https://cfpub.epa.gov/ecotox/search.cf)
	Bivalves	*Mytilus galloprovincialis*	72h EC_{50}	Settlement inhibition (Atl Sea)	2.4	1.85 - 3.03	T	Gutner-Hoch et al. (2018)	https://doi.org/10.3390/jmse6010006
	Bivalves	*Mytilus galloprovincialis*	72h EC_{50}	Settlement inhibition (Red Sea)	4.2	1.4 - 12.2	T	Gutner-Hoch et al. (2018)	https://doi.org/10.3390/jmse6010006

Biocide	Organism	Species	Endpoint	Parameter	Value (mg/L)	CI 95% (mg/L)	Target	Reference	DOI
	Bivalves	*Mytilus galloprovincialis*	72h LC_{50}	Mortality (Atl Sea)	3.2	2.57 - 4.13	T	Gutner-Hoch et al. (2018)	https://doi.org/10.3390/jmse6010006
	Bivalves	*Mytilus galloprovincialis*	72h LC_{50}	Mortality (Red Sea)	15.5	6.0 - 39.9	T	Gutner-Hoch et al. (2018)	https://doi.org/10.3390/jmse6010006
	Bryozoans	*Bugula neritina*	72h EC_{50}	Settlement inhibition (Red Sea)	0.02	0.01 - 0.05	NT	Gutner-Hoch et al. (2018)	https://doi.org/10.3390/jmse6010006
	Bryozoans	*Bugula neritina*	72h EC_{50}	Settlement inhibition (Med Sea)	0.05	0.02 - 0.1	NT	Gutner-Hoch et al. (2018)	https://doi.org/10.3390/jmse6010006
	Crustaceans	*Artemia salina*	24h LC_{50}	Mortality	1.37	0.38 - 4.87	NT	Gutner-Hoch et al. (2019)	https://doi.org/10.1016/j.envpol.2019.05.031
	Echinoderms	*Paracentrotrus lividus*	48h EC_{50}	Larval development	0.002	0.001 - 0.004	NT	Gutner-Hoch et al. (2019)	https://doi.org/10.1016/j.envpol.2019.05.031
	Crustaceans	*Artemia salina*	24h LC_{50}	Mortality	3.17	2.45 - 3.88	NT	Koutsaftis and Aoyama (2007)	https://doi.org/10.1016/j.scitotenv.2007.07.023
	Crustaceans	*Heptacarpus futilirostris*	96h LC_{50}	Mortality	0.12	92.3 - 157	NT	Mochida et al. (2006)	https://doi.org/10.1897/05-688R.1
	Fish	*Pagrus major*	96h LC_{50}	Mortality	98.2	60.5 - 159	NT	Mochida et al. (2006)	https://doi.org/10.1897/05-688R.2
	Macroalgae	*Hormosira banksii*	48h EC_{50}	Germination	0.21	n.a.	T	Myers et al. (2006)	http://doi.org/10.1016/j.marpolbul.2006.01.010
	Macroalgae	*Hormosira banksii*	72h EC_{50}	Germination	0.19	n.a.	T	Myers et al. (2006)	http://doi.org/10.1016/j.marpolbul.2006.01.011
	Macroalgae	*Hormosira banksii*	48h EC_{50}	Growth inhibition	0.31	n.a.	T	Myers et al. (2006)	http://doi.org/10.1016/j.marpolbul.2006.01.012
	Macroalgae	*Hormosira banksii*	72h EC_{50}	Growth inhibition	0.24	n.a.	T	Myers et al. (2006)	http://doi.org/10.1016/j.marpolbul.2006.01.013
	Crustaceans	*Penaeus japonicus*	96h LC_{50}	Mortality	0.16	n.a.	NT	Yamada (2006)	https://doi.org/10.1007/698_5_056

Table S2: Lowest effect and lethal concentration (EC/LC50) of antifouling biocides to marine organisms; "T "= Target; "NT" = Non Target

Table S3: Antifouling biocides quotient risk (RQ) for the water compartment. Red cells indicate ecological risk (RQ>1) and green cells indicate no ecological risk (RQ<1). Table includes the following values used to calculate the RQ: Lowest median lethal or effect biocidal concentration ($L(E)C_{50}$), maximum measured environmental concentration (MEC) of each biocide in a given country or the predicted environmental concentration (PEC; in the absence of MEC values in the literature), the predicted no effect concentration (PNEC) derived through deterministic (det) and probabilistic (prob) approaches and, respective assessment factors (AF_{det}; AF_{prob}) and the RQ calculated using both PNEC values (RQ_{det} and RQ_{prob}). n.a.: data not available. References for the lowest $L(E)C_{50}$ values : [a] Devilla et al. (2005); [b] Dupraz et al. (2018); [c] Bao et al. (2011); [d] EPA (2000); [e] Arrhenius et al. (2014); [f] Koryakova (1993); [g] Bellas et al. (2006); [h] Johansson et al. (2012); [i] Hano et al. (2017); [j] Hagen et al. (2010).

Biocide	L(E)C_{50}	MEC/PEC	Country	PNECdet	PNECprob	AFdet	HC5	RQdet	RQprob	MEC_Reference	DOI/Ref
Chlorothalonil	0.5[j]	0.01	France	5×10^{-4}	0.52	1000	2.6	21.8	0.02	(ACE, 2002)	Assessment of Antifouling Agents in Coastal Environments (1998-2002) (bodc.ac.uk)
		1.38	UK					2760	2.6	(Voulvoulis et al., 2000)	https://doi.org/10.1016/S0025-326X(00)00034-5
Cu_2O	20[f]	32.12	PEC	2×10^{-3}	n.a.	10000	n.a.	16060	n.a.	(European Chemical Agency [ECHA], 2016b)	ECHA Assessment report, Dicopper oxide, Regulation (EU) n°528/2012
CuPT	0.7[c]	0	PEC	7×10^{-4}	0.1	1000	0.5	1.1	0.01	(European Chemical Agency [ECHA], 2015a)	ECHA Assessment report, Copper Pyrithione, Regulation (EU) n°528/2012
CuSCN	9.6[d]	8.64	PEC	9.6×10^{-4}	n.a.	10000	n.a.	9000	n.a.	(European Chemical Agency [ECHA], 2016a)	ECHA Assessment report, Copper thiocyanate, Regulation (EU) n°528/2012
DCOIT	0.4[a]	3×10^{-3}	Sweden	4×10^{-4}	0.21	1000	1.06	7.5	0.01	(Readman, 2006)	https://doi.org/10.1007/698_5_047
		6×10^{-3}	Korea					15	0.02	(Kim et al., 2014)	https://doi.org/10.1016/j.marpolbul.2013.10.043
		0.01	Japan					27.5	0.05	(Mochida et al., 2015)	https://doi.org/10.1016/j.envpol.2015.05.012
		0.05	Greece					122.5	0.2	(Sakkas et al., 2002)	https://doi.org/10.1007/BF02987576
		0.28	Denmark					707.5	1.3	(Steen et al., 2004)	https://doi.org/10.1016/j.chemosphere.2004.06.043
		3.7	Spain					9250	17.4	(Martínez et al., 2001)	https://doi.org/10.1080/09593332208618258
Dichlofluanid	0.01[h]	0.02	Korea	2×10^{-5}	0.58	500	2.9	1088.5	0.04	(Lee et al., 2011)	https://doi.org/10.1016/j.jhazmat.2010.10.048
		0.02	Portugal					1100	0.04	(Gonzalez-Rey et al., 2015)	https://doi.org/10.1016/j.marpolbul.2015.04.029
		0.04	Greece					1800	0.06	(Hamwijk et al., 2005)	https://doi.org/10.1016/j.chemosphere.2005.01.072
		3.37	Spain					168500	5.8	(Martínez et al., 2001)	https://doi.org/10.1080/09593332208618258
Diuron	0.42[b]	0.03	Iran	4.2×10^{-4}	0.25	1000	1.25	69.2	0.1	(Saleh et al., 2014)	https://doi.org/10.1016/j.chroma.2014.06.057
		0.03	Japan					73.8	0.1	(Kaonga et al., 2016)	https://doi.org/10.1016/J.CHEMOSPHERE.2015.11.100
		0.09	Albania					223.5	0.3	(Manzo et al., 2014)	https://doi.org/10.1039/c3em00724c

Biocide	L(E) C_{50}	MEC/PEC	Country	PNEC det	PNECprob	AFdet	HC5	RQ det	RQ prob	MEC_Reference	DOI/Ref
		0.26	Belgium					626.1	1	(Wille et al., 2011)	https://doi.org/10.1016/j.chroma.2011.10.039
		0.27	France					638.1	1	(Caquet et al., 2013)	https://doi.org/10.1007/s11356-012-1171-y
		0.29	Malaysia					678.5	1.1	(Ali et al., 2014)	https://doi.org/10.1016/j.marpolbul.2014.05.049
		0.37	UK					871.4	1.4	(Gatidou et al., 2007)	https://doi.org/10.1016/j.envint.2006.07.002
		0.48	Italy					1130.9	1.9	(Di Landa et al., 2006)	https://doi.org/10.1016/j.marpolbul.2006.05.027
		1.13	Netherlands					2690.4	4.5	(Lamoree et al., 2002)	https://doi.org/10.1016/S0021-9673(02)00878-6
		1.36	Korea					3238.1	5.4	(Kim et al., 2014)	https://doi.org/10.1016/j.marpolbul.2013.10.043
		2.16	Australia					5142.8	8.6	(Reitsema, 2008)	Reitsema, T. (2008). *Antifouling biocides in Perth coastal waters: a snapshot at select areas of vessel activity - Technical Series Report No. 1*.
		2.19	Spain					5214.2	8.7	(Martínez et al., 2001)	https://doi.org/10.1080/09593332208618258
		7.8	Brazil					18571.4	31.2	(Diniz et al., 2014)	10.5935/0103-5053.20130289
Medetomidine	1[g]	0	PEC	1×10^{-4}	n.a.	10000	n.a.	4.2	n.a.	(European Chemical Agency [ECHA], 2015b)	ECHA Assessment report, Medetomidine, Regulation (EU) n°528/2012
Tolylfluanid	22[e]	0.17	PEC	2.2×10^{-2}	2.53	1000	12.65	7.7	0.07	(European Chemical Agency [ECHA], 2014)	ECHA Assessment report, Tolylfluanid, Regulation (EU) n°528/2012
Zineb	29[i]	0.14	PEC	0,03	n.a.	1000	n.a.	4.9	n.a.	(New Zealand EPA, 2012)	New Zealand EPA. (2012). *Antifouling paints reassessment. Preliminary Risk Assessment. New Zealand Government.*
ZnPT	0.4[c]	3.3×10^{-5}	UK	4×10^{-4}	0.05	1000	0.25	0.08	0.001	(MacKie et al., 2004)	https://doi.org/10.1016/j.aca.2004.01.033

Chapter 2

Ecotoxicological effects of the antifouling biocide DCOIT in neotropical organisms.

Article 1 - Degradation kinetics of antifouling biocides in sediment during the spiking equilibrium phase

Buno Galvão de Campos[a], Fernando Perina[a], Fiamma Abreu[b], Gilberto Filman[b] Denis Abessa[a]

[a]*São Paulo State University (UNESP), Praça Infante Dom Henrique, s/n, Zipcode: 11330-900 São Vicente, São Paulo, Brazil*

[b] *Rio Grande Federal University (FURG), Av. Itália s/n, 7 Campus Carreiros, C.P. 474, 96201-900, Rio Grande, RS, Brazil*

Corresponding Author: *Bruno Galvão de Campos, São Paulo State University (UNESP), Praça Infante Dom Henrique, s/n, Zipcode: 11330-900 São Vicente, São Paulo, Brazil. E-mail: b.campos@unesp.br*

Status*:* Waiting feedbacks from coauthors to proceed with submission as short communication to *Soil and Sediment Contamination*

Abstract

The new generation antifouling biocides, as well as other emerging contaminants, are not yet included in the sediment quality guidelines neither present protocols for sediment spiking. Most of these biocides present short half-lives in water, but little information regarding its degradation in sediments is available. Thus, there is a need to stablish the reliable duration of the equilibrium phase for sediment spiking, prior to sediment toxicity testing, in order to determine the real concentrations of exposure during ecotoxicological tests. The objective of the present study was to evaluate the degradation of DCOIT, Irgarol, Diuron and dichlofluanid during a spiking equilibrium phase of 24 hours in three different time intervals (0, 6 h and 24 h) and concentrations (10 $ng.g^{-1}$, 100 $ng.g^{-1}$ and 1000 $ng.g^{-1}$), by applying kinetic degradation models. The models presented a better fit for 1000 $ng.g^{-1}$ treatments, in which the half-lives of DCOIT and Diuron were < 5 h, dichlofluanid < 2 h, and Irgarol < 6h. Our results also indicate that excepting the dichlofluanid, the degradation rates of the other antifouling biaoces were reduced dramatically after 6 hours of equilibrium. Therefore, an equilibrioum phase oh 24h (or

superior to 6 h) is considered viable for sediment spiking. Our findings represent valuable information to guide future sediment toxicity tests with such compounds.

Keywords. DT50, modeling, ecotoxicology, sediment, pollution, fortification

1. Introduction

Antifouling paints are specially designed to cope with biofouling in human-made structures through the continuous releases of biocides, thus creating a protective chemical barrier against target species (Yebra et al., 2004). The lack of a systematic assessment of the environmental risk of these biocides may allow the emergence of new threatens to the marine environment, as recently recognized for Irgarol 1051, which was banned in 2017 from the EU due to its persistence and high toxicity (Campos et al., 2021). Along with Irgarol, the literature also reports that other biocides such as DCOIT, dichlofluanid, chlorothalonil, diuron, ZnPT, among others, present harmful effects to non-target organisms (Martins et al., 2018). In addition, assessments of their toxicity on benthic species and environmental monitoring in marine sediments are scarce, representing an important gap for the hazard and environmental risk assessment of these compounds.

The sediment compartment represents an important route of contaminant exposure toward marine organisms and consequently affects the whole ecosystem (Chapman et al., 2002; Maranho et al., 2009). Benthic organisms contribute to the conversion of energy, mass, and nutrients between the benthic and pelagic zones, a process also knows as benthic-pelagic coupling. (Marcus and Marcus, 1998) In addition, sediment bioturbators such as Polychaeta, amphipods, and copepods are considered ecosystem engineers, contributing to the maintenance of marine biodiversity and ecosystem functioning (Caliman et al., 2013).

The toxicity assessment of xenobiotics towards benthic species is usually carried out through the contamination of pristine sediment in a process known as sediment spiking or fortification. After the spiking, the test organism is exposed to the contaminated sediment. There are three main guidelines for sediment spiking, which were respectively proposed by the USEPA (2001), Environment Canada (1995), and ASTM (2008). All these guidelines recommend an equilibrium phase, where the newly contaminated sediment is kept refrigerated and in the dark for a certain period allowing the system to reach chemical equilibrium or a steady phase. The equilibrium phase duration may vary specifically according to the

degradation rate and physicochemical nature of the contaminant; this equilibrium phase may range from 24 hours for metals to months for organic contaminants with high K_{ow} values.

Antifouling biocides are not yet included in sediment quality guidelines and they also have not been considered in sediment spiking protocols, which hampers the assessment of their ecological risks. Most of these biocides have short half-lives in water and no (or little) information regarding sediments. Therefore, during a sediment spiking procedure, an appropriate period for the equilibrium phase remains unknown, and this uncertainty can drastically influence the determination of effective concentration during toxicity tests. The objective of the present study was to evaluate the half-live and degradation kinetics of DCOIT, Irgarol, Diuron, and Dichlofluanid (Figure 1), in a spiked sediment during an equilibrium phase of 24 hours. Our findings may guide future sediment toxicity tests with such compounds.

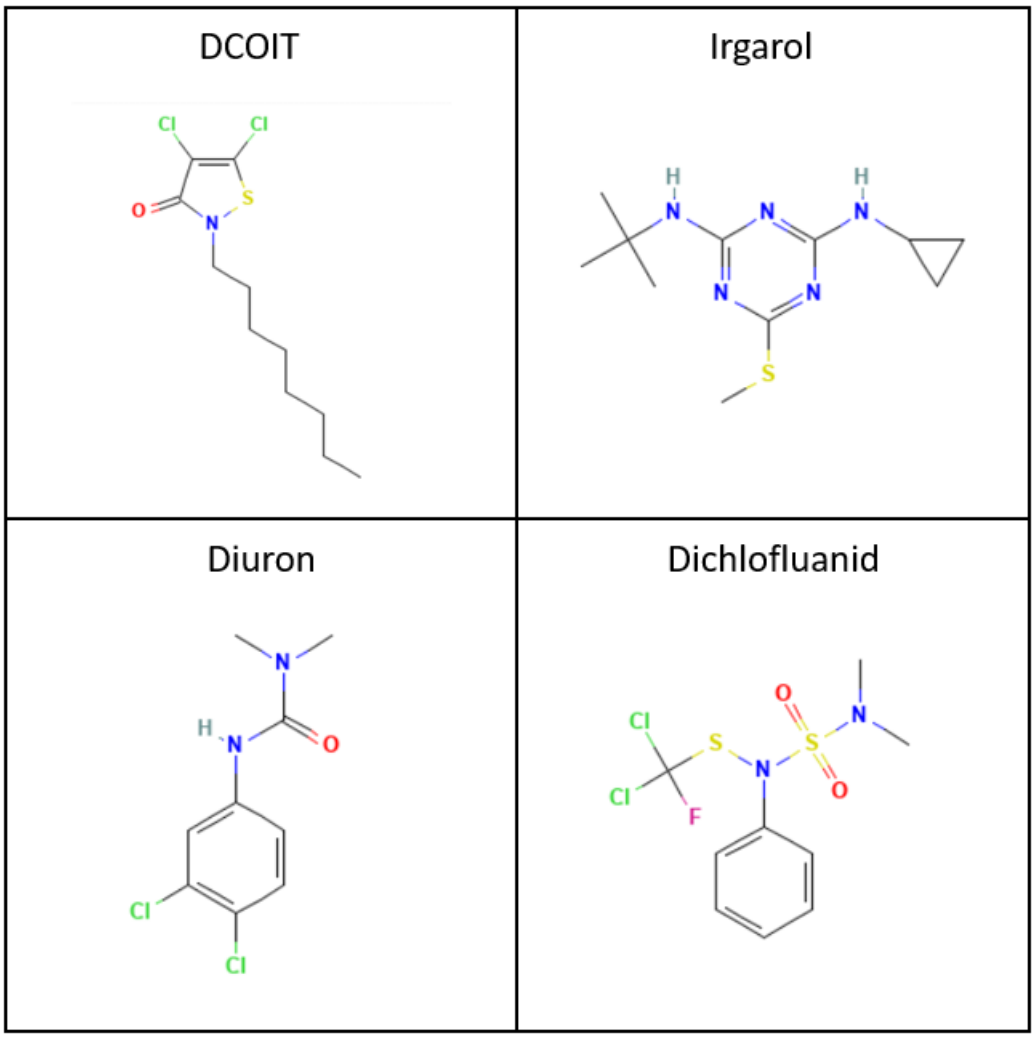


Figure 1: 2D Chemical structure of DCOIT, Irgarol, Diuron, and Dichlofluanid (source: Pubchem®)

2. Material and Methods

2.1 Chemical

The DCOIT (4,5-dichloro-2-n-octyl-4-isothiazolin-3-one), Irgarol 2(-metiltio-4-terbutilamino-6-ciclopropilamino-s-triazina), Diuron (1-dimethylurea) and dichlofluanid (N-[dichloro(fluoro)methyl]sulfanyl-N-(dimethylsulfamoyl)aniline), was purchased from Sigma Aldrich (Brazil) with purity > 99%. Stock solutions were prepared with ultrapure water (20ml) and 800 µl acetone of HPLC-grade (Sigma Aldrich, Brazil) as cosolvent. The stock solutions were used to further spike the sediment.

2.2 Sediment Spiking Procedure

Spiking of antifouling biocides was performed in natural estuarine sediment (water content = 18.91%, organic matter = 3.99%, $CaCO_3$ = 1.24%, sand = 94.76% (very coarse sand = 0.02%, coarse sand = 0.03%, medium sand = 1.93%, fine sand = 46.23%, very fine sand = 41.02%, silt and clay = 5.5%) sampled in the South of the Lagamar Protected Area located in Cananeia (SP, Brasil). This region has been considered a reference site due to its high biodiversity, low status of contamination, and absence relevant of anthropogenic impacts (Campos et al., 2016; Cruz et al., 2014).

The sediment spiking technique was applied using the slurry method (USEPA, 2001). Three aliquots of 300 g of wet sediment were contaminated with DCOIT, Irgarol, Diuron, and Dichlofluanid from stock solutions, diluted in acetone, in the following concentrations: 10 $ng.g^{-1}$, 100 $ng.g^{-1}$, and 1000 $ng.g^{-1}$ (e.g. the 10 $ng.g^{-1}$ sediment aliquot contains all four biocides in the corresponding concentration). The sediment was mixed for homogenization during 30min using a glass rod. For equilibrium, the sediment was kept in dark, at 4°C, for 24h. A control sediment (no biocide added) also went through all the processes.

2.3 Degradation Kinetics

During the spike equilibrium phase, three aliquots (surface, middle, and bottom) of 2 g of sediment were taken in three distinct time intervals: T0 - just after the contamination (with no equilibrium); T6 - 6 hours from contamination; and T24 - 24 hours form contamination, for the respective tested concentration. These 3 aliquots representing different areas of the sediments' containers were taken to test the homogeneity of the spike method and to evaluate the biocides degradation over time. Before quantification, each aliquot ware cooled at -80°C for 30min, then lyophilized for subsequent extraction and analyses.

2.4 Extraction and Liquid Chromatography analysis

Extraction of the DCOIT, Irgarol, Diuron, and Dichlofluanid was performed according to the procedures described by Abreu et al. (2020), where 1 g of dry sediment were weighted into 25 mL glass vials. Samples were spiked with 100 µL of Atrazine D5 (100 $ng.L^{-1}$,surrogate standard), after which 10 mL of acetonitrile was added. Samples were then mixed for 1 min, sonicated (50 ◦C for 30 min), and then centrifuged (4000 rpm for 7 min). This step was repeated three times. The resulting supernatants were combined and evaporated (Syncore®) to 1 mL. The extracts were cleaned by solid-phase extraction (SPE) using C18 cartridges previously activated with 4 mL of ethyl acetate and ultrapure water (Milli-Q®). The extracts were diluted with 50 ml of ultrapure water, passed through SPE cartridges, dried for 1 h, and then eluted 2 times using 2 mL of ethyl acetate. The eluates were decreased by volume under nitrogen flow to 1 mL before being transferred to a new vial with methanol. All obtained APPs and sediment extracts were also analyzed in triplicate. The samples were analyzed by gas chromatography using a Perkin Elmer Clarus 500MS equipped with a mass spectrometer.

The quality assurance and quality control (QA/QC) procedures were based on regular analysis of blanks, spiked matrices, and certified reference material (CRM – PACS-3/National Research Council of Canada, Ottawa, Canada). Limits of detection (LOD) and quantification (LOQ), calculated by the signal to noise ratio, for Diuron and Irgarol the LD and LQ were 0.5 and 1 $ng.g^{-1}$ respectively, for DCOIT it was 1 and 3 $ng.g^{-1}$, and for dichlofluanid 1 and 5 $ng.g^{-1}$.

2.5 Statistical analyses and degradation kinetics modeling

To verify the success of the homogenization process, the coefficient of variance (CV) between the surface, middle, and bottom of the spiking container was calculated for each biocide, as presented in formula I.

$$CV = \left(\frac{SD}{Mean\ concentration\ (surface, middle, bottom)}\right) * 100\% \qquad \text{(I)}$$

CV = coefficient of variance; SD = standard deviation

The time taken for a 50% decline in mass or concentration (DT50 or half-life) of each biocide was calculated through degradation kinetics models using the “xxDeg” R package, based on the NAFTA (NAFTA, 2015) and US EPA (US EPA, 2015) guidelines for degradation kinetics of pesticides in environmental media. For DCOIT and Dichlofluanid the degradation kinetics was calculated for the 1000 $ng.g^{-1}$ treatment, and the following models were evaluated: Bi-exponential model (DFOP) and the First-Order Multi-Compartment model (FOMC). For

Irgarol and Diuron all three concentration treatments (10, 100, and 1000 $ng.g^{-1}$) were assessed by DFOP, FOMC, Single first-order kinetics (SFO), and Hockey-stick model (HS).

Due to the limited time intervals (3) the model fitting could not be assessed statically, just by the comparison of the residuals and thought visual inspection (both are also considered according to the guidelines). The presented models were chosen based on the fitting. We acknowledge that the absence of the statistical fitting validation may be a limitation, however, our results and discussion provide a first glance at the degradation of such compounds in sediment, providing an important data that can be used in further and more detailed studies.

3. Results and Discussion

The concentrations of antifouling biocides in each time as well as the CV for the homogenization in each treatment are presented in the table 1. Dichlofluanid presented the worst homogenization with CV > 30% indicating heterogenicity of the data. For DCOIT, Irgarol and Diuron, the results were similar, with better homogenization seen at the 1000 $ng.g^{-1}$ and 100 $ng.g^{-1}$. The worst CV were found at 10 $ng.g^{-1}$ with CV>30% for T0 and T24 for all biocides. Overall, these results indicate that spiking procedures with higher concentrations are easier to be achieved and homogenized (Figure 2). The USEPA (2001) guideline suggests a 4 h continuous homogenization, or 60 seconds twice a day for one week. These two methods are impractical for some antifouling biocides due to their short half-lives.

Table 1: concentrations of antifouling biocides in T0, 6 and 14h after spiking under equilibrium condition. a, b, and c indicate the coefficient of variance between the aliquots from surface, middle and bottom.

Antifouling Biocides	Nominal concentration ($ng.g^{-1}$)	Time		
		T0	6 h	24 h
DCOIT (LD = 1; LQ = 3)	Blank	<LQ	<LQ	<LQ
	10	3.5 ± 1.1[b]	<LQ	<LQ
	100	4.88	3.5 ± 0.3[a]	3.5 ± 0.8[b]
	1000	7616.8 ± 1226.2[a]	3480.7 ± 776.6[b]	2558.9 ± 337.6[a]
Diuron (LD = 0.5; LQ = 1)	Blank	<LQ	<LQ	<LD
	10	22.6 ± 7.7[c]	15.2 ± 1.1[a]	13.8 ± 4.7[c]
	100	214.6 ± 61[b]	194.2 ± 14.6[a]	174.5 ± 29.2[a]
	1000	4694.3 ± 241.4[a]	1977.6 ± 337[b]	1712.6 ± 177.0[a]
Irgarol (LD = 0.5; LQ = 1)	Blank	<LQ	<LQ	<LQ
	10	26.2 ± 7[c]	18. ± 2.8[a]	16.8 ± 5.4[c]
	100	180.9 ± 14.6[a]	166.3 ± 35.7[a]	159.2 ± 30.8[c]
	1000	4882.5 ± 268.8[a]	2369.2 ± 584.4[c]	2029.4 ± 244.7[a]

Dichlofluanid (LD = 1; LQ = 5)	Blank	<LQ	<LD	<LQ
	10	<LD	<LQ	<LD
	100	<LQ	<LQ	<LQ
	1000	1189.5 ± 460.4[c]	157.7 ± 36[c]	16.9 ± 7.4[c]

a = CV ≤ 15% = homogeneous data;
b = 30% ≤CV> 15%; = average dispersion;
c= CV> 30% = heterogeneous data;

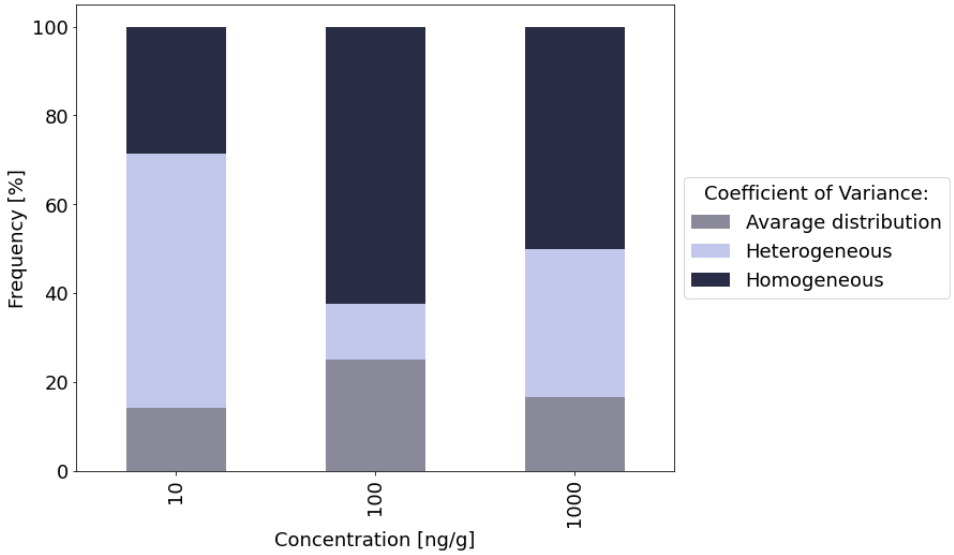


Figure 2: Coefficient of variance (CV) between surface, middle, and bottom of the spiking container for each tested concentration (10 ng.g^{-1}, 100 ng.g^{-1} and 100 ng.g^{-1}) . CV represents the success of the homogenization process, where CV ≤ 15% = homogeneous data; 30% ≤ CV > 15% = average dispersion; CV> 30% = heterogeneous data.

The antifouling biocides presented a fast-initial degradation phase followed by a slower one, a classic pattern of bi-phasic degradation kinetics. In the present study, the single first-order kinetics (SFO) and 3 bi-phasic degradation models (HS, DFO, and FOMC models) were evaluated. Data were only presented for models that could run the available dataset.

The HS model consists of two sequential first-order curves, it assumes that the biocide concentration initially declines according to a first-order kinetics with a constant rate k1, and at a certain point in time, the constant rate changes to a different value (k2). The FOMC model considers the sediment matrix heterogeneous, and this is accounted for the model by dividing the soil into many sub-compartments each with a different first-order k1. The DFOP model deals with the non-linear degradation by calculating two degradation coefficients which are later integrated. (FOCUS, 2006).

The tested models calculated inconsistent half-life values for biocides that didn't reach ≥50% of degradation. This pattern occurred mainly in the lowest concentrations (10 - 100 ng.g-1) where observed degradation over the 24 h ranged from 0% to 36% (far below the 50%

threshold). For these cases, we considered that the model's results were unreliable, and that new analyses would require more sampling points and longer duration to be evaluated. Even so, we think that it is important to share this data with the scientific community, which may provide important information and insights for future work. On the other way, for all biocides at 1000 $ng.g^{-1}$ the degradation was greater than 50% during the experiment, and the models were consistent, so we considered these data much more accurate and reliable.

DCOIT was not detected at 10 $ng.g^{-1}$, and presented no degradation at 100 ng.g-^{1} during 24 h. At 1000 $ng.g^{-1}$, compared to T0, T6 and T24 presented degradation rates of 54% and 66%, respectively. Figure 3 contains the degradation kinetics for DCOIT. The half-life ranged from 2.38 h for the DFOP model to 4.87 h for the HS model.

Thomas et al. (2003) corroborates our results, by calculating a half-life <0.5 days for DCOIT. Due to the Hight log Koc of DCOIT (4.19), it tends to bind and be partitioned to the sediment, which may act as a reservoir for DCOIT (Chen & Lam, 2017). DCOIT has already been detected in marine sediments worldwide (Campos, et al. 2021) where concentrations have reached 281 $ng.g^{-1}$in some harbors, as observed in Korea by Lee et al. (2015).

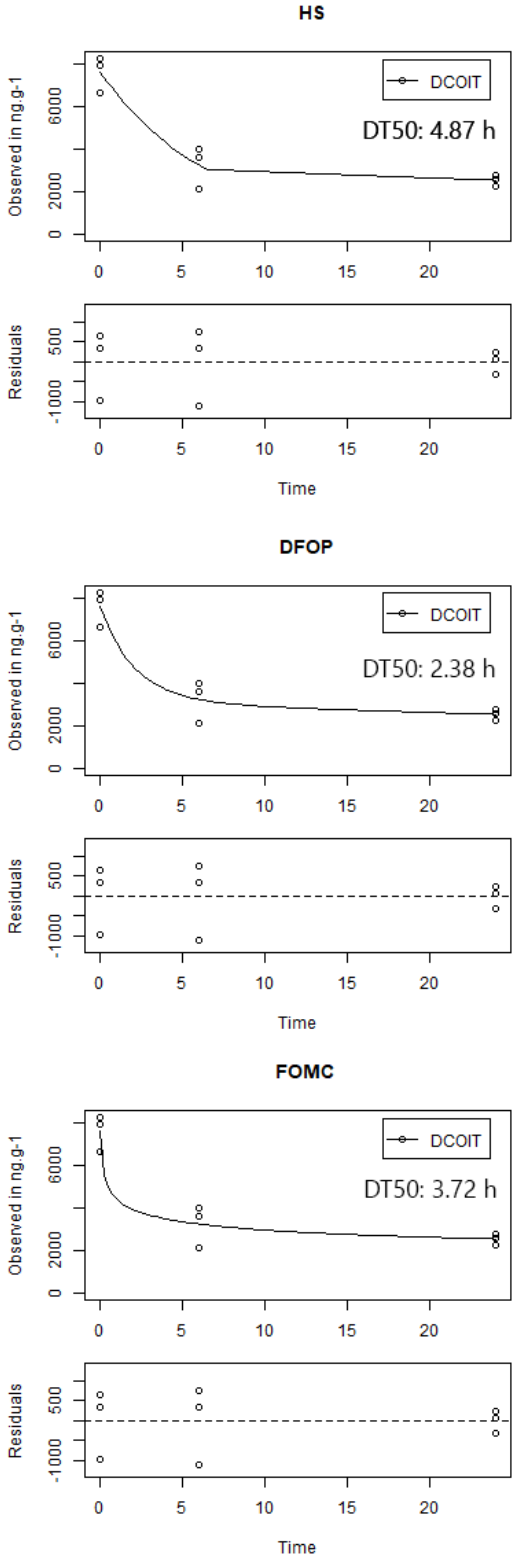


Figure 3: Degradation kinetics of DCOIT (1000 ng.g-1), under Hockey-stick model (HS), Bi-exponential model (DFOP) and the Gustafson and Holden model (FOMC) with their respective time taken for a 50% decline in concentration (half-life = DT50)

As DCOIT, diuron presented a bi-phasic degradation kinetics (figure 4). In the 10 ng.g^{-1} the degradation rates at T6 and T24, compared to T0, were 33% and 39% respectively. In 100 ng.g^{-1} such rates were 10 % and 19% respectively, and in 1000 ng.g^{-1} they were 58% and 64%. The degradation kinetics model for 10 ng.g^{-1} indicated a half-life of 493 h by the FOMC model, 70.8 h by the DFOP model, and 39.9 h by the SFO, due to the high residue (difference between measured and estimated concentration). In the 100 ng.g^{-1} just the SFO model could calculate the half-life (84.7 h, cf. Figure 5), which we considered unreliable due to the observed degradation, indicating that this model did not suit well for the dataset. For the 1000 ng.g-1 treatment the FOMC, DFOP, and HS model presented more similar half-live results, determined as 1.15, 2.97, and 4 h respectively (figure 6).

Diuron has a well-documented persistence in the marine environment (Faÿ et al., 2018), with a half-life in seawater ranging from 1 month to 1 year depending on the environmental conditions (Dafforn et al., 2011), biodegradation is the main route of degradation. Diuron high Koc of 485 indicates a high sediment adsorption capacity and, therefore, a heterogeneous repartition in soil (Giacomazzi and Cochet, 2004). Thomas et al. (2003) determined through a pseudo-first order kinetic model that Diuron have an anaerobic half-life of 14 days in marine sediments. The highest diuron concentration in coastal sediments (0.14 µg.g^{-1}) was found in Korean harbor areas (Lam et al., 2017).

Irgarol at 10 ng.g^{-1} presented degradation rates of 31% and 39% at T6 and T24 respectively, compared to T0. These data indicate that after 6h the degradation rate reduced, and the degradation rate reached a “steady” state (Figure 7). The FOMC and DFOP model indicate half-lives of 5379 h (7.3 months) and 109 h (4.5 days) respectively. For the 100 ng.g^{-1} Irgarol degraded 9% and 19% at T6 and T24 compared to T0, and exhibited the same pattern of equilibrium after 6 h. The FOMC and SFO model indicated half-lives of 6.9x10^{8} h and 145 h respectively (figure 8), as the observed concentrations did not reach the 50% degradation threshold during the experiment in addition to the limited time points, the model could not estimate the half-life properly thus resulting in inconsistent half-life values. Considering that the SFO model for 100 ng.g^{-1} gave a similar half-life value compared to the DFOP model for 10 ng.g^{-1}, we considered the 145h from the SFO was more reliable than the value produced by the DFOP. At least at 1000 ng.g^{-1}, T6 and T24 degraded 51% and 58% compared to T0. The half-lives for the three tested models were consistent, and the results indicated respective values of 4.5 h (FOMC model), 5.2h (DFOP model), and 5.5 h (HS model) (figure 9)

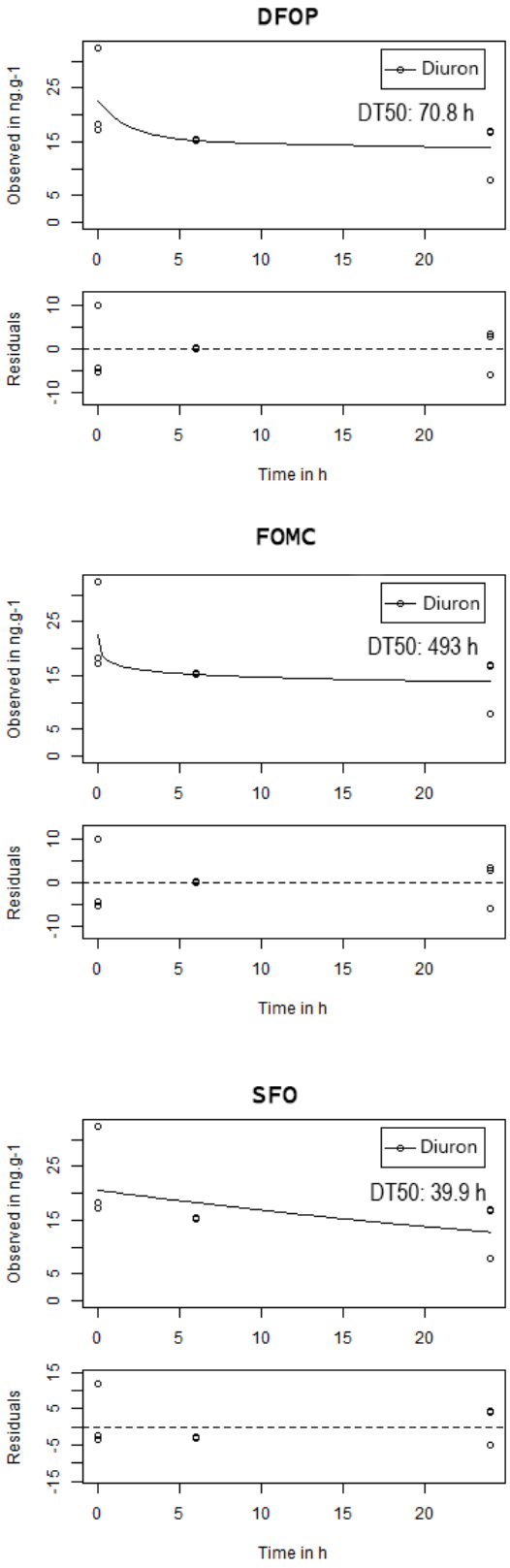


Figure 4: Degradation kinetics of Diuron for 10 ng.g^{-1} Spiking, under Single first-order kinetics (SFO), Bi-exponential model (DFOP) and the Gustafson and Holden model (FOMC) with their respective time taken for a 50% decline in mass or concentration (half-life = DT50)

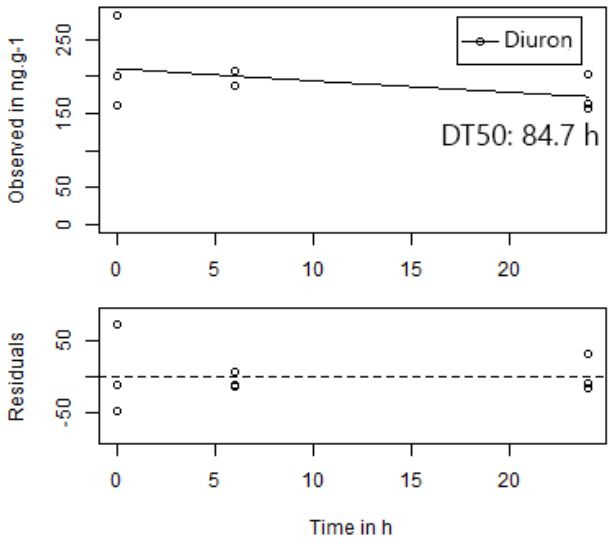


Figure 5: Degradation kinetics of Diuron for 100 ng.g^{-1} Spiking, under Single first-order kinetics (SFO) with their respective time taken for a 50% decline in mass or concentration (half-life = DT50)

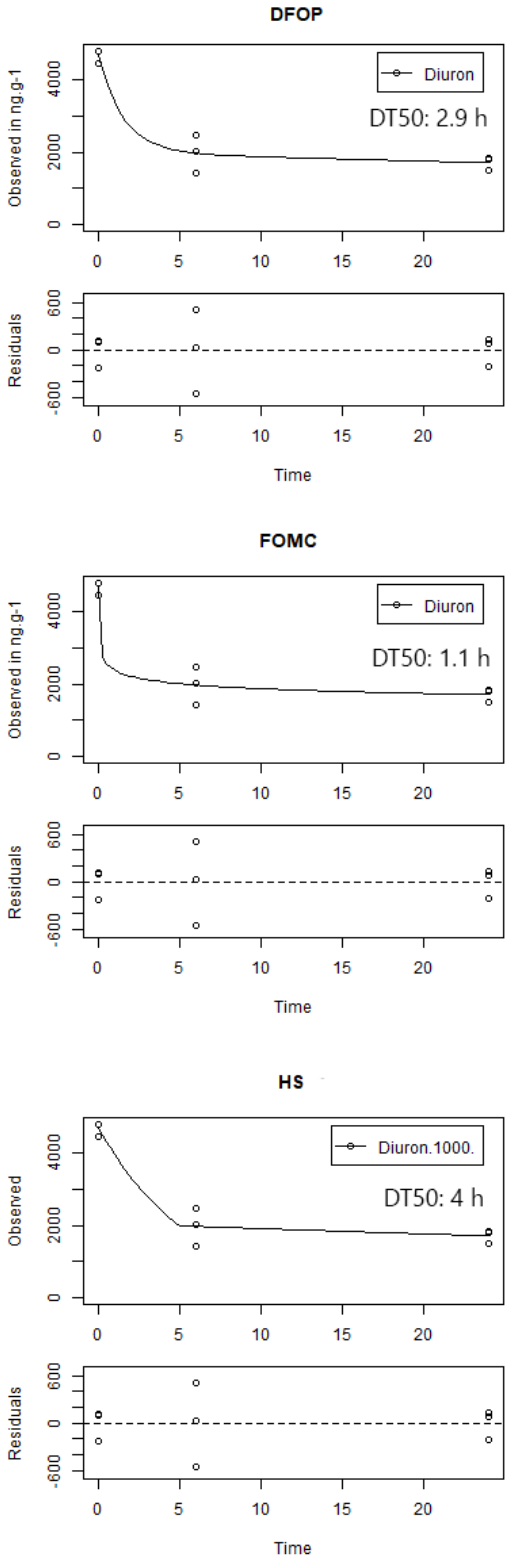


Figure 6: Degradation kinetics of Diuron for 1000 ng.g^{-1} Spiking, under Hockey-stick model (HS), Bi-exponential model (DFOP) and the Gustafson and Holden model (FOMC) with their respective time taken for a 50% decline in mass or concentration (half-life = DT50)

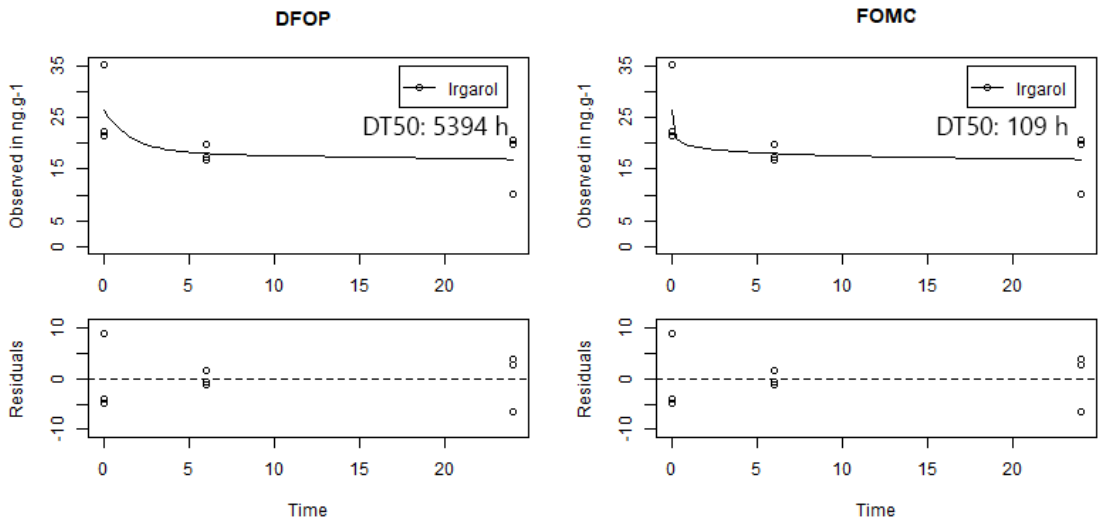


Figure 7: Degradation kinetics of Irgarol (10 ng.g^{-1}), Bi-exponential model (DFOP) and the Gustafson and Holden model (FOMC) with their respective time taken for a 50% decline in mass or concentration (half-life = DT50)

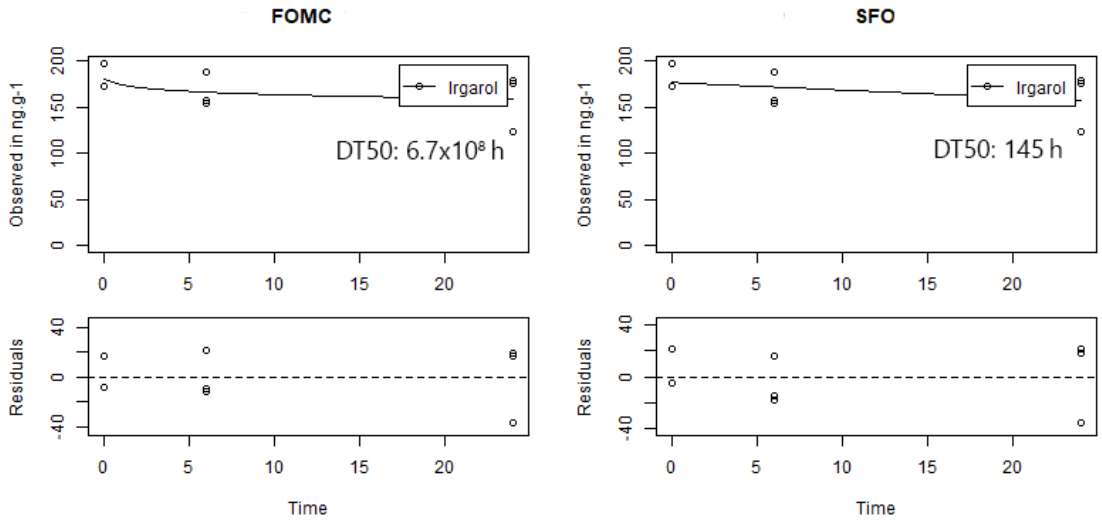


Figure 8: Degradation kinetics of Irgarol (100 ng.g^{-1}), under first-order kinetics (SFO) and and the Gustafson and Holden model (FOMC) with their respective time taken for a 50% decline in mass or concentration (half-life = DT50)

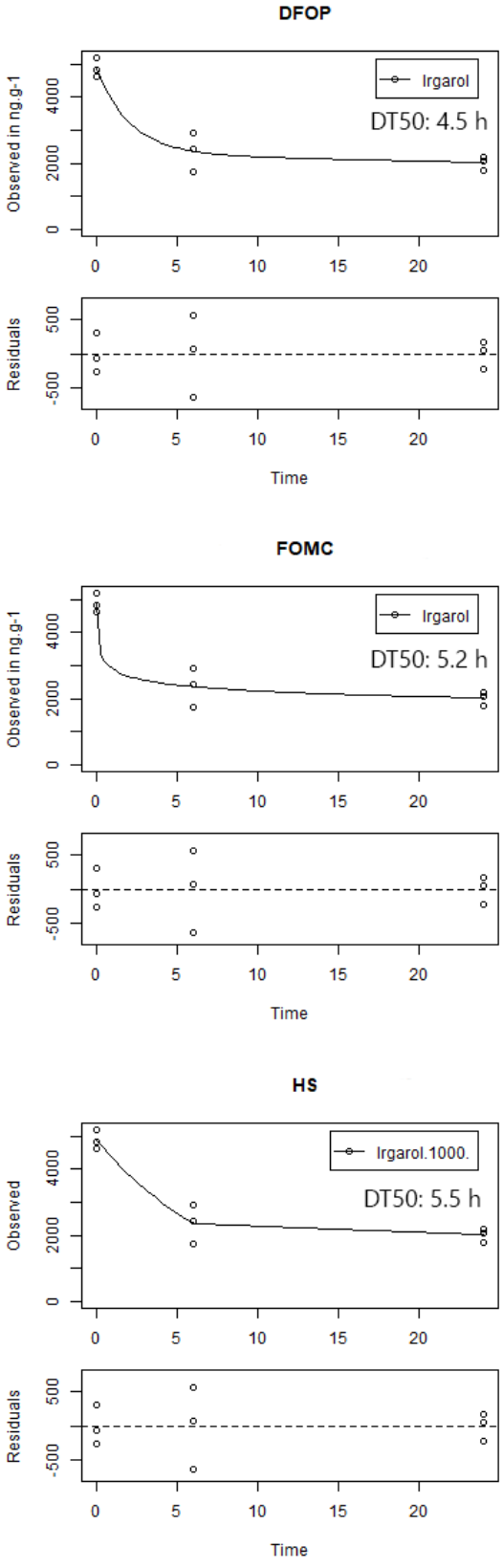


Figure 9: Degradation kinetics of Irgarol (1000 ng.g^{-1}), under Hockey-stick model (HS), Bi-exponential model (DFOP) and the Gustafson and Holden model (FOMC) with their respective time taken for a 50% decline in mass or concentration (half-life = DT50)

In sea-water Irgarol exhibits a relatively long half-life of up to 350 days (Omae, 2003). Thomas et al. (2003) observed no degradation of Irgarol during 12 days in marine sediment. In addition, Irgarol has been detected in sediments word-wide in concentrations up to 89 μg kg−1 as found in a Brazilian harbor (Viana et al. 2019)

Our data indicates that the Irgarol half-life varies significantly depending on the spiked concentration, even with the same sediment and equilibrium parameters (temperature and light). Nevertheless, the results obtained at the 1000 $ng.g^{-1}$ treatment were more consistent. In addition, all treatments indicates that after 6 h Irgarol degradation slows down, and the concentration stabilizes (at least up to the 24h analyzed). Considering such estabilization, an equilibrium phase period of 24h should be adequate for sediment spiking procedures. Yet, biocides degradation is highly susceptible to biodegradation and dependent on the physicochemical properties of the media (Thomas 2001). Fine sediments with a high content of organic matter are highly adsorbent and tend to be anoxic, with an anaerobic microbial community. Coarse sediments tend to have lower organic matter content and adsorption capacity, which may result in xenobiotics dissolved on the interstitial water (Campos *et al*. 2015). Accordingly, Gatidou *et al.* (2007) observed a strong correlation between antifouling biocides concentrations such as Irgarol and the sediment physicochemical properties, namely a positive correlation to particles <63 μm, and negatively correlation to pH and organic carbon. In the present study, the sediment tested was sandy, with low content of organic matter. Thus, we highlight that the behavior of such biocides in muddy and/or organically rich sediments may differ than that reported in the present study.

Dichlofluanid was only detected at 1000 ng.g-1, with degradation rates of 87% and 99% at T6 and T24 respectively. The FOMC and DFOP presented similar half-lives of 1.436 and 1.92 h respectively. Dichlofluanid was the biocide with the lowest half-life. This compound has low solubility in water (< 2 $mg.L^{-1}$) and Log Koc of 3.7 (Wezel and Vlaardingen, 2004). Despite its low solubility, accumulation in sediment is unlikely to occur, due to its rapid degradation in water (half-life of 1.2 h in seawater). Hamwijk et al. (2005) observed half-lives ranging between 1.2 and 3 h in water-sediment systems at 20°C and pH 7.5 - 8.1. Thomas et al. (2003) corroborates our results, by the determination of half-lives of <0.5 days for Dichlofluanid. Despite its rapid degradation, the highest observed environmental concentrations of Dichlofluanid in coastal sediments range from 0.016 $\mu g.g^{-1}$ in Brazil (Abreu et al., 2020) to 0.8 $\mu g.g^{-1}$ in Malaysia (Mukhtar et al., 2019).

The tested antifouling biocides showed bi-phasic degradation kinetics with two distinct degradation rates (K1 and K2) divided by a breakpoint (observed to be within 6h). The K1 corresponded to a sharp and exponential degradation rate, after the breakpoint, the degradation rate (K2) reduced drastically and assumed a linear pattern. Therefore, we considered 24h a viable time for the spike equilibration phase, as after 6h the concentrations tends to stabilize and then be more reliable for ecotoxicological testing (apart from dichlofluanid that completely degraded within 24h).

4. Conclusion

Our data indicate that the 24 h equilibrium phase during the spiking procedure is adequate for DCOIT, Irgarol, and Diuron, which presented a significant reduction of the degradation after 6h (breaking point). However, a researcher studying the fate and effects of these compounds should consider the initial concentration and the degradation rate the compound will undergo during the process. At 1000 $ng.g^{-1}$ the half-lives of DCOIT and Diuron were < 5 h, while the half-life for dichlofluanid was < 2 h and for Irgarol it was < 6h. Regarding Dichlofluanid, in 6h it presented a degradation >90%, thus indicating that the 24 h period of equilibrium is not suitable for this compound.

5. References

Abreu, F. E. L., Natália, J., Braga, Í., & Fillmann, G. (2020). Are antifouling residues a matter of concern in the largest South American port ? 398(January). https://doi.org/10.1016/j.jhazmat.2020.122937

ASTM Internationa. (2008). Standard Guide for Collection, Storage, Characterization, and Manipulation of Sediments for Toxicological Testing and for Selection of Samplers Used to Collect Benthic Invertebrates (E 1391 – 03 (2008).

Caliman, A., Carneiro, L. S., Leal, J. J. F., Farjalla, V. F., Bozelli, R. L., & Esteves, F. de A. (2013). Biodiversity effects of benthic ecosystem engineers on the spatial patterns of sediment CH4 concentration in an urban Neotropical coastal lagoon. Acta Limnologica Brasiliensia, 25(3), 302–314. https://doi.org/10.1590/s2179-975x2013000300009

Campos, B. G. de, Buruaem, L., Figueiredo, G. De, Pauly, E., Carolina, A., Cruz, F., Monte, N., Irene, L., Paula, A., Rodrigues, D. C., Machado, W., Moledo, D., & Abessa, D. S. (2019). Integrating multiple lines of evidence of sediment quality in a tropical bay. Marine Pollution Bulletin, 146(July), 925–934. https://doi.org/10.1016/j.marpolbul.2019.07.051

Campos, B. G., Cruz, A. C. F., Buruaem, L. M., Rodrigues, A. P. C., Machado, W. T. V, & Abessa, D. M. S. (2016). Using a tiered approach based on ecotoxicological techniques to assess the ecological risks of contamination in a subtropical estuarine protected area. Science of the Total Environment, 544, 564–573. https://doi.org/10.1016/j.scitotenv.2015.11.124

Campos, Bruno Galvão de, Figueiredo, J., Perina, F., Abessa, D. de S., Loureiro, S., & Martins, R. (2021). Occurrence, effects and environmental risk of antifouling biocides (EU PT21): Are marine ecosystems threatened? Critical Reviews in Environmental Science and Technology, in press. https://doi.org/10.1080/10643389.2021.1910003

Chapman, P. M., Ho, K. T., Munns, W. R., Solomon, K., & Weinstein, M. P. (2002). Issues in sediment toxicity and ecological risk assessment. Marine Pollution Bulletin, 44(4), 271–278. https://doi.org/10.1016/S0025-326X(01)00329-0

Chen, L., & Lam, J. C. W. (2017). SeaNine 211 as antifouling biocide: A coastal pollutant of emerging concern. Journal of Environmental Sciences, 61, 68–79. https://doi.org/10.1016/j.jes.2017.03.040

Cruz, A. C. F., Davanso, M. B., Araujo, G. S., Buruaem, L. M., Santaella, S. T., de Morais, R. D., & Abessa, D. M. S. (2014). Cumulative influences of a small city and former mining activities on the sediment quality of a subtropical estuarine protected area. Environmental Monitoring and Assessment, 186(11), 7035–7046. https://doi.org/10.1007/s10661-014-3908-1

Dafforn, K. A., Lewis, J. A., & Johnston, E. L. (2011). Antifouling strategies: History and regulation, ecological impacts and mitigation. Marine Pollution Bulletin, 62(3), 453–465. https://doi.org/10.1016/j.marpolbul.2011.01.012

Faÿ, F., Horel, G., Linossier, I., & Vallée-réhel, K. (2018). Effect of biocidal coatings on microfouling: In vitro and in situ results. 114(2018), 162–172. https://doi.org/10.1016/j.porgcoat.2017.10.017

FOCUS. (2006). Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 2.0 Fate Studies .

Gatidou, G., Thomaidis, N. S., & Zhou, J. L. (2007). Fate of Irgarol 1051, diuron and their main metabolites in two UK marine systems after restrictions in antifouling paints. Environment International, 33(1), 70–77. https://doi.org/10.1016/j.envint.2006.07.002

Giacomazzi, S., & Cochet, N. (2004). Environmental impact of diuron transformation: A review. In Chemosphere (Vol. 56, Issue 11, pp. 1021–1032). Elsevier Ltd. https://doi.org/10.1016/j.chemosphere.2004.04.061

Hamwijk, C., Schouten, A., Foekema, E. M., Ravensberg, J. C., Collombon, M. T., Schmidt, K., & Kugler, M. (2005). Monitoring of the booster biocide dichlofluanid in water and marine sediment of Greek marinas. Chemosphere, 60(9), 1316–1324. https://doi.org/10.1016/j.chemosphere.2005.01.072

Lam, N. H., Jeong, H. ho, Kang, S. dong, Kim, D. J., Ju, M. J., Horiguchi, T., & Cho, H. S. (2017). Organotins and new antifouling biocides in water and sediments from three Korean Special Management Sea Areas following ten years of tributyltin regulation: Contamination profiles and risk assessment. Marine Pollution Bulletin, 121(1–2), 302–312. https://doi.org/10.1016/j.marpolbul.2017.06.026

Lee, M. R. N., Kim, U. J., Lee, I. S., Choi, M., & Oh, J. E. (2015). Assessment of organotin and tin-free antifouling paints contamination in the Korean coastal area. Marine Pollution Bulletin, 99(1–2), 157–165. https://doi.org/10.1016/j.marpolbul.2015.07.038

Maranho, L. A., Abreu, I., Santelli, R., Cordeiro, R. C., Soares-gomes, A., & Moreira, L. B. (2009). Sediment toxicity assessment of Guanabara Bay , Rio de Janeiro , Brazil . 2009(56), 851–855.

Marcus, N. H., & Marcus, N. H. (1998). Minireview: The importance of benthic-pelagic coupling and the forgotten role of life cycles in coastal aquatic systems. Limnology and Oceanography, 43(5), 763–768. https://doi.org/10.4319/lo.1998.43.5.0763

Martins, S. E., Fillmann, G., Lillicrap, A., & Thomas, K. V. (2018). Review: Ecotoxicity of organic and organo-metallic antifouling co-biocides and implications for environmental hazard and risk assessments in aquatic ecosystems. Biofouling, 34(1), 34–52. https://doi.org/10.1080/08927014.2017.1404036

Mukhtar, A., Mohamat-Yusuff, F., Zulkifli, S., Harino, H., Ismail, A., & Inoue, K. (2019). Concentration of Organotin and Booster Biocides in Sediments of Seagrass Area from Sungai Pulai Estuary, South of Johor, Malaysia. Environments, 6(2), 26. https://doi.org/10.3390/environments6020026

NAFTA. (2015). Guidance for Evaluating and Calculating Degradation Kinetics in Environmental Media. https://www.epa.gov/sites/production/files/2015-09/documents/degradation-kin.pdf

Omae, I. (2003). Organotin antifouling paints and their alternatives. Applied Organometallic Chemistry, 17(2), 81–105. https://doi.org/10.1002/aoc.396

Thomas, K. (2001). The environmental fate and behaviour of antifouling paint booster biocides: A review. Biofouling, 17(1), 73–86. https://doi.org/10.1080/08927010109378466

Thomas, K. V., McHugh, M., Hilton, M., & Waldock, M. (2003). Increased persistence of antifouling paint

biocides when associated with paint particles. Environmental Pollution, 123(1), 153–161. https://doi.org/10.1016/S0269-7491(02)00343-3

US EPA. (2001). Methods for Collection, Storage and Manipulation of Sediments for Chemical and Toxicological Analyses:Technical Manual (EPA-823-B-01-002).

US EPA. (2015). Standard Operating Procedure for Using the NAFTA Guidance to Calculate Representative Half-life Values and Characterizing Pesticide Degradation. https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/standard-operating-procedure-using-nafta-guidance

Wezel, A. P. Van, & Vlaardingen, P. Van. (2004). Environmental risk limits for antifouling substances. 66, 427–444. https://doi.org/10.1016/j.aquatox.2003.11.003

Yebra, D. M., Kiil, S., & Dam-Johansen, K. (2004). Antifouling technology - Past, present and future steps towards efficient and environmentally friendly antifouling coatings. Progress in Organic Coatings, 50(2), 75–104. https://doi.org/10.1016/j.porgcoat.2003.06.001

Article 2 – A preliminary study on multi-level biomarkers response of the tropical oyster *Crassostrea brasiliana* to the antifouling biocide 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT).

Bruno Galvão de Campos [a], Mayana Karoline Fontes [a], Paloma Kachel Gusso-Choueri [b], Gabriela Pustiglione Marinsek [a], Caio Rodrigues Nobre [a], Beatriz Barbosa Moreno , Fiamma Eugenia Lemos Abreu [e], Gilberto Fillmann [e] Renata de Britto Mari [a], Denis Moledo de Souza Abessa [a]

[a] *Bioscience Institute, São Paulo State University (UNESP), Praça Infante Dom Henrique, s/n, 11330-900, São Vicente, SP, Brazil*

[b] *Department of Ecotoxicology, Santa Cecília University, Santos, São Paulo, Brazil*

[c] *Institute for Advanced Studies of Ocean, São Paulo State University (UNESP), Av. João Francisco Bensdorp, 1178, 11350-011, São Vicente, SP, Brazil*

[d] *Instituto do Mar, Universidade Federal de São Paulo (UNIFESP), Rua Maria Máximo, 168, 11030-100, Santos, SP, Brazil*

[e] *Instituto de Oceanografia, Universidade Federal do Rio Grande (FURG), Av. Itália s/n, km 8, Campus Carreiros, 96203-900, Rio Grande, RS, Brazil*

Corresponding Author: *Bruno Galvão de Campos, São Paulo State University (UNESP), Praça Infante Dom Henrique, s/n, Zipcode: 11330-900 São Vicente, São Paulo, Brazil. E-mail: b.campos@unesp.br*

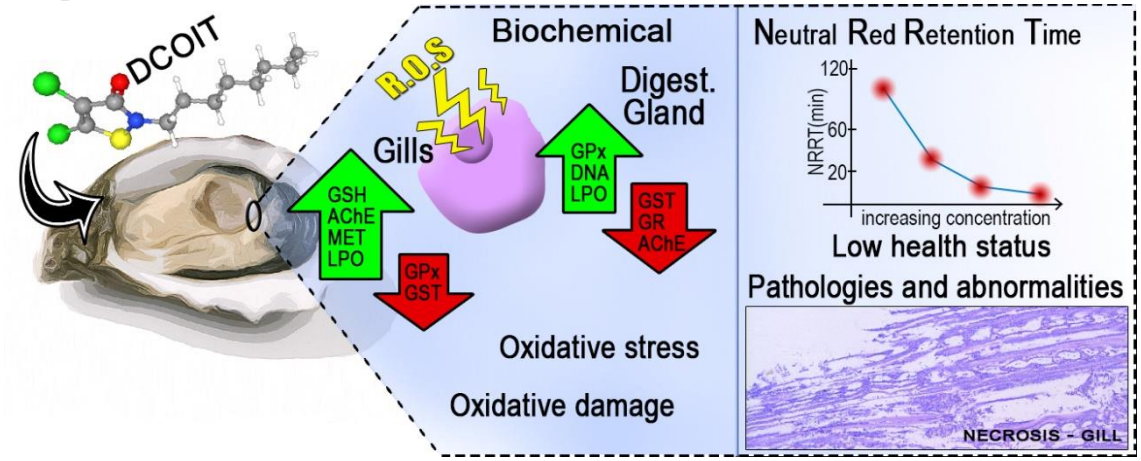
Status*:* Published in the *Marine Pollution Bulletin*

DOI*:* https://doi.org/10.1016/j.marpolbul.2021.113241

Highlights

- DCOIT caused biochemical, cellular, and histopathological effects *on C. brasiliana*
- Digestive gland and gills presented tissue-specific responses at biochemical level
- Modulation of antioxidant enzymes led to oxidative stress and lipid peroxidation
- DCOIT damaged lysosomal membranes and increases histopathological pathologies
- Environmental concentrations of DCOIT are enough to affect *C. brasiliana*

Graphical Abstract



Abstract

This study investigated the sublethal effects of environmentally relevant concentrations of DCOIT on the neotropical oyster *Crassostrea brasiliana*. Gills and digestive glands of animals exposed to increasing concentrations of DCOIT were analyzed for biochemical, cellular, and histopathological responses. Exposure to DCOIT (0.2 to 151 µg L^{-1}) for 120h triggered oxidative stress in both tissues (through the modulation of GPX, GST, GSH and GR), which led to damage of membrane lipids (increase of LPO and reduction of the NRRT). DCOIT increased histopathological pathologies in gills, such as necrosis, lymphocyte infiltration and epithelial desquamation. This study showed that short term exposure to environmental concentrations of DCOIT causes negative effects on *C. brasiliana* at biochemical, physiological, and histological levels. Therefore, the use of DCOIT as a booster biocide in antifouling paints should be further assessed, as it may cause environmental hazards to marine organisms.

Keywords: Sea-Nine, bivalve, toxicity, Neutral red, histopathology, environmental hazard

1. Introduction

The antifouling biocide 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT), commercially known as Sea-Nine 211™, was developed to replace organotin-based paints, which were banned in 2008 by the International Maritime Organization (IMO) due to their persistent and bioaccumulative nature and endocrine disrupting capability (Chen and Lam, 2017a). Although the United States Environmental Protection Agency once considered DCOIT as an environmentally friendly antifouling due to its rapid degradation (USEPA, 1996), DCOIT has been detected worldwide in water and sediment, as observed in table 1.

Table 1: Environmental concentrations of DCOIT in seawater (ng L^{-1}) and sediment (ng g^{-1})

Country	Matrix	Concentration	Reference
Spain	Water	2,600 – 3,700	Martínez and Barceló, 2001
Denmark		5 - 283	Steen et al., 2004
Japan		<LOD - 100	Tsunemasa et al., 2006
Korea		<LOD - 6	Kim et al., 2014
Greece		<6.4 - 49	Sakkas et al., 2002
Korea	Sediment	<LOD - 281	Kim et al., 2014
Brazil		<0.2 - 63.6	Abreu et al., 2021
Japan		<0.03 - 110	Harino et al., 2007

<LOD – below limit of detection

The half-life of DCOIT depends on environmental conditions, and ranges from 2.6 days in natural seawater without sunlight at 30°C (Harino et al., 2005), up to not noticeable degradation after 7 days in artificial non-sterile saltwater at 25°C (Figueiredo et al., 2019).

Several studies have demonstrated that DCOIT has a high and non-selective toxicity to marine organisms (Chen and Lam, 2017). Several effects were reported on aquatic organisms, such as growth inhibition of bacteria and photosynthetic species, acute toxicity on micro and macroinvertebrates, and embryotoxicity on mollusks, echinoderms and fish (Campos et al., 2021), immunosuppression (Cima et al., 2008), oxidative stress (Cima et al., 2013) and reproductive and endocrine disruption (Chen et al., 2017). Nevertheless, the mechanism of action explaining how DCOIT induces a variety of negative effects is still unclear. DCOIT presents a predicted non-effect concentration (PNEC) of 0.2 µg L^{-1} in water (Figueiredo et al., 2020) and 0.97 ng g^{-1} dw in sediment (Abreu et al., 2021), while the estimated effect concentration values (EC_{50}) range from 0.4 µg L^{-1} for the coccolithophore *Emiliania huxleyi* (Devilla et al., 2005) to 3.25 mg L^{-1} for the polychaete *Hediste diversicolor* (Figueiredo et al., 2019). Despite the existence of toxicity data for marine organisms of temperate regions exposed to DCOIT, few studies have addressed its adverse sub-lethal effects to tropical and subtropical species (Fonseca et al., 2020; Gabe et al., 2021).

Although no relevant bioaccumulation of DCOIT was observed in fish (Jacobson and Willingham, 2000), its relatively high K_{ow} (2.8 - 6.4) (Chen and Lam, 2017a) suggests that this compound has potential to bioaccumulate in animal tissues. Thus, in order to predict potential ecological risks caused by DCOIT to marine ecosystems, it is desirable to identify and comprehend its potential subchronic effects, as well as its mode of action (MoA). The present

study evaluated the effects on the neotropical bivalve *Crassostrea brasiliana* exposed to increasing concentrations of DCOIT, assessing the responses in two soft-tissues (gills and digestive gland) at different levels of biological organization (biochemical, cellular, and histopathological).

2. Material and Methods

2.1 Chemicals and exposure solutions

The concentrations of DCOIT (CAS number 64359-81-5, purity ≥ 99%, Sigma-Aldrich®) used in the bioassays (0.2 µg L^{-1}, 57 ± 7.1 µg L^{-1}, and 151 ± 0.8 µg L^{-1}) were prepared by diluting a stock solution (real concentration of 90 ± 8 mg L^{-1} prepared in acetone) in brackish water (salinity:15). These concentrations comprise the highest environmental concentration of 3.7 µg L^{-1} found by Martínez et al.(2001), as well as the average effect concentration (EC_{50}) of DCOIT to marine bivalves (101 ± 0.3 µg L^{-1}) (Bellas, 2006; Environmental Protection Agency [EPA], 1992; Willingham and Jacobson, 1996).

2.2 Chemical quantification

The real concentration of DCOIT (stock solution, and water concentrations at the end of the experiment) was measured in duplicate according to Harino et al. (2005) with modifications. Briefly, 1 mL of the DCOIT stock solution was diluted in 50 mL of MiliQ water and extracted by solid phase extraction (SPE) using C-18 cartridges (6 mL, 500 mg, Supelco®, Sigma-Aldrich). The analyte was eluted with ethyl acetate (2 × 2 mL), and the eluates were elevated to 800 mL. One milliliter of the extract was fortified with PCB30 (10 ng mL^{-1}) as an internal standard and analyzed by gas-phase chromatography with electron capture detector (Perkin Elmer Clarus 500 GC-ECD) (chromatogram presented as Supplementary material – Fig. S1). The oven temperature was set as 2 min at 80 ◦C, then to 320 ◦C at a rate of 15℃ min^{-1} (hold for 5 min). Quantification was performed by a calibration curve using successive dilutions of a DCOIT stock solution in ethyl acetate (100 µg L^{-1}).

Quality assurance and quality control (QA/QC) procedures were based on the analysis of procedural blanks and standard reference material. Recoveries from the internal standard were > 90%. LOQ and LOD were 0.9 µg L^{-1} and 0.3 µg L^{-1}, respectively, calculated as the average of the equipment noise plus 10 times the standard deviation for LOQ and 3 times the standard deviation for LOD.

The nominal concentration of the exposure solutions was calculated based on the measured concentration of the stock solution.

2.3 Oyster acquisition and acclimation

Adult oysters (8.2 cm ± 1.1) were obtained from an oyster farming located in Cananéia, São Paulo, Brazil, in a marine protected area. This region was considered as reference site due to its high biodiversity and absence of anthropogenic impacts (Campos et al, 2019, Cruz et al., 2014). Besides, a recent study showed low levels of metals and PAHs and lack of toxicity in sediments from Cananéia (Moreira et al., 2021). In laboratory, the oysters were acclimated for 72h under controlled clean conditions (constant aeration, photoperiod of 16h:8h (L:D), temperature of 25 ± 2 °C, and daily fed *ad libitum* with the green microalgae *Tetraselmis* sp.).

To certify the health status of the organisms used in the bioassays, the lysosomal membrane stability (LMS) assay was performed in 12 organisms from the acclimating tank (T0), prior to the beginning of the experiment. LMS was determined by Neutral Red Retention Time (NRRT) method (Lowe and Pipe, 1994).

2.4 Biomarker assay

2.4.1 Oyster exposure

After the acclimation, three replicates containing 4 individuals of *C. brasiliana (n=12)* were prepared per treatment in 5 L glass aquaria each. Both water (negative control with artificial brackish water) and solvent controls (0.04% acetone, corresponding to the highest solvent concentration used in the exposure treatments) followed the same experimental design. The experiments lasted 120h (with constant aeration, temperature 25 ± 1ºC, salinity 15, and photoperiod 16:8 h (light:dark)). Physicochemical parameters were monitored at the beginning and the end of the exposure. Considering the degradation of DCOIT, a complete water renewal and recontamination of the media were performed every 24 h. The new test solutions were prepared at the time of use by diluting aliquots of the stock solution in brackish water. At the end of the exposure, two aliquots of 50 mL were taken from each treatment to DCOIT quantification.

After 120 h of exposure, 300 – 500 µL of hemolymph of each individual was withdrawn from their posterior adductor muscle for the Neutral Red Retention Time Assay (NRRT). Then, each oyster was weighed and dissected. Gills were removed for both histopathological and biochemical analyses and digestive glands were separated for biochemical biomarkers. For histopathological analysis, 5 individuals of each treatment were randomly selected and the posterior region of the first gill lamella was removed and fixed in ALFAC solution (ethyl alcohol (85%), formaldehyde (10%) and glacial acetic acid (5%), the remaining lamellas were

used for biochemistry. For biochemistry the tissues were transferred to cryogenic tubes and stored at -80ºC.

2.4.2 Biochemical biomarkers

Gills and digestive gland soft tissue samples were homogenized with Tris-HCL buffer (Tris 50 mM; EDTA 1 mM; DTT 1 mM; Surose 50 mM; KCl a 150 mM, PMSF 1 mM, pH 7,6) at 4 %w/v. Then, an aliquot was separated for total protein quantification, lipid peroxidation (LPO) and DNA damage. LPO was spectrophotometrically evaluated (λ_{ex} 516 nm; λ_{em} 600 nm) through the thiobarbituric acid reactive substances determination (TBARS), according to Oakes and Kraak (2003). LPO results were expressed as µg of TBARS /mg protein. DNA damage was measured by the alkaline precipitation assay (Olive, 1988) using fluorescence (λ_{ex} 360 nm; λ_{em} 450 nm) to assess DNA strand breaks (Gagné et al., 1995) after staining with Hoechst dye. Results were expressed as µg of DNA strands/mg protein. Total protein was determined for both aliquots and tissues, according to Bradford (1976).

The remaining homogenate were centrifuged at 12000 g for 20 min at 4 °C, and the supernatant was then used for the quantification of total protein content, reduced glutathione (GSH), the activity of glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST) and acetylcholinesterase (AChE) enzymes, and the mitochondrial respiratory activity (MET). GSH contents were measured by the determination of sulfhydryl groups as described by Sedlak & Lindsay (1968). GST, GPx, and GR activities were determined spectrophotometrically at 340 nm, following the protocols developed by McFarland et al. (1999) and the adaptations to microplate proposed by Frasco and Guilhermino (2002). AChE activity was spectrophotometrically measured at 415 nm by the colorimetric method of Ellman et al. (1961). Results were expressed as nmol of substrate hydrolyzed per min per mg protein in the case of CAT, GPx, GR and GST and nmol of substrate hydrolyzed per min per mg protein for AChE activity. MET was determined only in the gill soft tissues through the isolation of mitochondria using the reduction of p-iodonitrotetrazolium dye method (Packard, 1971; Smolders et al., 2004). Results were expressed as RFU/min/mg protein.

2.4.3 Cytological biomarkers

The stability of lysosomal membranes of hemocytes was evaluated using the NRRT Assay (Lowe and Pipe, 1994). The hemolymph was withdrawn from the posterior adductor muscle of living bivalves. The hemocytes were stained with the neutral red dye (40 µL) and

incubated in the dark for 15 min. The test's endpoint was the time when the dye loss of lysosomes was evident in at least 50% of the hemocytes, which is evidenced by abnormalities of the cells' color and shape. The retention time was calculated in relation to the longest time the dye remained retained.

2.4.4 Morphological biomarkers - Gills

The posterior region of the first gill lamella was removed and fixed in the ALFAC solution for 16h. Thus, gills tissues were dehydrated in increasing percentages of alcohol and diaphanized in xylol. Gills were embedded using Paraplast Plus® (Sigma-Aldrich), submitted to semi-serial cross-sections with 5 μm thickness and finally stained using the Hematoxylin and Eosin (HE) technique, for general evaluation of organ histopathological alterations (Bernet et al., 1999, with adaptations).

The histopathological index was quantified according to Bernet et al. (1999), using a pre-established importance factor (w) according to the severity of injury, as follows: (1) lesions with minimal pathological importance, (2) intermediate lesions, and (3) lesions with high pathological significance that are usually irreversible and enough to cause partial or total loss of the organ function. Lesions found in the gills were classified according to a score (a) ranging from 0 to 6, depending on degree and extent of injury, with 0 representing the absence of lesions and 6 a very frequent occurrence. Then, the importance factor (w) was multiplied by the score (a) to calculate the change score (x). To calculate the histological index (HI), an overall pathological status of the organ, the change score (x) of all anomalies were summed, as described by the following formula: ($HI = \sum (w * a)$).

2.5 Data integration and statistical analysis

The solvent and water control were merged into a single control group since they were not statistically different as observed by the Two-way ANOVA (variable 1: biomarkers, variable 2: controls (water and solvent); no statistical difference was observed between controls in each biomarker: $p=0.18$ for gills and $p=0.91$ for digestive gland).

For the biochemical biomarkers, the results of all treatments were analyzed through ANOVA, followed by the Bonferroni test for post hoc comparisons (or its equivalent non-parametric test, Kruskal-Wallis followed by the Dunn's test, when data failed to pass homoscedasticity and normality tests) to allow comparisons between treatments. Statistical differences were considered significant when $p \leq 0.05$.

The results obtained were integrated by using a principal component analysis (PCA, with a cut-off value of 0.45). The data sets obtained for gills and digestive glands were analyzed separately, and the matrices consisted of the concentrations tested versus the endpoints of each biomarker. The R package "factoextra" was used to generate the PCAs.

3. Results

3.1 Biochemical biomarkers

The real concentrations of DCOIT at the end of the experiment were <0.3 µg L^{-1} (<LOD), 57 µg L^{-1}, and 151 µg L^{-1} respectively, indicating that the time between water changes was adequate to maintain the concentrations constant.

The results of biochemical biomarkers in gills soft tissues exposed to DCOIT are shown in figure 1. The activity of GPX was significantly increased after exposure to 0.2 µg L^{-1} and inhibited after exposure to 57 µg L^{-1} and 151 µg L^{-1} of DCOIT. Although a significant inhibition of GST activity was observed in all concentrations, the average inhibition was greater than 90% compared to control in the gills of the organisms exposed to 57 µg L^{-1} and 151 µg L^{-1} of DCOIT. Intracellular levels of GSH were significantly increased at 57 and 151 µg L^{-1} of DCOIT, while MET activity and LPO levels were significantly increased in all DCOIT concentrations. AChE activity was significantly higher than the control at 0.2 µg L^{-1} of DCOIT. In the gills, there was no DNA damage nor significant differences in GR activity.

The results of biochemical biomarkers in digestive glands are shown in figure 2. GPX activity was significantly increased after exposure to all concentrations, while GST was inhibited (>60% inhibition compared to control) in all concentrations. Intracellular levels of GSH were reduced at 151 µg L^{-1} of DCOIT, whilst GR activity was inhibited at 57 and 151 µg L^{-1} of DCOIT. DNA damage was observed at 57 and 116 µg L^{-1} of DCOIT, LPO levels were significantly higher at 0.2 µg L^{-1} and 151 µg L^{-1}, and an inhibition of AChE activity was observed at 151 µg L^{-1} at DCOIT.

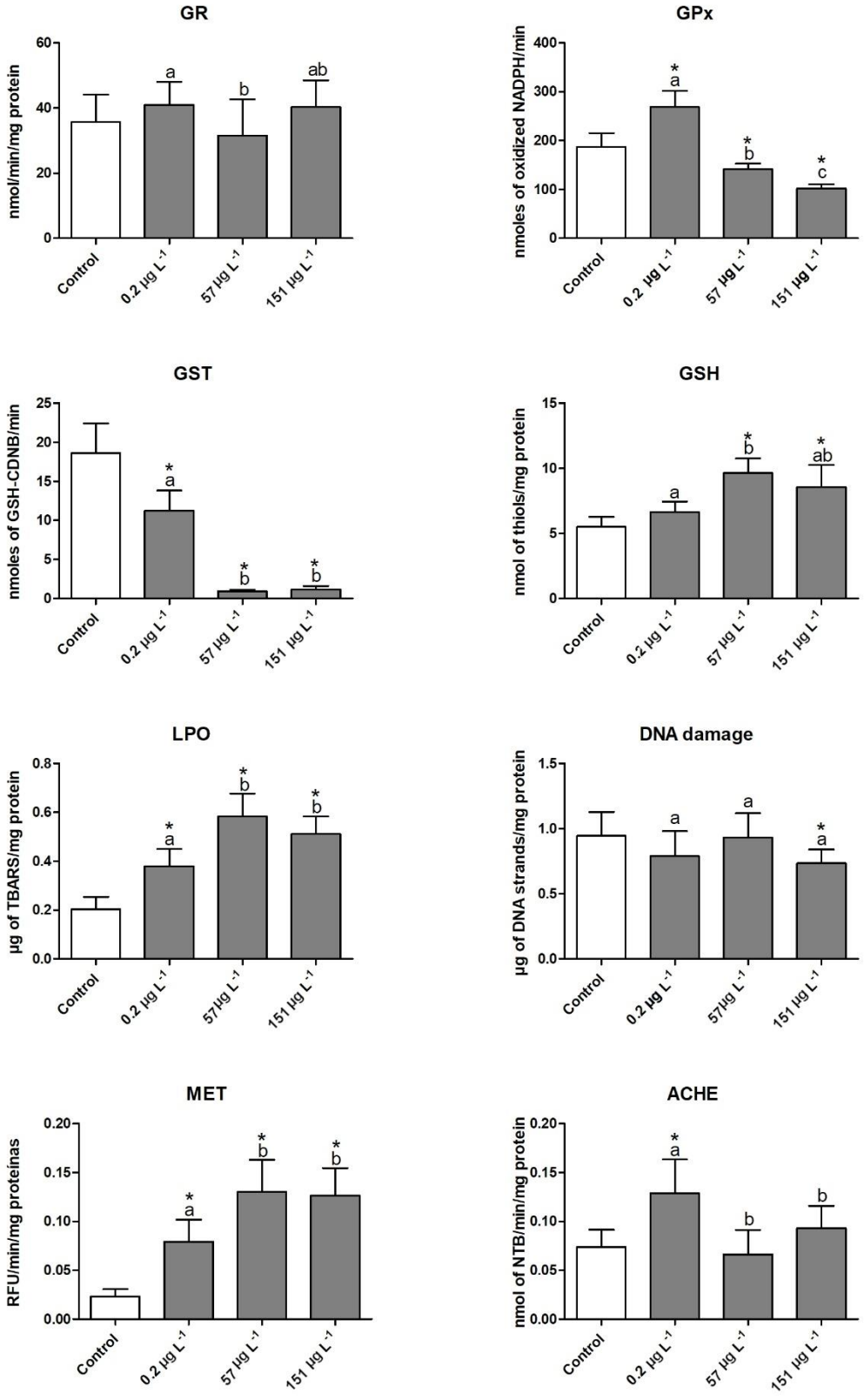


Figure 1: Biochemical biomarkers measured in gills of *Crassostrea brasiliana* exposed to waterborne DCOIT. Values are expressed as means and standard deviations (N = 12). * - significantly different from control ($p \leq 0.05$); a,b and c indicates significantly difference between treatments.

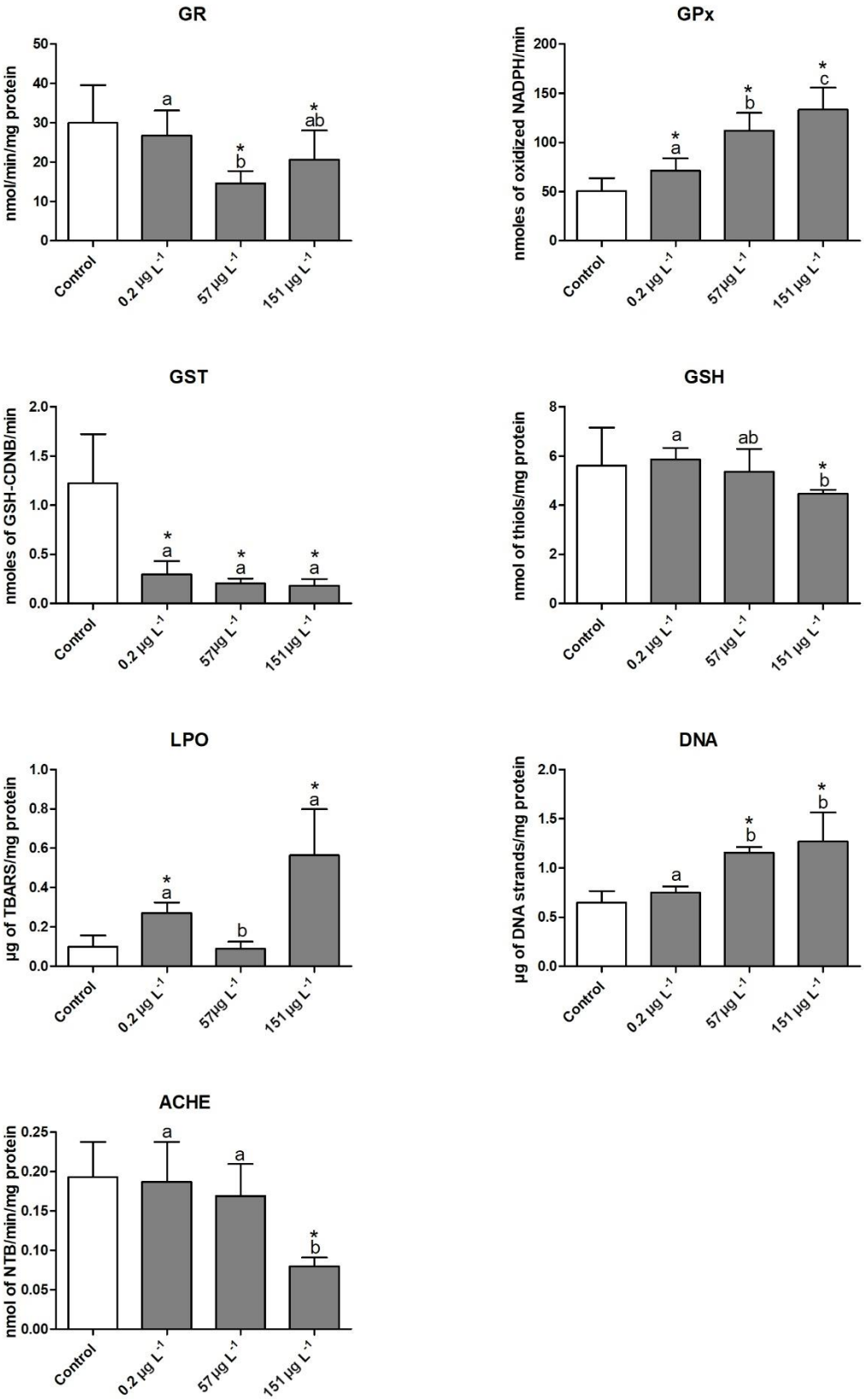


Figure 2: Biochemical biomarkers measured in digestive glands of *Crassostrea brasiliana* exposed to waterborne DCOIT. Values are expressed as means and standard deviations (N = 12). * - significantly different from control ($p \leq 0.05$); a,b and c indicates significantly difference between treatments.

3.2 Cytological biomarkers – NRRT

The lysosomal membrane stability of the hemocytes analyzed, measured by the NRRT, was significantly

reduced after exposure to all concentrations of DCOIT (figure 3). Compared to the control group (100.5 ±16 min), NRRT was reduced by 66% (33.7 min) at 0.2 µg L^{-1}, 92% (7.5 min) at 57 µg L^{-1}, and 98% (1.8 min) at 151 µg L^{-1}.

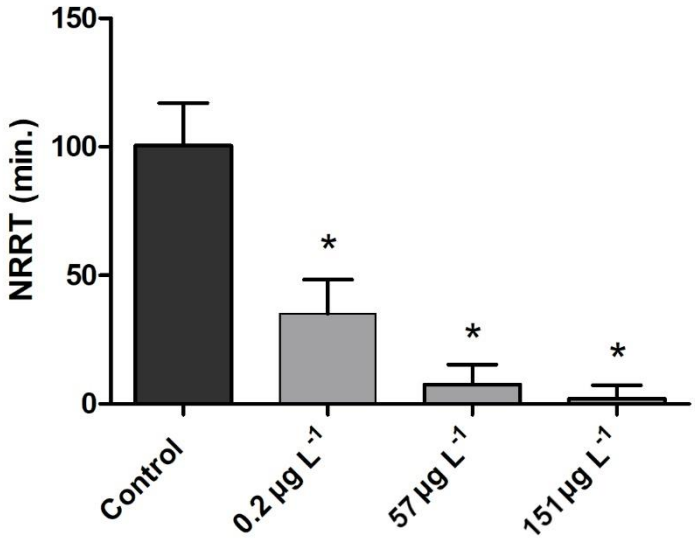


Figure 3: Neutral Red Retention time (NRRT) in hemocytes of *Crassostrea brasiliana* exposed to different concentrations of DCOIT. The results are expressed as means and their respective standard deviations (N = 12). * - significantly different from the control.

3.3 Histopathological biomarkers

The most common pathologies found in the organisms exposed to DCOIT were necrosis, leucocitary infiltration, epithelial desquamation and HAC (infiltration of hyperchromatic anaplastic cells) (Figure 4), followed by hyperplasia, lamellar degeneration, parasitism and lamellar fusion. The histopathological analyses revealed that necrosis occurrence increased (in average) 11% at 0.2 µg L^{-1}, 60% at 57 µg L^{-1} and 133% at 151 µg L^{-1} of DCOIT in comparison to the control. Leucocitary infiltration also increased in comparison to control, ranging from 15% (0.2 µg L^{-1}) to 38% (151 µg L^{-1}). The occurrence of epithelial desquamation increased substantially only at 57 µg L^{-1} (105%) compared to control. The presence of HAC (infiltration of hyperchromatic anaplastic cells) was observed in all concentrations in the following rates: 12% at 0.2 µg L^{-1}, 60% at 57 µg L^{-1} and 66% at 151 µg L^{-1}.

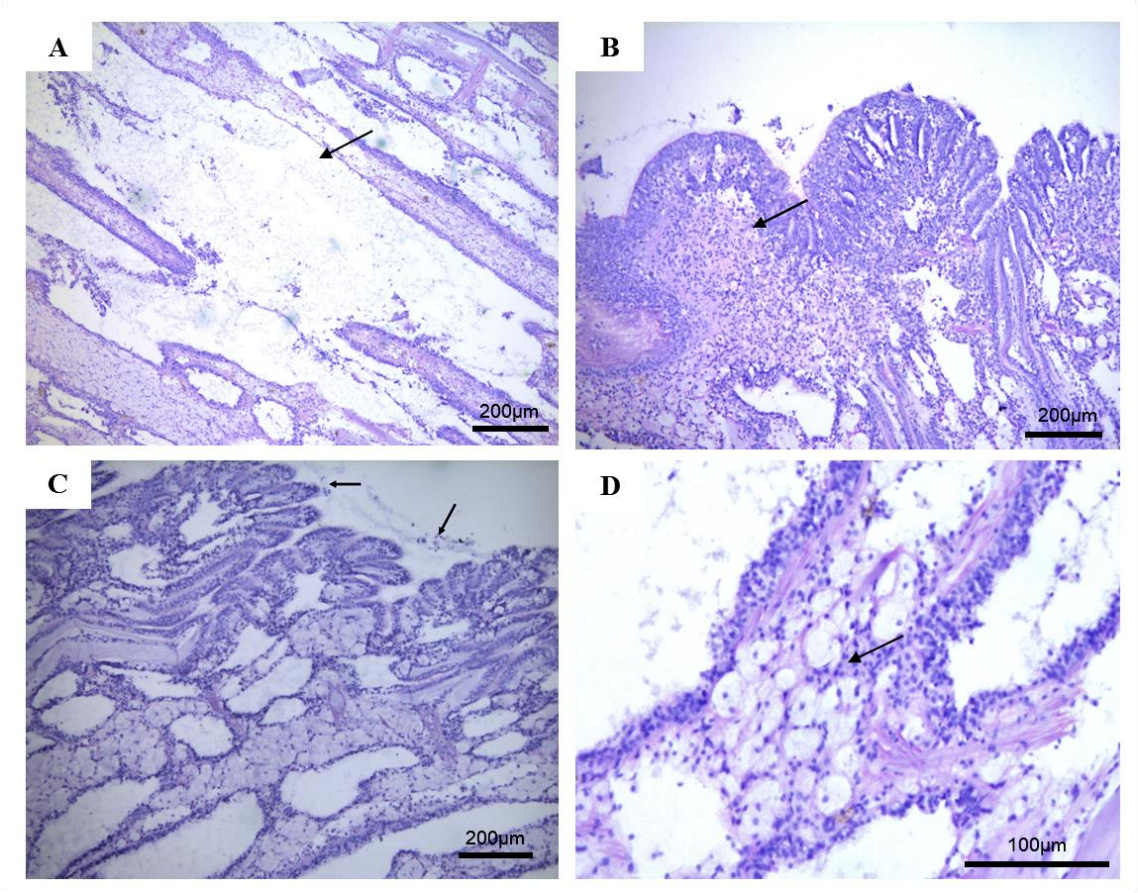


Figure 4: Most common alterations and pathologies observed in the gills of oysters (*Crassostrea brasiliana*) exposed to DCOIT. A: necrosis; B: leucocitary infiltration; C: epithelial desquamation; D: HAC - infiltration of hyperchromatic anaplastic cells.

In all treatments, the values of histopathological index (HI), were significantly higher than that obtained in the control, showing a dose dependent pattern (figure 5). The gills of oysters exposed to 0.2 µg L^{-1} and 57 µg L^{-1} of DCOIT exhibited a HI of 25.5±3.5 and 24.6±5, respectively, while the gills of animals exposed to 151 µg L^{-1} of DCOIT presented a HI of 37.3±6.7, which is, on average, 80.2% and 168.5% higher than the control, respectively.

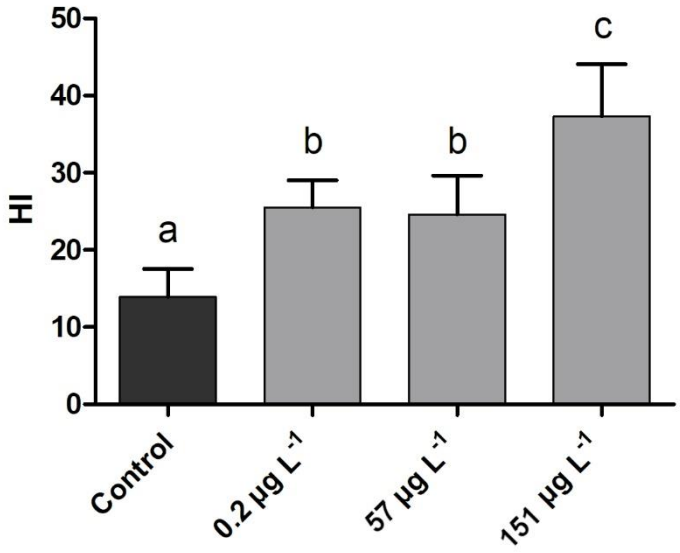


Figure 5: Histopathological index value (HI) of gill tissues of *Crassostrea brasiliana* exposed to DCOIT. Lower values indicate healthy tissues. Values are expressed as means and their respective standard deviations. * Different letters (a, b and c) indicate significant statistical differences (ANOVA: $p \leq 0.05$).

3.4 Data Integration

The PCA performed with data for the gills revealed that the first 2 principal components (PCs) explained 93% of the variances (figure 6). PC-1 explained 59% of the variances and indicated that the most relevant variables GPX and GST are negatively correlated to LPO, GSH, MET and HI, demonstrating a positive correlation between the LPO and histopathological damage, while PC-2 accounted for 34% of the variances and the most relevant variables AChE, GR and HI are negatively correlated to DNA damage (table 2).

Table 2: Eigenvalues and Factor Loadings of the PCA performed with data for the gills. Red values indicate loadings > 0.45.

Eigenvalues		
	Eigenvalue	Cumulative %
Factor 1	5.28	58.68
Factor 2	3.09	93.08
Factor Loadings		
Variable	PC1	PC2
GPX	-0.85	-0.11
GST	-0.99	0.06
LPO	0.96	0.02
DNA	-0.11	0.96
GSH	0.97	0.17
GR	-0.24	-0.96
MET	0.97	-0.12
AChE	-0.3	-0.85
HI	0.74	-0.66

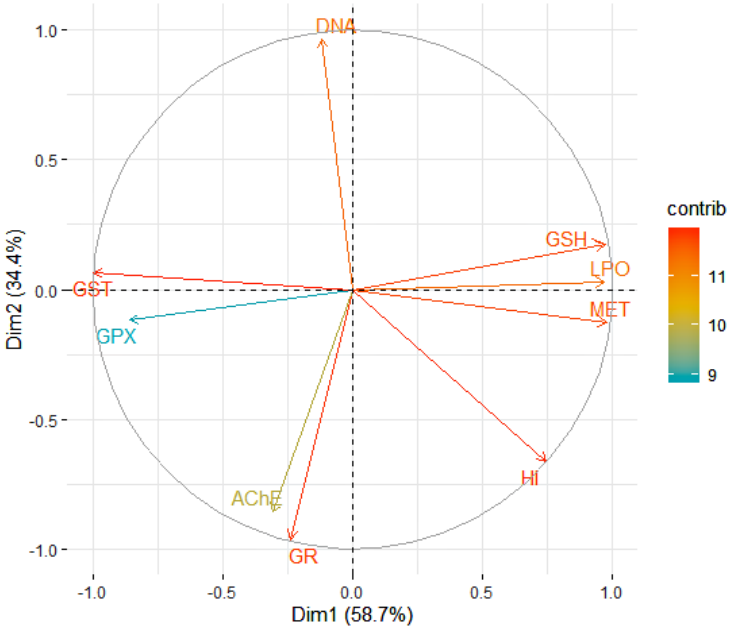


Figure 6: Principal component analysis (PCA) performed for the gills, integrating the biochemical (DNA damage, GSH, LPO, GR, AChE, MET, GPX and GST) and histological (HI) biomarkers data. "Contrib" indicates the total contribution in % of a given variable, explaining the variations retained by PC1 (Dim1) and PC2 (Dim2).

The PCA performed with data for the digestive glands showed that the first 2 PCs explained 92.9% of the variances (figure 7 and table 3). PC-1 explained 74% of the variances and showed that the most relevant variables GST, GSH, GR and AChE are negatively correlated

to GPX, LPO and DNA damage, while PC-2 accounted for 18.2% of total variances and indicated a positive correlation of the most relevant variables LPO and GR.

Table 3: Eigenvalues and Factor Loadings of the PCA performed with data for digestive gland. Red values indicate loadings > 0.45.

Eigenvalues		
	Eigenvalue	Cumulative %
Factor 1	5.23	74.83
Factor 2	1.27	92.98
Factor Loadings		
Variable	PC1	PC2
GPX	0.98	-0.13
GST	-0.75	0.41
LPO	0.75	0.58
DNA	0.97	-0.15
GSH	-0.89	-0.33
GR	-0.71	0.67
AChE	-0.92	-0.37

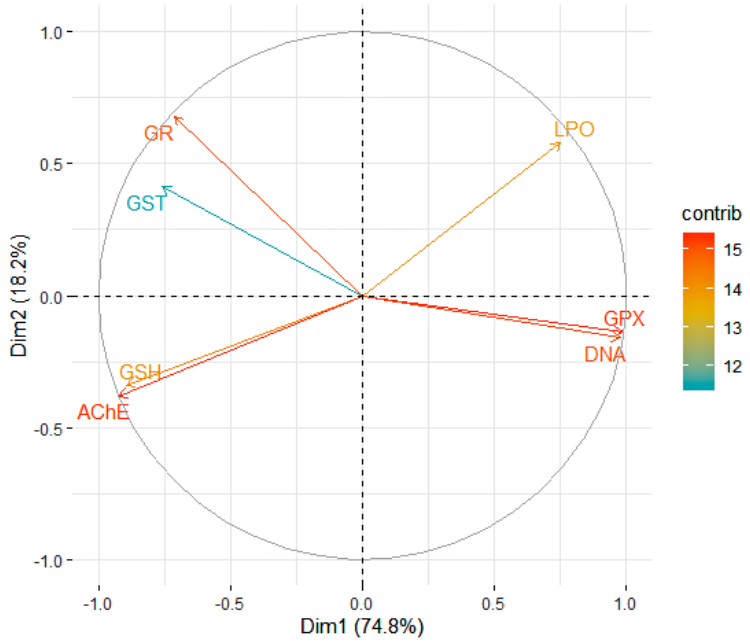


Figure 7: Principal component analysis (PCA) performed for the digestive gland, integrating the biochemical (DNA damage, GSH, LPO, GR, AChE, GPX and GST) biomarkers data. "Contrib" indicates the total contribution in % of a given variable, explaining the variations retained by PC1 (Dim1) and PC2 (Dim2).

4. Discussion

DCOIT was created specifically to be used as an antifouling biocide in 1996 (Cima et al., 2008), and at the beginning it was considered to have negligible effects on marine environments due to its short half-life, as promulgated by Jacobson and Willingham (2000). However, DCOIT has been detected in Mediterranean, Atlantic and Pacific waters, with concentrations ranging from 0.003 µg L^{-1} in coastal waters of Sweden (Readman, 2006) to 3.7 µg L^{-1} in a Spanish marina (Martínez & Barceló, 2001). According to Chen and Lam, (2017) DCOIT can be considered a pseudo-persistent pollutant due its continuous environmental input. In this sense, the present study demonstrated that environmentally relevant concentrations of DCOIT can produce adverse effects at biochemical, cytological, and histological levels in the neotropical oyster *C. brasiliana*.

4.1 Biochemical effects in the gills

In the gills, DCOIT markedly inhibited the GST activity, indicating that the detoxification and elimination system may be compromised, which can also explain the high GSH concentrations. According to Borković-Mitić et al. (2013), the inhibition of GST activity could occur either by direct action of the xenobiotic or indirectly, via the production of ROS that interact directly with the enzyme, depleting its substrate GSH, and/or through downregulation of GST genes through different mechanisms. Gabe et al. (2021) observed downregulation of mRNA levels of the microsomal GST (MGST-like) genes in *P. perna* mussels after 96 h of DCOIT exposure.

The increased activity of MET indicates an intensification in energy expenditure and/or an increase in cellular metabolism, which, according to Gagne et al. (2007), is expected in organisms exposed to xenobiotics, since the detoxification and metabolism of such compounds requires energy.

The observed increase of AChE activity may indicate that signaling of cellular apoptosis was stimulated. The increase of AChE expression or activity is commonly detected in apoptotic cells after or during apoptotic stimuli in vitro and in vivo (Zhang et al., 2002). According to these authors, AChE plays a critical role in the process of cell apoptosis, being responsible for the activation of caspase-9 (also known as initiating caspase). Thus, it can be considered an important regulator and marker of cellular death. In addition, the results of PCA performed in gills soft tissues also showed a positive relationship between AChE and histological abnormalities.

4.2 Biochemical effects in the digestive gland

In the digestive gland, the significant induction of GPx activity indicates an augmented depuration of intracellular reactive oxygen species (ROS), concomitantly with lipid peroxidation (as observed). Although ROS was not measured in the present study, Chen et al. (2014) found high concentrations of ROS in the liver of fish exposed to 3 µg L^{-1} of DCOIT for 28 days. Furthermore, the observed elevation of the mitochondrial electron transport activity in the gills can also lead to a systemic increase in production of ROS. As for the gills, GST concentrations in the digestive gland were significantly reduced indicating an inhibition of the detoxification and depuration system, at least through this metabolic pathway.

The results of LPO also indicated damage to cell membranes. Similarly, DNA damage was detected exhibiting a dose dependent pattern. These results suggest oxidative stress in digestive glands of oysters exposed to DCOIT, as seen in the PCA by the positive association of GPX activity, DNA damage, and LPO. According to the literature, oxidative stress has also been reported for corals (Cima et al., 2013), fish (Chen et al., 2014b; Ito et al., 2013), and ascidians (Cima et al., 2008) exposed to sublethal concentrations of DCOIT.

The results showing DNA damage in oysters exposed to DCOIT are concerning because such damage may lead to a range of effects, including chromosomic instability, changes in the expression of proteins that play important roles in the homeostasis, mutations, induction of carcinogenesis, or cell apoptosis (Parolini et al., 2017). Although Cima et al. (2008) detected DNA damage in tunicate hemocytes exposed to DCOIT (0.1 to 10 µg L^{-1}) for 60 min, little information regarding genotoxicity caused by DCOIT to marine organisms is available.

Supporting the hypothesis of oxidative stress and failure in the neutralization of reactive oxygen species, the integrated results for the digestive gland indicated a positive correlation among LPO, DNA damage, and GPX, suggesting that the increased levels of ROS caused lipoperoxidation and inhibition of GST.

Regarding AChE, although mollusks do not have a centralized nervous system like vertebrates, their digestive gland has a network of fibers and nerve cells that play multiple functions, including the control of stomach physiology (Lobo-da-Cunha, 2019; Usheva et al., 2006). Inhibition of AChE activity, as seen after exposure to the highest DCOIT concentration, results in a constant supply of acetylcholine, characterizing neurotoxicity (Ricciardi et al., 2006). Inhibition of AChE in marine invertebrates (mysids) exposed to DCOIT was previously described by Do et al. (2018). In addition, inhibition of AChE activity in digestive glands of

bivalves has been considered a reliable endpoint to assess toxicity (Damiens et al., 2004; de Souza et al., 2018; Stara et al., 2020). In marine vertebrates, DCOIT also inhibits AChE, as observed by Chen et al. (2014b) in brain tissues of *Oryzias latipes* (teleost) exposed to a concentration of 2.55 µg L^{-1} for 29 days. Moreover, Mochida et al. (2010) also observed neurotoxicity in a bovine nerve cell culture.

4.3 Cytological and Histopathological effects

Lysosomes are cellular organelles capable of accumulating contaminants, being involved in the degradation processes of xenobiotics (Martínez-Gómez et al., 2015). If lysosomal membranes are destabilized due to ROS or contaminants, digestive enzymes may leach into the cytosol, leading to the cell injuries or even its death (Dailianis et al., 2003). Thus, the stability of lysosomal membranes is considered an important biomarker of the initial stages of damage (Aguirre-Martínez et al., 2015), which can often be related to the oxidative stress, since ROS can cause lipid peroxidation and DNA damage. The neutral red is a cationic dye which can enter in viable cells by passive diffusion or pinocytosis across the plasmatic membrane (Cima and Ballarin, 2012). In health cells, lysosomes take up and retain larger quantities of neutral red for a longer period of time compared to damaged cells (Martínez-Gómez et al., 2008). In the present study, the hemocytes of oysters exposed to all tested concentrations of DCOIT showed a reduction of NRRT. Possibly, the production of ROS caused structural damage of lysosomal membranes by lipid oxidation, altering their permeability, possibly through the impairment of the H^+ ion pump (Lowe and Pipe, 1994). The biochemical biomarkers results (GPx, LPO and DNA damage) corroborates with the NRRT supporting the evidence of oxidative stress. Similar results were observed by Koro et al. (2015) in bivalves *Perna perna* exposed to 0.1 to 10 µg L^{-1} of TBT. Ascidians exposed to Irgarol (10, 100, 200, 500 µM), another antifouling biocide, also presented severe damage to their lysosomal membranes (Cima and Ballarin, 2012). The loss of lysosomal membrane stability induced by the exposure to DCOIT can also affect the immunological response, nutrition, and reproduction of these organisms (Moore et al., 2006).

In bivalves, as in other aquatic organisms, the gills are the first organs to come into contact and be affected by contaminants. Besides, they may play a role in xenobiotic detoxification (Fontes et al., 2017). The morphology and histology of gill tissues may provide information on the response to xenobiotics (Nogueira et al., 2017), since damage of gill structures often affects the physiology of the organism, with implication to their health. The

current study was the first applying histopathological techniques to assess the effects of DCOIT on oysters. The histopathological index showed an overall poor health condition in the exposed organisms, which worsens following a dose-dependent pattern. Increasing rates of severe pathologies were evidenced, and included necrosis, leucocitary infiltration and epithelial desquamation. Su et al. (2019) reported damage of gills and hyperchromatic cells in the shrimp *Litopenaeus vannamei* exposed to DCOIT (15 - 30 µg L^{-1}), while Su et al. (2018) observed that damaged gills negatively affected the respiration of fish exposed to DCOIT (≤300 µg L^{-1}). In the present study, PCA results indicated an association of histopathological damage and LPO, demonstrating that the oxidative damage at cellular level can lead to tissue pathologies and abnormalities after short periods of exposure (up to 120h). The observed PCA relationship between HI and AChE activity highlighted the process of apoptosis, the leucocitary infiltration activates the pro-apoptotic BCL2 genes, which increases the activity of AchE responsible to form the apoptosome (Mohammadzadeh et al. 2022). Thus, the results suggested that histological changes were closely associated with biochemical alterations that occurred after the exposure to DCOIT.

Based on the presented findings and supported by the risk assessments performed by Abreu et al. (2021) and Campos et al. (2021), environmental concentrations of DCOIT can impose risk to the marine biota, especially in areas within high traffic of vessels, with recurrent inputs of antifouling substances such as DCOIT (Abreu et al., 2020). Biomarkers of energy metabolism, genotoxicity and lysosomal stability have proven to be ecologically relevant and god indicators of the general physiological conditions, with a high correlation to individual and population-level effects (Mouneyrac and Amiard-Triquet, 2013). Thus, the integration of endpoints from multilevel biomarkers can improve ecological risk assessment and provide evidence for environmental management and regulatory decisions. Moreover, the analysis of subchronic and sublethal effects, such as biomarkers, may provide the early detection of ecological disturbances, before critical effects occur.

5. Conclusions

The present study demonstrated that the oyster *Crassostrea brasiliana* is adversely affected when exposed to environmentally relevant concentrations of DCOIT. The effects on the gills and digestive glands were different. In gills, there was histopathological damage, but the biochemical biomarkers indicated lack of effects on the activity of the antioxidant system (which explains the oxidative stress and the damage observed). In digestive glands, there were

an induction of the antioxidant defense system. Hemocytes presented damage of their lysosomal membranes. The results suggest that coastal areas close to ports and marinas may present ecological risk to biota due to the DCOIT.

6. Acknowledgments

The authors would like to thank the FINEP - Financiadora de Estudos e Projetos (Proc. No 1111/13 – 01.14.0141.00), CAPES - Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Grant #88881.156405/2017-01), and CNPq - Conselho Nacional de Desenvolvimento de Científico e Tecnológico (Grant #456372/2013-0) for the financial support. B. G. Campos (Grants #2017/10211-0) and P. K. G. Choueri (Grants #2017/04970-5), and D. M. S. Abessa (Grants #2020/03004-0) thank the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for the financial support. F. E. L. Abreu was sponsored by CAPES – finance code 001, while G. Fillmann (PQ #314202/2018-8) and D.M.S. Abessa (PQ #308533/2018-6) are research fellows of CNPq.

7. References

Abreu, F.E.L., Martins, S.E., Fillmann, G., 2021. Ecological risk assessment of booster biocides in sediments of the Brazilian coastal areas. Chemosphere 276, 130155. https://doi.org/10.1016/j.chemosphere.2021.130155

Abreu, F.E.L., Natália, J., Braga, Í., Fillmann, G., 2020. Are antifouling residues a matter of concern in the largest South American port ? 398. https://doi.org/10.1016/j.jhazmat.2020.122937

Aguirre-Martínez, G. V., Owuor, M.A., Garrido-Pérez, C., Salamanca, M.J., Del Valls, T.A., Martín-Díaz, M.L., 2015. Are standard tests sensitive enough to evaluate effects of human pharmaceuticals in aquatic biota? Facing changes in research approaches when performing risk assessment of drugs. Chemosphere 120, 75–85. https://doi.org/10.1016/j.chemosphere.2014.05.087

Bellas, J., 2006. Comparative toxicity of alternative antifouling biocides on embryos and larvae of marine invertebrates. Sci. Total Environ. 367, 573–585. https://doi.org/10.1016/j.scitotenv.2006.01.028

Bernet, D., Schmidt, H., Meier, W., Burkhardt-Holm, P., Wahli, T., 1999. Histopathology in fish: proposal for a protocol to assess aquatic pollution. J. Fish Dis. 22, 25–34. https://doi.org/10.1046/j.1365-2761.1999.00134.x

Borković-Mitić, S., Pavlović, S., Perendija, B., Despotović, S., Gavrić, J., Gačić, Z., Saičić, Z., 2013. Influence of some metal concentrations on the activity of antioxidant enzymes and concentrations of vitamin e and SH-groups in the digestive gland and gills of the freshwater bivalve Unio tumidus from the Serbian part of Sava River. Ecol. Indic. 32, 212–221. https://doi.org/10.1016/j.ecolind.2013.03.024

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Campos, B.G. de, Figueiredo, J., Perina, F., Abessa, D. de S., Loureiro, S., Martins, R., 2021. Occurrence, effects and environmental risk of antifouling biocides (EU PT21): Are marine ecosystems threatened? Crit. Rev. Environ. Sci. Technol. in press. https://doi.org/10.1080/10643389.2021.1910003

Campos, B. G. de, Buruaem, L., Figueiredo, G. De, Pauly, E., Carolina, A., Cruz, F., Monte, N., Irene, L., Paula,

A., Rodrigues, D. C., Machado, W., Moledo, D., & Abessa, D. S. (2019). Integrating multiple lines of evidence of sediment quality in a tropical bay. Marine Pollution Bulletin, 146(July), 925–934. https://doi.org/10.1016/j.marpolbul.2019.07.051

Chen, L., Au, D.W.T., Hu, C., Peterson, D.R., Zhou, B., Qian, P.Y., 2017. Identification of Molecular Targets for 4,5-Dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT) in Teleosts: New Insight into Mechanism of Toxicity. Environ. Sci. Technol. 51, 1840–1847. https://doi.org/10.1021/acs.est.6b05523

Chen, L., Lam, J.C.W., 2017a. SeaNine 211 as antifouling biocide: A coastal pollutant of emerging concern. J. Environ. Sci. 61, 68–79. https://doi.org/10.1016/j.jes.2017.03.040

Chen, L., Ye, R., Xu, Y., Gao, Z., Au, D.W.T., Qian, P.Y., 2014a. Comparative safety of the antifouling compound butenolide and 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT) to the marine medaka (Oryzias melastigma). Aquat. Toxicol. 149, 116–125. https://doi.org/10.1016/j.aquatox.2014.01.023

Chen, L., Zhang, H., Sun, J., Wong, Y.H., Han, Z., Au, D.W.T., Bajic, V.B., Qian, P.Y., 2014b. Proteomic changes in brain tissues of marine medaka (Oryzias melastigma) after chronic exposure to two antifouling compounds: Butenolide and 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT). Aquat. Toxicol. 157, 47–56. https://doi.org/10.1016/j.aquatox.2014.09.010

Cima, F., Ballarin, L., 2012. Immunotoxicity in ascidians: Antifouling compounds alternative to organotins. III - The case of copper(I) and Irgarol 1051. Chemosphere 89, 19–29. https://doi.org/10.1016/j.chemosphere.2012.04.007

Cima, F., Bragadin, M., Ballarin, L., 2008. Toxic effects of new antifouling compounds on tunicate haemocytes. I. Sea-Nine 211TM and chlorothalonil. Aquat. Toxicol. 86, 299–312. https://doi.org/10.1016/j.aquatox.2007.11.010

Cima, F., Ferrari, G., Ferreira, N.G.C., Rocha, R.J.M., Serôdio, J., Loureiro, S., Calado, R., 2013. Preliminary evaluation of the toxic effects of the antifouling biocide Sea-Nine 211 in the soft coral Sarcophyton cf. glaucum (Octocorallia, Alcyonacea) based on PAM fluorometry and biomarkers. Mar. Environ. Res. 83, 16–22. https://doi.org/10.1016/j.marenvres.2012.10.004

Cruz, A. C. F., Davanso, M. B., Araujo, G. S., Buruaem, L. M., Santaella, S. T., de Morais, R. D., & Abessa, D. M. S. (2014). Cumulative influences of a small city and former mining activities on the sediment quality of a subtropical estuarine protected area. Environmental Monitoring and Assessment, 186(11), 7035–7046. https://doi.org/10.1007/s10661-014-3908-1

Dailianis, S., Domouhtsidou, G.P., Raftopoulou, E., Kaloyianni, M., Dimitriadis, V.K., 2003. Evaluation of neutral red retention assay, micronucleus test, acetylcholinesterase activity and a signal transduction molecule (cAMP) in tissues of Mytilus galloprovincialis (L.), in pollution monitoring. Mar. Environ. Res. 56, 443–470. https://doi.org/10.1016/S0141-1136(03)00005-9

Damiens, G., His, E., Gnassia-Barelli, M., Quiniou, F., Roméo, M., 2004. Evaluation of biomarkers in oyster larvae in natural and polluted conditions. Comp. Biochem. Physiol. - C Toxicol. Pharmacol. 138, 121–128. https://doi.org/10.1016/j.cca.2004.05.010

de Souza, P.R., de Souza, K.S., de Assis, C.R.D., de Araújo, M.C., Silva, K.C.C., de Fátima Xavier da Silva, J., Ferreira, A.C.M., da Silva, V.L., Adam, M.L., de Carvalho, L.B., de Souza Bezerra, R., 2018. Acetylcholinesterase of mangrove oyster Crassostrea rhizophorae: A highly thermostable enzyme with promising features for estuarine biomonitoring. Aquat. Toxicol. 197, 109–121. https://doi.org/10.1016/j.aquatox.2018.02.008

Devilla, R., Brown, M., Donkin, M., Tarran, G., Aiken, J., Readman, J., 2005. Impact of antifouling booster biocides on single microalgal species and on a natural marine phytoplankton community. Mar. Ecol. Prog. Ser. 286, 1–12. https://doi.org/10.3354/meps286001

Do, J.W., Haque, N., Lim, H., Hwa, B., Lee, D., 2018. Constant exposure to environmental concentrations of the antifouling biocide Sea-Nine retards growth and reduces acetylcholinesterase activity in a marine mysid. Aquat. Toxicol. 205, 165–173. https://doi.org/10.1016/j.aquatox.2018.10.019

Ellman, G.L., Courtney, K.D., Andres, V., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88–95. https://doi.org/10.1016/0006-2952(61)90145-9

Environmental Protection Agency [EPA], 1992. Pesticide Ecotoxicity Database [WWW Document]. URL https://cfpub.epa.gov/ecotox/search.cf (accessed 6.29.20).

Figueiredo, J., Loureiro, S., Martins, R., 2020. Hazard of novel anti-fouling nanomaterials and biocides DCOIT and silver to marine organisms. Environ. Sci. Nano 7, 1670–1680. https://doi.org/10.1039/d0en00023j

Figueiredo, J., Oliveira, T., Ferreira, V., Sushkova, A., Silva, S., Carneiro, D., Cardoso, D.N., Gonçalves, S.F., Maia, F., Rocha, C., Tedim, J., Loureiro, S., Martins, R., 2019. Toxicity of innovative anti-fouling nano-based solutions in marine species. Environ. Sci. nano. https://doi.org/10.1039/C9EN00011A

Fonseca, V.B., Guerreiro, A. da S., Vargas, M.A., Sandrini, J.Z., 2020. Effects of DCOIT (4,5-dichloro-2-octyl-4-isothiazolin-3-one) to the haemocytes of mussels Perna perna. Comp. Biochem. Physiol. Part - C Toxicol. Pharmacol. 232, 108737. https://doi.org/10.1016/j.cbpc.2020.108737

Fontes, M.K., Gusso-choueri, P.K., Maranho, L.A., Moledo, D., Abessa, D.S., Mazur, W.A., Campos, B.G. De, Guimarães, L., Toledo, M.S. De, Lebre, D., Marques, J.R., Felicio, A., Cesar, A., Almeida, E.A., Dias, C., Pereira, S., Guimarães, L.L., Toledo, M.S. De, Lebre, D., Marques, J.R., 2017. A tiered approach to assess effects of diclofenac on the brown mussel Perna perna: A contribution to characterize the hazard. Water Res. 132, 361–370. https://doi.org/10.1016/j.watres.2017.12.077

Frasco, M.F., Guilhermino, L., 2002. Effects of dimethoate and beta-naphthoflavone on selected biomarkers of Poecilia reticulata. Fish Physiol. Biochem. 26, 149–156.

Gabe, H.B., Guerreiro, A. da S., Sandrini, J.Z., 2021. Molecular and biochemical effects of the antifouling DCOIT in the mussel Perna perna. Comp. Biochem. Physiol. Part - C Toxicol. Pharmacol. 239, 108870. https://doi.org/10.1016/j.cbpc.2020.108870

Gagne, F., Blaise, C., Andre, C., Pellerin, J., 2007. Implication of site quality on mitochondrial electron transport activity and its interaction with temperature in feral Mya arenaria clams from the Saguenay Fjord 103, 238–246. https://doi.org/10.1016/j.envres.2006.05.006

Gagné, F., Trottier, S., Blaise, C., Sproull, J., Ernst, B., 1995. Genotoxicity of sediment extracts obtained in the vicinity of a creosote-treated wharf to rainbow trout hepatocytes. Toxicol. Lett. 78, 175–4274. https://doi.org/https://doi.org/10.1016/0378-4274(95)03259-N

Harino, H., Mori, Y., Yamaguchi, Y., Shibata, K., Senda, T., 2005. Monitoring of antifouling booster biocides in water and sediment from the port of Osaka, Japan. Arch. Environ. Contam. Toxicol. 48, 303–310. https://doi.org/10.1007/s00244-004-0084-2

Harino, H., Yamamoto, Y., Eguchi, S., Kawai, S., Kurokawa, Y., Arai, T., Ohji, M., Okamura, H., Miyazaki, N., 2007. Concentrations of antifouling biocides in sediment and mussel samples collected from Otsuchi Bay, Japan. Arch. Environ. Contam. Toxicol. 52, 179–188. https://doi.org/10.1007/s00244-006-0087-2

Ito, M., Mochida, K., Ito, K., Onduka, T., Fujii, K., 2013. Induction of apoptosis in testis of the marine teleost mummichog Fundulus heteroclitus after in vivo exposure to the antifouling biocide 4,5-dichloro-2-n-octyl-3(2H)-isothiazolone (Sea-Nine 211). Chemosphere 90, 1053–1060. https://doi.org/10.1016/j.chemosphere.2012.08.052

Jacobson, A.H., Willingham, G.L., 2000. Sea-nine antifoulant: An environmentally acceptable alternative to organotin antifoulants. Sci. Total Environ. 258, 103–110. https://doi.org/10.1016/S0048-9697(00)00511-8

Kim, N.S., Shim, W.J., Yim, U.H., Hong, S.H., Ha, S.Y., Han, G.M., Shin, K.-H., 2014. Assessment of TBT and organic booster biocide contamination in seawater from coastal areas of South Korea. Mar. Pollut. Bull. 78, 201–208. https://doi.org/10.1016/j.marpolbul.2013.10.043

Lobo-da-Cunha, A., 2019. Structure and function of the digestive system in molluscs. Cell Tissue Res. https://doi.org/10.1007/s00441-019-03085-9

Lowe, D.M., Pipe, R.K., 1994. Contaminant induced lysosomal membrane damage in marine mussel digestive cells: an in vitro study. Aquat. Toxicol. 30, 357–365. https://doi.org/https://doi.org/doi:10.1016/0166-445X(94)00045-X

Martínez-Gómez, C., Benedicto, J., Campillo, J.A., Moore, M., 2008. Application and evaluation of the neutral red retention (NRR) assay for lysosomal stability in mussel populations along the Iberian Mediterranean coast. J. Environ. Monit. 10, 490–499.

Martínez, K., Barceló, D., 2001. Determination of antifouling pesticides and their degradation products in marine sediments by means of ultrasonic extraction and HPLC-APCI-MS. Fresenius. J. Anal. Chem. 370, 940–945. https://doi.org/10.1007/s002160100904

Martínez, K., Ferrer, I., Hernando, M.D., Fernández-Alba, A.R., Marcé, R.M., Borrull, F., Barceló, D., 2001. Occurrence of antifouling biocides in the spanish mediterranean marine environment. Environ. Technol. (United Kingdom) 22, 543–552. https://doi.org/10.1080/09593332208618258

McFarland, V.A., Inouye, L.S., Lutz, C.H., Jarvis, A.S., Clarke, J.U., McCant, D.D., 1999. Biomarkers of oxidative stress and genotoxicity in livers of field collected brown bullhead, Ameiurus nebulosus. Arch. Environ. Contam. Toxicol. 37, 236–241. https://doi.org/https://doi.org/10.1007/s002449900510

Mochida, K., Amano, H., Onduka, T., Kakuno, A., Fuji, K., 2010. Toxicity of 4,5-dichloro-2-n-octyl-3 2H - isothiazolone Sea-Nine 211 to two marine teleostean fishes. Japanese J. Environ. Toxicol. 13, 105–116.

Moore, M.N., Icarus Allen, J., McVeigh, A., 2006. Environmental prognostics: An integrated model supporting lysosomal stress responses as predictive biomarkers of animal health status. Mar. Environ. Res. 61, 278–304. https://doi.org/10.1016/j.marenvres.2005.10.005

Mouneyrac, C., Amiard-Triquet, C., 2013. Biomarkers of Ecological Relevance in Ecotoxicology, in: Férard, J.-F., Blaise, C. (Eds.), Encyclopedia of Aquatic Ecotoxicology. Springer Science+Business Media Dordrecht, pp. 92–107. https://doi.org/10.1007/978-94-007-5704-2

Nogueira, L., Mello, D.F., Trevisan, R., Garcia, D., da Silva Acosta, D., Dafre, A.L., de Almeida, E.A., 2017. Hypoxia effects on oxidative stress and immunocompetence biomarkers in the mussel Perna perna (Mytilidae, Bivalvia). Mar. Environ. Res. 126, 109–115. https://doi.org/10.1016/j.marenvres.2017.02.009

Oakes, K.D., Kraak, G.J. Van Der, 2003. Utility of the TBARS assay in detecting oxidative stress in white sucker (Catostomus commersoni) populations exposed to pulp mill effluent. Aquat. Toxicol. 63, 447–463. https://doi.org/https://doi.org/10.1016/S0166-445X(02)00204-7

Okoro, H.K., Snyman, R.G., Fatoki, O.S., Adekola, F.A., Ximba, B.J., Slabber, M.Y., 2015. Lysosomal membrane stability of the mussel, Mytilus galloprovincialis (L.), as a biomarker of tributyltin exposure. Bull. Environ. Contam. Toxicol. 94, 609–613. https://doi.org/10.1007/s00128-015-1526-4

Olive, P.L., 1988. DNA precipitation assay: A rapid and simple method for detecting DNA damage in mammalian cells. Environ. Mol. Mutagen. 11, 487–495. https://doi.org/https://doi.org/doi:10.1002/em.2850110409

Packard, T.T., 1971. The measurement of electron transport activity in marine phytoplankton. J. Mar. Res. 29, 234–244.

Parolini, M., Ghilardi, A., Della Torre, C., Magni, S., Prosperi, L., Calvagno, M., Del Giacco, L., Binelli, A., 2017. Environmental concentrations of cocaine and its main metabolites modulated antioxidant response and caused cyto-genotoxic effects in zebrafish embryo cells. Environ. Pollut. 226, 504–514. https://doi.org/10.1016/j.envpol.2017.04.046

Readman, J.W., 2006. Development, Occurrence and Regulationof Antifouling Paint Biocides: Historical Review and Future Trends. Antifouling Paint Biocides 1–15. https://doi.org/10.1007/698_5_047

Ricciardi, F., Binelli, A., Provini, A., 2006. Use of two biomarkers (CYP450 and acetylcholinesterase) in zebra mussel for the biomonitoring of Lake Maggiore (northern Italy). Ecotoxicol. Environ. Saf. 63, 406–412. https://doi.org/10.1016/j.ecoenv.2005.02.007

Sakkas, V.A., Konstantinou, I.K., Lambropoulou, D.A., Albanis, T.A., 2002. Survey for the occurrence of antifouling paint booster biocides in the aquatic environment of Greece. Environ. Sci. Pollut. Res. Int. 9, 327–332. https://doi.org/10.1007/BF02987576

Sedlak, J., Lindsay, R.H., 1968. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal. Biochem. 25, 192–205.

Smolders, R., Bervoets, L., Coen, W. De, Blust, R., 2004. Cellular energy allocation in zebra mussels exposed along a pollution gradient: linking cellular effects to higher levels of biological organization. Environ. Pollut. 129, 99–112. https://doi.org/https://doi.org/10.1016/j.envpol.2003.09.027

Stara, A., Pagano, M., Capillo, G., Fabrello, J., Sandova, M., Vazzana, I., Zuskova, E., Velisek, J., Matozzo, V., Faggio, C., 2020. Assessing the effects of neonicotinoid insecticide on the bivalve mollusc Mytilus galloprovincialis. Sci. Total Environ. 700, 134914. https://doi.org/10.1016/j.scitotenv.2019.134914

Steen, R.J.C.A., Ariese, F., Hattum, B. Van, Jacobsen, J., Jacobson, A., 2004. Monitoring and evaluation of the environmental dissipation of the marine antifoulant (DCOIT) in a Danish Harbor 57, 513–521. https://doi.org/10.1016/j.chemosphere.2004.06.043

Su, Y., Li, H., Xie, J., Xu, C., Dong, Y., Han, F., Qin, J.G., Chen, L., Li, E., 2019. Toxicity of 4 , 5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT) in the marine decapod Litopenaeus vannamei. Environ. Pollut. 251, 708–716. https://doi.org/10.1016/j.envpol.2019.05.030

Tsunemasa, N., Hashimoto, K., Yamaoka, Y., Ueno, H., Okamura, H., 2006. Contamination of an Alternative Antifoulant in Coastal Waters of Hiroshima Bay. rnal Environ. Chem. 16, 201–211.

USEPA, 1996. Sea-Nine, Green Chemistry Challenge Awards [WWW Document]. URL https://goo.gl/vxIQky

Usheva, L.N., Vaschenko, M.A., Durkina, V.B., 2006. Histopathology of the digestive gland of the bivalve mollusk Crenomytilus grayanus (Dunker, 1853) from southwestern Peter the Great Bay, Sea of Japan. Russ. J. Mar. Biol. 32, 166–172. https://doi.org/10.1134/S1063074006030047

Willingham, G.L., Jacobson, A.H., 1996. Designing an Environmentally Safe Marine Antifoulant. ACS Symp. Ser. 640, 224–233. https://doi.org/10.1021/bk-1996-0640.ch011

Zhang, X.J., Yang, L., Zhao, Q., Caen, J.P., He, H.Y., Jin, Q.H., 2002. Induction of acetylcholinesterase expression during apoptosis in various cell types. https://doi.org/10.1038/sj.cdd.4401034

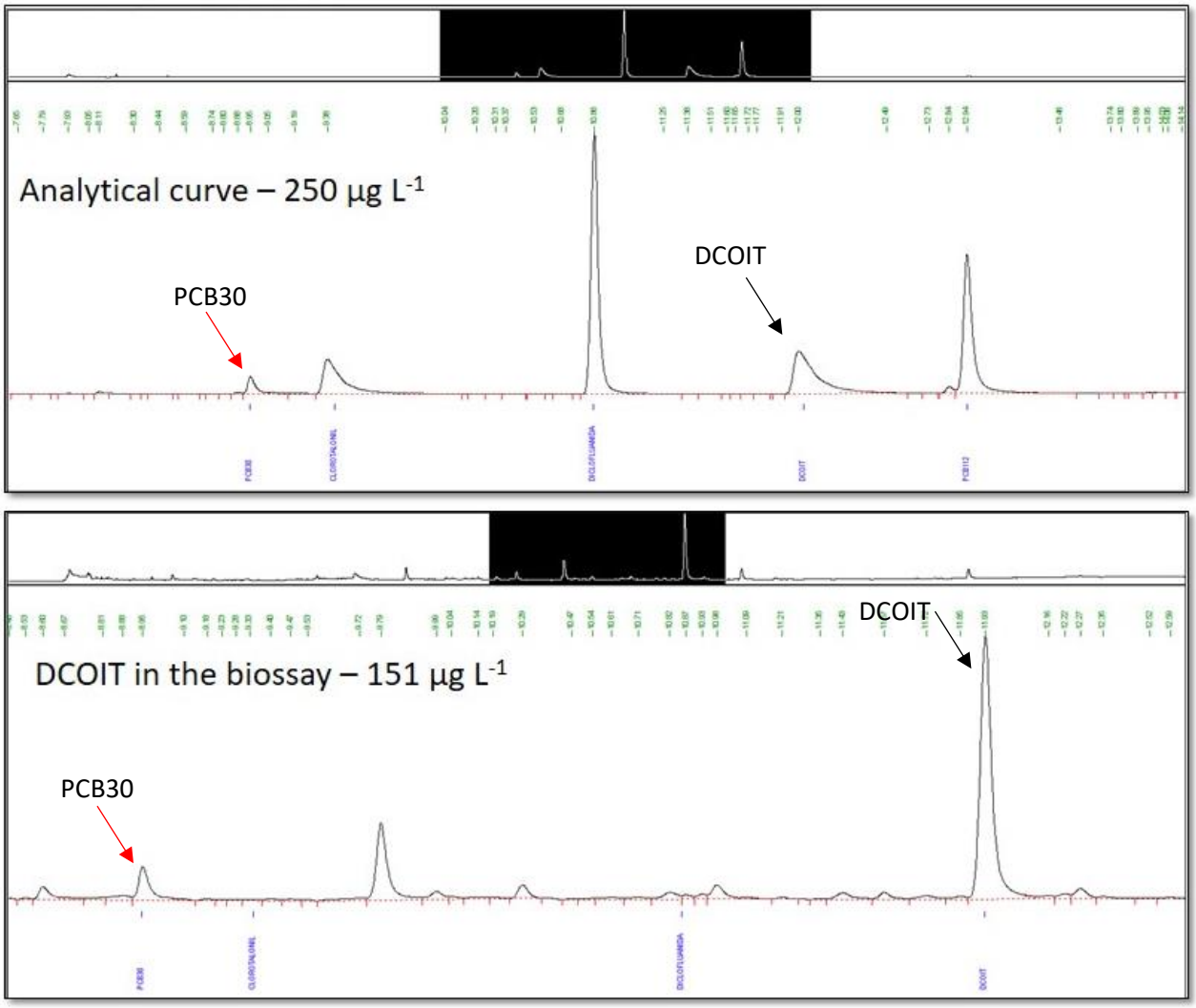


Figure S1: Analytical curve (250 µg L^{-1}) and water sample (151 µg L^{-1}) chromatogram showing the DCOIT (back arrow) and the PCB30 internal standard (red arrow).

Article 3 - Water and sediment toxicity and risk assessment of DCOIT towards neotropical organisms.

Bruno Galvão de Campos[a], Lucas Buruaem Moreira [a],Guacira de Figueiredo Eufrasio Pauly [a], Ana Carolina Feitosa Cruz [a], Fernando Perina [a], Fiamma Abreu[b], Gilberto Filman[b] Denis Moledo de Souza Abessa [a]

[a] *São Paulo State University (UNESP), Praça Infante Dom Henrique, s/n, Zipcode: 11330-900 São Vicente, São Paulo, Brazil*

[b] *Rio Grande Federal University (FURG), Av. Itália s/n, 7 Campus Carreiros, C.P. 474, 96201-900, Rio Grande, RS, Brazil*

Corresponding Author: *Bruno Galvão de Campos, São Paulo State University (UNESP), Praça Infante Dom Henrique, s/n, Zipcode: 11330-900 São Vicente, São Paulo, Brazil. E-mail: b.campos@unesp.br*

Status*:* Under consideration for submission to *Environmental Pollution*

Highlights

- Toxicity of DCOIT in water and sediment on neotropical organisms.
- ERA of DCOIT based on whole sediment toxicity.
- Tropical species are more sensitive to DCOIT than temperate animals.
- Potential environmental risks of DCOIT in sediments worldwide.

Abstract

DCOIT is an effective antifouling biocide, which presence in both environmental waters and sediments and toxicity towards nontarget species have been generating great concern. This study evaluated the toxicity of DCOIT in aqueous solutions on *Perna perna* (bivalve – embryonic development), *Echinometra lucunter* (sea-urchin - embryonic development), *Artemia* sp (crustacean - mortality), and in whole sediments on *Nitrocra* sp. (copepod – fecundity rate), and *Tiburonella viscana* (amphipod - mortality). The obtained data were used to calculate environmental hazards and risks which were compared to values obtained in temperate regions. The water toxicity can be summarized as follows: *P. perna* - EC_{50}: 8.3 µg/L, *E. lucunter* - EC_{50}: 33.9 µg/L, *Artemia sp* - LC_{50}: 163 µg/L, for sediment, *T. viscana* -LC_{50}: 0.56 µg/g, and *Nitrocra sp* - EC_{50}: 0.21 µg/g. DCOIT hazard, which was assessed based on the PNEC, for tropical pelagic organisms was 1.7-fold lower (more sensitive) compared to non-tropical species. For sediment, DCOIT presented a PNEC of 0.9 µg/kg and the RQs were >1 for Korea, Japan, Spain, Malaysia, Indonesia, Vietnam, and Brazil in areas with high

concentrations of DCOIT such as ports ship/boatyards, marinas, and maritime traffic zones. The presented data is important to support the establishment of policies and regulations for the booster biocides across the world.

Keywords: PNEC, Risk quotient, Sea-Nine, Biocide, Hazard, Antifouling

1. Introduction

Since the prohibition of organotin in antifouling paints, DCOIT (4,5-dichloro-2-n-octyl-4-iso- thiazolin-3-one) has become one of the most widely used alternative biocides (Lam et al., 2017). However, recent studies have shown that DCOIT is toxic to non-target species such as crustaceans, echinoderms and microalgae (Figueiredo et al., 2019). Despite its rapid degradation in water (13 days; Albanis et al., 2002) and sediment (<0.5 days; Thomas et al., 2003), in ports and areas with intensive traffic of vessels the DCOIT can be considered a pseudo persistent pollutant (Chen & Lam, 2017b), and it has been detected in water and sediment worldwide in concentration up to 3.7 mg/L in water (Spain - Martínez and Barceló, 2001) and 0.28 mg/g in sediment (Korea - Lee et al., 2015).

The fate and behavior of DCOIT in the marine environment are not yet fully understood, and just recently, studies have addressed its environmental risk to coastal areas (Abreu et al., 2021; Campos et al., 2021; Figueiredo et al., 2020; Martins et al., 2018a). However, most of the published environmental monitoring studies have been carried out in temperate areas (mainly Europe and Asia). The distribution of the tested species is also very restricted, and according to Campos et al. (2021) most of the tested species are restricted to the Mediterranean and Northeastern Atlantic or present cosmopolitan distribution. The lack of information on the environmental occurrence and toxicity of DCOIT in tropical areas represents an important source of uncertainty to environmental risk assessments conducted in these areas, and the predicted non-effect concentration (PNEC) and risk quotients (RQ) obtained using temperate data may not be suitable for tropical ecosystems.

Regarding sediment risk assessment and toxicity, as far as we are concerned, there is just one study that evaluates the risk of DCOIT in marine sediment (Abreu et al., 2021). However, due to the limited information on sediment toxicity most of the available information on the DCOIT's effects concentration come from freshwater species and the PNEC has been frequently calculated based on the water toxicity values and sediment phisicoquemical properties.

Considering the fate and behavior of DCOIT in tropical ecosystems, important questions were raised. What is the sediment toxicity of DCOIT towards benthic species? Are these species at risk? Are tropical or neotropical species more sensitive to DCOIT compared to temperate or cosmopolitan species? Are the available PNECs suitable for tropical or neotropical species? To respond these questions, the present study aimed to, for the first time, assess the whole sediment toxicity of DCOIT on the following tropical benthic species: The amphipod *Tiburonella viscanna* and the copepod *Nitocra* sp. It also aimed to evaluate the risk and hazards related with the contamination of marine sediments with the DCOIT using whole sediment toxicity data, and to evaluate the water toxicity of DCOIT toward the following neotropical species: embryos of the brown mussel *Perna perna*, eggs and embryos of echinoderm (*Echinometra lucunter)*, nauplii of the crustacea *Artemia* sp. Finally, this investigation aimed to evaluate and compare the PNECs obtained to and temperate species.

2. Material and Methods

2.1 Toxicity tests in aqueous solutions

The toxic effects of DCOIT were assessed for three marine invertebrates: the microcrustacean *Artemia sp*, and embryos of the bivalve *Perna perna* and the sea urchin *Echinometra lucunter*. The nominal exposure concentrations were prepared by diluting the stock solution of 96 mg/L (real concentration) in 0.45 µm filtered artificial seawater. The stock solution was prepared with acetone as cosolvent. Physicochemical properties (salinity, pH and dissolved oxygen) were measured at the beginning and end of experiments.

Individuals of *E. lucunter* and *P. perna* were collected from reference areas from the coast of São Paulo (BR) and transported to laboratory. Before the experiment, the organisms were acclimated for 72 h, with no food supply, with continuous aeration at 25±2 ºC and a photoperiod of 16:8 (light:dark). *Artemia* cysts were bought in the local fish store. The experiments had a negative (seawater) and acetone (solvent) control.

For *Artemia* sp., the acute toxicity test was carried out following the procedures described by Veiga and Vital (2002). Ten 48-hour old nauplii were allocated in four replicates containing 10 ml of DCOIT solution. The test concentrations (nominal) were 963 µg/L 722 µg/L, 481 µg/L, 240 µg/L, 96 µg/L and 10 µg/L, and were chosen based on the literature (Figueiredo et al., 2019; Jung et al., 2017) and previous experiments. The mortality was checked at the end of the 48 h exposure.

For *E. lucunter* and *P. perna* the chronic toxicity was assessed though the embryo-larval development test proposed by ABNT NBR 15350 (2006) and Zaroni et al. (2005)respectively. For both organisms, gametes spawning was induced and then followed by in vitro fertilization. Approximately 500 fertilized eggs were added in glass test tubes containing 10 ml of DCOIT solution; 4 replicates were prepared for each treatment. For *E. lucunter* the tested concentrations were: 96 µg/L, 72 µg/L, 48 µg/L, 24 µg/L, 12 µg/L, 5 µg/L, 0.09 µg/L, and 0.009 µg/L, while for *P. perna* the tested concentrations were: 481 µg/L, 241 µg/L, 96 µg/L, 72 µg/L, 48 µg/L, 25 µg/L, 12 µg/L, 5 µg/L, 0.9 µg/L, and 0.09 µg/L. At the end of the exposures (≈ 36 hours for *E. lucunter*, and ≈ 48 hours for *P. perna*), the experiments were finished by the addition of neutralized formaldehyde, and the embryos were analyzed through microscope for morphological abnormalities and delayed development.

2.2 Whole sediment toxicity test

The acute whole sediment toxicity test with the amphipod *Tiburonella viscana* was performed as described by Melo and Abessa (2002) and ABNT (2016).The test was assembled in 1L polyethylene test chambers with a 2 cm layer of spiked sediment (approximately 150 g) and 700 ml of artificial seawater. Ten organisms, in quadruplicate, were exposed to the following concentrations: 65 ng/g, 107 ng/g, 409 ng/g, 15557 ng/g and 111401 ng/g, under controlled temperature (25±2 ºC), constant aeration and 13:10 light: dark photoperiod. After 10 days of exposure the mortality was checked. The used sediment was collected in the Lagamar Protected Area, close to the Ilha do Cardoso State Park (Cananéia, State of São Paulo, Brazil). This region was considered as reference site due to its high biodiversity and absence of anthropogenic impacts (Cruz et al., 2014; Campos et al., 2016).

The chronic toxicity test was assessed for the copepod *Nitocra* sp. as described by Lotufo and Abessa (2002). Four replicates were assembled in polyethylene test chambers containing 4g of sediment and 20 ml of brackish water (salinity 20). Then 10 ovigerous females per replicate were exposed for 7 days under constant temperature (25 ± 2 °C) and 12h:12h (light: dark) photoperiod. At the end of the experiment the contents of each test chamber were fixed with formaldehyde (4%) and dyed with Rose-Bengal (0.1%). Offspring and adults were counted to determine the fertility rate.

2.3 Analytical procedures

The measured concentration of DCOIT on water stock solution and sediment were performed in duplicate according to Harino et al. (2005). Briefly, for the sediment, 1 g of sediment sample was spiked with 10 ng/ml of atrazine as an internal standard, and 15 mL of acetonitrile. The samples were sonicated for 30 min and centrifuged at 4000 rpm for 7 min. The supernatants were extracted by solid phase extraction (SPE) using C-18 cartridges (6 mL, 500 mg, Supelco®, Sigma-Aldrich). The analyte was eluted with ethyl acetate (2 x 2 mL).

For water, the quantification on the stock solution, followed the same procedure as for the sediment supernatants abovementioned. The analytes were analyzed by gas chromatography (GC-ECD) using a Perkin Elmer Clarus 500MS equipped with mass spectrometer. The limits of quantification (LOQ), was calculated by the signal to noise ratio, for sediment it was 3 ng/g and for water 0.94 µg/L. QA & QC was based on regular analysis of blanks, spiked matrices, and certified reference material (CRM – PACS-3/National Research Council of Canada, Ottawa, Canada).

2.4 Statistical analysis

The fertility rate of *Nitocra* sp. was converted to percentage based on the control value. Effect and lethal concentrations for 50% of the population (EC/LC50) were calculated with the GRAPHPAD PRISM v5 software, using the non-linear regression equation that best fits the data. The non-effect concentration (NOEC) and lowest effect concentration (LOEC) values were assessed after the One-way analysis of variance (ANOVA) followed by the Dunnett's t'-test. Data transformation (i.e. logarithm transform) was carried out for non-parametic data. If these transformations did not fulfil the aim for normality and homoscedasticity, a Kruskal-Wallis was performed followed by a Dunn's test.

2.5 Hazard and risk assessment

Risk quotients (RQ) can be calculated from both the predicted non effect concentration (PNEC) and the measured environmental concentration (MEC), but because the RQs may greatly differ from region to region depending on the MEC, it becomes difficult to compare the risks posed by the DCOIT to tropical and temperate organisms. To eliminate this source of variability we decided to evaluate the risk of DCOIT only through the PNEC, and this approach is also known as hazard assessment (Chapman et al., 2002; Power & Lanno, 1997).

The PNEC for tropical species was derived from the probabilistic approach, which requires ideally 8 taxonomic groups and/or the main trophic levels represented, applied in a probabilistic distribution function, which output is the 5% hazardous concentration (HC5), a threshold that is expected to protect 95% of the ecosystem's species (TGD, 2003; Staples et al., 2008). The HC5 was calculated based on L/E/IC50 (lethal, effect, and inhibition concentration) using the R software. Species with a geographical distribution restricted to tropical end non-tropical (temperate and polar) areas were analyzed separately. When more than one toxicity data was available for a species, the geometric average was used instead of multiple values. The probabilistic PNEC was derived as follows:

$$PNECprob = \frac{HC5}{(AF = 5)}$$

Regarding the hazard and risk assessment of the sediment, due to the limited toxicity data available for benthic organisms, the PNEC was calculated through the deterministic approach (TGD, 2003):

$$PNECdet = \frac{lowestEfectConc.}{(AF = 100)}$$

The NOECs dataset include values from this study and the literature. The assessment factor (AF) of 100 was applied according to the TGD (2003) guideline as there is at least one chronic toxicity test available. Then the RQ was derived from as follows (TGD, 2003):

$$RQ = \frac{MEC}{PNEC}$$

3. Results

3.1 Seawater and whole-sediment toxicity

In the experiment with *Artemia* sp., the temperature, dissolved oxygen, and pH values ranged from 22.3 ^{o}C to 24 ^{o}C, 7.1 to 7.8, and 3.3 mg/L to 6.8 mg/L respectively (physicochemical properties available as supplementary material). Both the negative and acetone controls presented 100% of survival. The $LC_{50\text{-}48h}$ and $LC_{10\text{-}48h}$ values were 163 (135-

169) µg/L and 86 (61-121) µg/L, respectively (figure 1). The respective LOEC and NOEC were 96.3 and 9.63 µg/L.

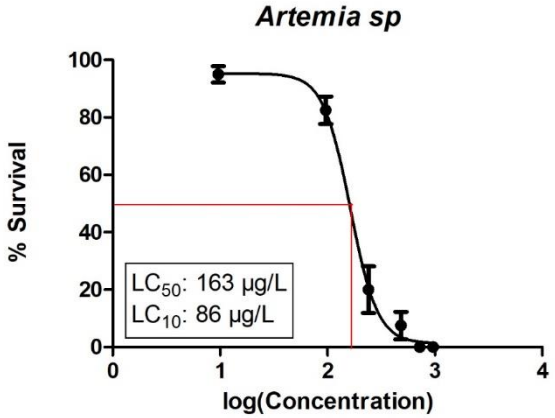


Figure 1: Toxicity of DCOIT on the survival of *Artemia sp*.

During the *E. lucunter* embryo-larval development test, the temperature, dissolved oxygen, and pH values ranged from 22.3 °C to 24.7 °C, 7 to 7.7, and 4.3 mg/L to 5.9 mg/L respectively (physicochemical properties available as supplementary material). There was no statistical difference between the negative and acetone control treatments ($p>0.5$). The $EC_{50\text{-}36h}$ and $EC_{10\text{-}36h}$ values were 33.9 (17-65) µg/L and 9.3 (5-17) µg/L respectively (Figure 2). The LOEC and NOEC were 24 and 12 µg/L respectively.

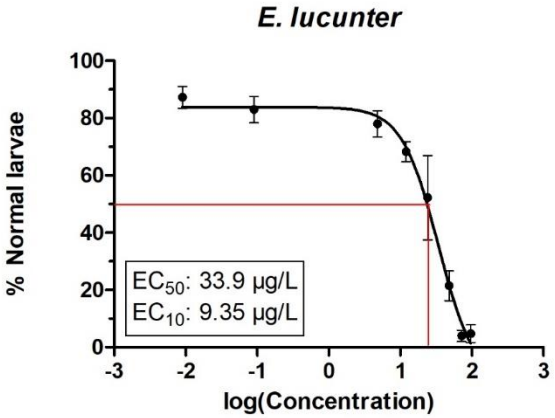


Figure 2: Toxicity of DCOIT on the embryo-larval development of *Echinometra lucunter*.

During the embryo-larval development test of *P. perna* the temperature, dissolved oxygen, and pH values ranged from 24 °C to 25.2 °C, 7.2 to 7.6, and 5.1 mg/L to 6.6 mg/L respectively (physicochemical properties available as supplementary material). The negative and acetone control presented no statistical difference ($p>0.5$). The $EC_{50\text{-}48h}$ and $EC_{10\text{-}48h}$ values

were 8.3 (7-9) µg/L and 3.1 (2-4) µg/L respectively (figure 3). The LOEC and NOEC were 12 and 4.8 µg/L respectively.

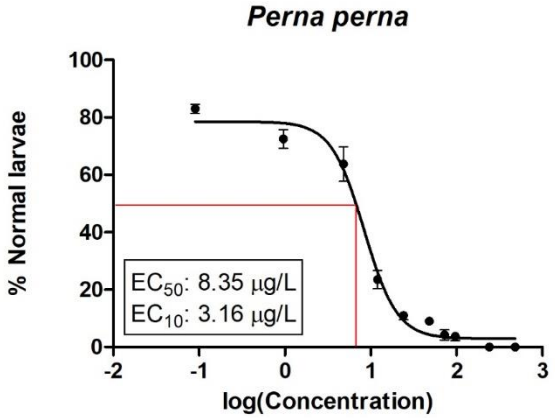


Figure 3: Toxicity of DCOIT on the embryo-larval development of *Perna perna.*

For whole sediment, the acute toxicity test of *T. viscana* the temperature, dissolved oxygen, and pH values ranged from 24.3 °C to 25 °C, 7.7 to 8.2, and 3.4 mg/L to 6.1 mg/L respectively (physicochemical properties available as supplementary material). The negative (with non-contaminated sediment) and acetone controls presented no statistical difference ($p>0.5$). the $LC_{50\text{-}10d}$ and $LC_{10\text{-}10d}$ values were 0.5 (0.1-2.6) µg/g and 0.024 (0.005-0.1) µg/g respectively (Figure 4). The LOEC and NOEC were 0.1 µg/g and 0.06 µg/g respectively.

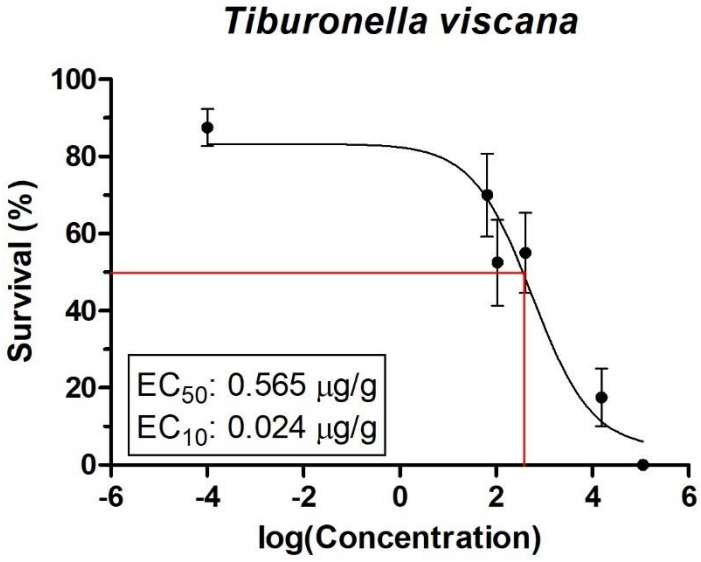


Figure 4: Toxicity of DCOIT on the survival of *Tiburonella viscana* amphipods.

For the chronic toxicity test of copepod *Nitocra* sp. the temperature, dissolved oxygen, and pH values ranged from 24 °C to 25 °C, 7.2 to 8.3, and 4 mg/L to 4.9 mg/L respectively

(physicochemical properties available as supplementary material). The negative (with non-contaminated sediment) and acetone control presented no statistical difference ($p>0.5$). The $LC_{50\text{-}10d}$ and $LC_{10\text{-}10d}$ values were 0.2 (0.009-0.48) µg/g and 0.009 (0.004-0.02) µg/g respectively (Figure 5). The LOEC and NOEC were 111.4 µg/g and 15.5 µg/g respectively.

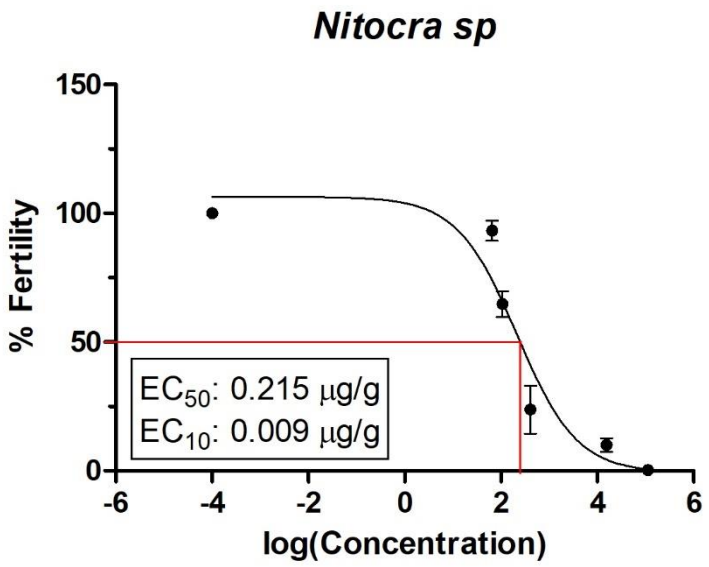


Figure 5: Toxicity of DCOIT on the fertility of *Nitocra* sp. copepods.

3.2 Environmental hazard and risk assessment

The SSDs derived from L/E/IC50 are shown in the figure 6 and the table 1 for tropical species and figure 7 and table 2 for non-tropical (temperate and polar) species. Both datasets included at least one macroalgae, bivalve, echinoderm, crustacean, polychaetae and fish species. The HC5 observed for tropical and non-tropical species were 0.99 µg/L and 1.7 µg/L respectively. The macroalgae *Ulva intestinalis* was the most sensitive tropical species. The echinoderm *Glyptocidaris crenularis* was the most sensitive non-tropical species. The non-tropical species showed a PNEC of 0.34 µg/L, which was 70% higher than the PNEC calculated for tropical species (0.19 µg/L).

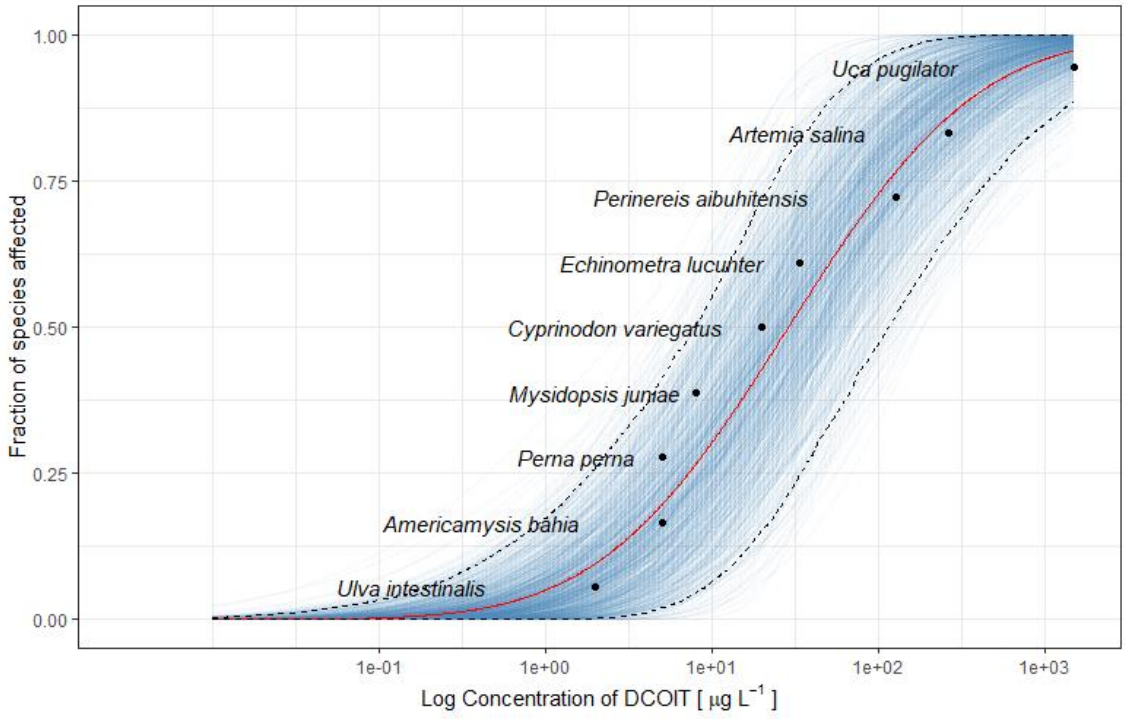


Figure 6: SSD distribution of DCOIT for tropical species

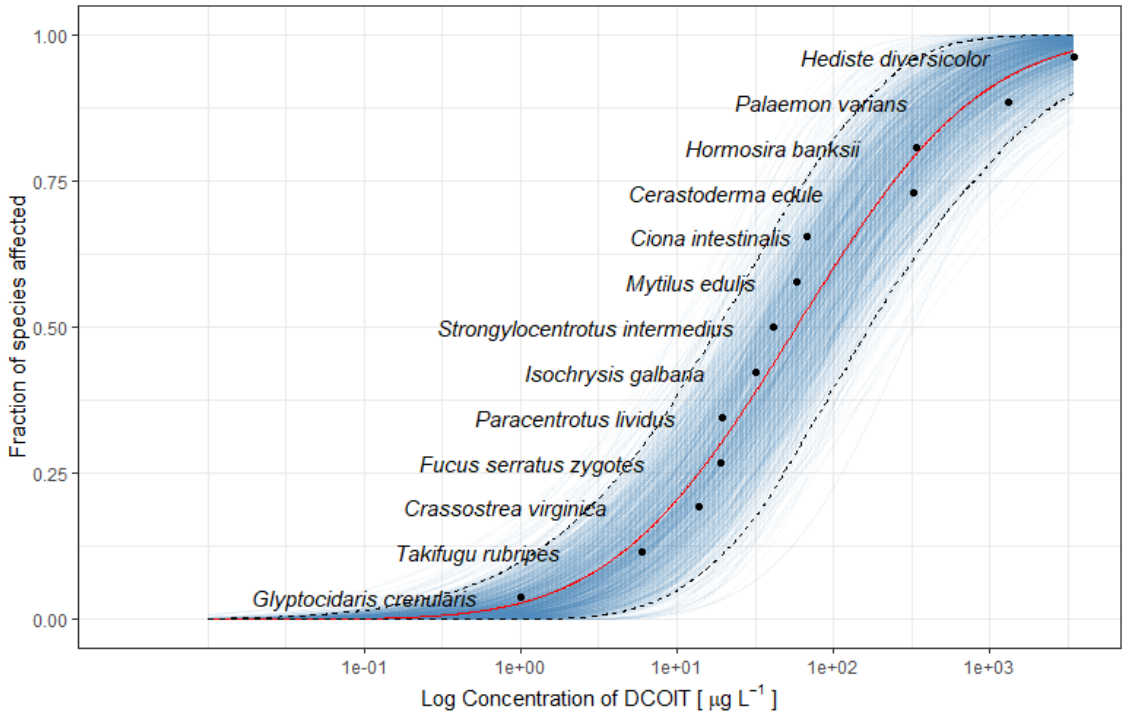


Figure 7: SSD distribution of DCOIT for temperate and polar species

Regarding the sediment, the obtained deterministic PNEC was 0.09 µg/kg by applying the lowest obtained NOEC of 9.7 µg/kg for the polychaeta *Perinereis nuntia* (Onduka et al., 2013). The RQ ranged from 0.9, in Thailand (Harino, Ohji, et al., 2006) to 2893 in Korea (Lee

et al., 2015) as presented in table 3. For all locations excepting Thailand the RQ values were above 1 indicating probable ecological risks.

Table 1: Toxicity data for tropical species used to calculate the HC5 and PNEC.

Organism	Species	Endpoint	Parameter	Value (ug/L)	PNEC(ug/L)	Reference
Crustaceans	*Americamysis bahia*	96h LC_{50}	Mortality	5		(Shade et al., 1993)
Fish	*Cyprinodon variegatus*	96h LC_{50}	Mortality	23		EPA (1992)
Fish	*Cyprinodon variegatus*	96h LC_{50}	Mortality	17		(Shade et al., 1993)
Crustaceans	*Mysidopsis juniae*	96h LC_{50}	Mortality	8		(Jesus et al., 2021)
Polychaete	*Perinereis aibuhitensis*	24h LC_{50}	Mortality	268		(Eom et al., 2019)
Polychaete	*Perinereis aibuhitensis*	96h LC_{50}	Mortality	142		(Eom et al., 2019)
Polychaete	*Perinereis aibuhitensis*	14d LC_{50}	Mortality	55		(Eom et al., 2019)
Macroalgae	*Ulva intestinalis*	120h EC_{50}	(Chlorophyll A concentration)	2	0.19	(Jacobson & Willingham, 2000)
Crustaceans	Artemia salina	48h LC_{50}	Mortality	318		(Jung et al., 2017)
Crustaceans	Artemia salina	48h LC_{50}	Mortality	163		Present study
Crustaceans	Artemia salina	48h LC_{50}	Mortality	351		(Figueiredo et al., 2019)
Echinoderma	Echinometra lucunter	36h EC_{50}	Embryo-Larval Development	33.9		Present study
Bivalves	Perna perna	48h EC_{50}	Embryo-Larval Development	8.35		Present study
Bivalves	Perna perna	48h EC_{50}	Embryo-Larval Development	12.4		(Santos et al., 2020)
Bivalves	Perna perna	72h EC_{50}	Byssus threads	96.1		(Santos et al., 2020)
Bivalves	Perna perna	40min EC_{50}	Fertilization	0.063		(Santos et al., 2020)
Crustaceans	*Uca pugilator*	96h LC_{50}	Mortality	1700		EPA (1992)
Crustaceans	*Uca pugilator*	96h LC_{50}	Mortality	1310		(Shade et al., 1993)

Table 2: Toxicity data for temperate and polar species used to calculate the HC5 and PNEC.

Species	Endpoint	Parameter	Value (ug/L)	PNEC(ug/L)	Reference
Isochrysis galbana	72h EC_{50}	Growth inhibition	32		(Figueiredo et al., 2019)
Mytilus edulis adult	48h EC_{50}	Immobilization	851		(EPA1992)
Mytilus edulis embryo	48h EC_{50}	Immobilization	11		(Bellas, 2006)
Mytilus edulis embryo	48h EC_{50}	Immobilization	411		(European Chemical Agency [ECHA], 2014)
Mytilus edulis embryo	48h EC_{50}	Immobilization	3		(EPA1992)
Paracentrotus lividus	48h EC_{50}	Larval growth	25		(Bellas, 2006)
Paracentrotus lividus	48h EC_{50}	Larval growth	20		(Bellas, 2007)
Paracentrotus lividus	48h LC_{50}	Mortality	25		Figueiredo et al. (2019)
Paracentrotus lividus	48h EC_{50}	Larval development	12		(Bellas, 2006)
Paracentrotus lividus	48h EC_{50}	Larval development	19		(Bellas, 2007)
Palaemon varians	96h LC_{50}	Mortality	1310		(Figueiredo et al., 2019)
Cerastoderma edule	96h LC_{50}	Mortality	325		(Figueiredo et al., 2019)
Hediste diversicolor	96h LC_{50}	Mortality	3430		(Figueiredo et al., 2019)
Ciona intestinalis	24h EC_{50}	Embryonic development	105	0.34	(Bellas, 2006)
Ciona intestinalis	24h EC_{50}	Larval settlement	43		(Bellas, 2006)
Crassostrea virginica	48h EC_{50}	Immobilization	12		(European Chemical Agency [ECHA], 2014)
Crassostrea virginica	48h EC_{50}	Immobilization	9		(EPA1992)
Crassostrea virginica	48h EC_{50}	Immobilization	24		(Willingham & Jacobson, 1996)
Fucus serratus zygotes	24h EC_{50}	Germination percentage	19		(Braithwaite & Fletcher, 2005)
Glyptocidaris crenularis	53h EC_{50}	Larval development	1		(Xu et al., 2011)
Hormosira banksii	48h EC_{50}	Germination	340		(Myers et al., 2006)
Hormosira banksii	48h EC_{50}	Rhizoid growth	430		(Myers et al., 2006)
Strongylocentrotus intermedius	50h EC_{50}	Embryonesis success	14		(Wang et al., 2011)
Strongylocentrotus intermedius	50h EC_{50}	Embryonesis success	32		(Wang et al., 2011)
Strongylocentrotus intermedius	50h EC_{50}	Embryonesis success	57		(Wang et al., 2011)
Strongylocentrotus intermedius	50h EC_{50}	Embryonesis success	114		(Wang et al., 2011)

Takifugu rubripes	96h LC_{50}	Mortality	6	(European Chemical Agency [ECHA], 2014)

Table 3: Sediment risk assessment data, with measured environmental concentrations (MEC), deterministic predict environmental concentration (PNEC) and Risk Quotient (RQ)

Country	Site	MEC (ng/g)	PNECdet	RQ	Reference
Korea	Coasts and Harbors	281	0.097	2896.9	(Lee et al., 2015)
Japan	Osaka Port	110		1134.0	(Harino et al., 2007)
Japan	Hiroshima Bay	55		567.0	(Mochida et al., 2015)
Japan	Otsuchi Bay	150		1546.4	(Harino, Ohji, et al., 2006)
Spain	Catalonia	4		41.2	(Martínez & Barceló, 2001)
Malaysia	Coast	4.2		43.3	(Harino & Langston, 2009)
Thailand	Coast	0.09		0.9	(Harino & Langston, 2009)
Indonesia	Coast	150		1546.4	(Harino, Midorikawa, et al., 2006)
Viatnam	Coast	1.3		13.4	(Harino et al., 2012)
Panama	Coast	123.4		1272.2	(Batista-andrade et al., 2018)
Korea	Bays and harbors	5.5		56.7	(Kim et al., 2014)
Brazil	Santos harbor	74.6		769.1	(Fiamma Eugênia Lemos Abreu et al., 2020)
Brazil	Vitória ES	64		659.8	(Abreu et al., 2021)

4. Discussion

According to Campos et al. (2019), the use of a group of organisms with different ecological strategies and sensitivities, as performed by the present study by using bivalves, echinoderms, and crustaceans in different life stages, provides an accurate view on how the xenobiotics are affecting the overall marine community. Acute ecotoxicological testes are important to define critical levels of contamination. On the other hand, chronic toxicity testes are suitable to detect and prevent early impacts in more realistic and common conditions observed in low and moderately impacted areas.

The obtained toxicity data for *E. lucunter* and *P. perna* are within the sensitivity reported in the literature for these taxa. Bellas (2006) and Santos et al. (2020) found a similar toxicity for the echinoderm *Paracentrotus lividus* (48h EC_{50}: 12.1 µg/L) and the bivalve *P. perna* (48h EC_{50}: 12.4 µg/L). On the contrary, for *Artemia sp.* Figueiredo et al. (2019) found a 48h LC_{50} 2-fold higher than the present study (318 µg/L). The EC_{50s} for sediment were one magnitude higher compared to the water values and represents the first toxicity data obtained for whole sediment for DCOIT. Nevertheless, compared to our data, Onduka et al. (2013) observed sediment toxicity at lower concentrations by testing the growth of the polychaete *Perinereis nuntia* exposed to DCOIT through dietary and sediment for 14 days, with a median lethal concentration of 110 µg/kg. However, considering the sediment degradation of DCOIT of <0.5 days Thomas et al., 2003) it is expected that the main route of contamination at Onduka et al. (2013) experiment was via dietary instead of sediment.

The hazard and risk assessments of DCOIT to tropical areas are scarce mainly due to the limited availability of information about toxicity and measured environmental concentrations data in the literature. Most of the studies estimating the environmental hazards and risks of DCOIT have not included whole sediment toxicity data for marine organisms nor are focused on tropical species (Campos et al., 2021; Figueiredo et al., 2020; Martins et al., 2018b). Moreover, some sediment ERAs have limitations because they are based on one taxonomic group, use toxicity data with freshwater species to estimate risks in marine environments, or use physicochemical characteristics of the DCOIT to estimate the PNEC (Abreu *et al.* 2021). The present study, for the first time, evaluated the hazard of DCOIT to tropical species and compared its toxicity to non-tropical species, generating hazard and risk quotients of DCOIT to benthic species by considering whole sediment toxicity to the effect characterization.

Based on our results, tropical species appeared to be more sensitive in comparison to non-tropical ones. Non-tropical species showed a HC5 and PNEC approximately 1.7-fold higher.

This pattern has been observed for the sediment quality guidelines, as Choueri et al. (2009) demonstrated that tropical species were more sensitive to metals and HPAs compared to equivalent organisms from temperate regions. According to Souza et al. (2015) the overall difference in the sensitivity to xenobiotics are related to the organism basal metabolic rate. According to Pörtner (2012) environmental regimes, such as the temperature, consist of an important variable that directly influence the metabolism of aquatic organisms. For instance, organisms that leave in warm conditions need to adapt their physiology and metabolism to deal with oxygen limitation, on the other hand, under cold conditions, metabolic rates tend to fall and high oxygen solubility in the water and body fluids causes oxygen availability to be in excess. According to Somero (2012) the climate adaptation starts at the transcriptomic and proteomic levels and has implications to the whole-organism functional capacity, such as energy budget, metabolism, membrane, and protein structures, and all these structures and processes also play an important role in the detoxification and toxicodynamic of xenobiotics.

The PNECs for tropical species obtained in this study are similar to those PNECs obtained in other studies that have not identified the toxicity data according to the geographical distribution of the test-species. Figueiredo et al. (2020) and Campos et al. (2021) reported PNEC values of 0.2 µg/L. These data indicates that hazard and risk assessments made with a representative dataset of different trophic levels, merging tropical, cosmopolitan, and temperate species were suitable to generate hazard and risk endpoint for both regions. Apparently, the values of tropical species decrease the toxicity thresholds, as a result of the higher sensitivity of tropical organisms.

Although DCOIT presents a relatively short half-life in seawater (13.3 days, Sakkas et al., 2002), it can be considered a pseudo-persistent contaminant in areas with high traffic of vessels due to the constant input rates. In such cases, DCOIT has recurrent occurrence, and high concentrations have been reported (e.g: Spain: 3.7 ug L-1, Martínez and Barceló (2001); Denmark: 0.2 ug L-1, Steen et al. (2004); Japan: 0.1 ug L-1, Tsunemasa et al., 2006). According to our data, in these areas, DCOIT may represent a risk to the local biota, as corroborated by other authors (Campos et al., 2021; Chen & Lam, 2017b; Figueiredo et al., 2020b; Martins, Fillmanna, et al., 2018).

The obtained PNEC and RQs for sediment indicate that DCOIT poses a high risk to the sediment-dwelling community in areas with a high concentration of DCOIT such as ports ship/boatyards, marinas, and maritime traffic zones. These findings were also corroborated by Abreu et al. (2021) which estimated that DCOIT could pose risk to 47% of 113 evaluated coastal

sites in Brazil. Compared to other antifouling biocides the PNEC of the DCOIT in sediment was lower than Dichlofluanid (PNEC = 16.6 µg/kg) and higher than Irgarol, Chlorothalonil, and Diuron (PNECs of 0.4 µg/kg , 2.4 µg/kg, and 4.3 µg/kg respectively) (Abreu et al., 2021). However, the toxicity data and MECs for these antifouling biocides are scarce, which increase the uncertainty of the risks and hazards estimated to the DCOIT. Thus, efforts to monitor and assess the toxicity of these biocides in the sedimentary compartment are urgent to refine and improve the available environmental risks in coastal areas.

We acknowledge that the available environmental risk assessments of DCOIT in sediment are not ideal due to the limited toxicity data. However, the present study represents an important step to generate endpoints of hazard and risk that efficiently protect the services and functions of coastal environments, being is important to support the development of regulation policies in the field of booster biocides across the world.

5. Conclusion

This study showed that DCOIT causes negative effects on the embriolvarval estage to 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). When added to the whole sediment DCOIT caused negative effects on the amphipod *T. viscana* at 565 µg/kg (LC50), while the copepod *Nitocra* sp. was affected from 215 µg/kg (EC50). The water hazard assessment based on toxicity data of tropical species indicates that tropical marine organisms are 1.7 more sensitive to DCOIT compared to non-tropical species. The present study provides the first risk assessment based on whole sediment toxicity data for bentic species, which revealed that as for planktonic and pelagic species, DCOIT may also pose risk for sediment dweller organisms specially in areas with high traffic of vassels. The presented findings can be useful in a regulatory context, and to better characterize the risk associated to the use of antifouling paints based on DCOIT.

6. Acknowledgments

B. G. Campos (Grants #2017/10211-0) and P. K. G. Choueri (Grants #2017/04970-5), and D. M. S. Abessa (Grants #2020/03004-0) thank the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for the financial support. and D.M.S. Abessa (PQ #308533/2018-6) are research fellows of CNPq. We also thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the financial support (Grant #88881.156405/2017–01).

7. References

ABNT, 2006. ABNT/NBR15350 – Ecotoxicologia aquática – Toxicidade crônica de curta duração – Método de ensaio com ouriço-do-mar (Echinodermata: Echinoidea).

ABNT. 2016. NBR 15638:2016. Ecotoxicologia aquática - Toxicidade aguda - Método de ensaio com anfípodos marinhos e estuarinos em sedimentos. São Paulo: ABNT. 19p.

Abreu, F., Martins, S.E., Fillmann, G., 2021. Ecological risk assessment of booster biocides in sediments of the Brazilian coastal areas. Chemosphere 276, 130155. https://doi.org/10.1016/j.chemosphere.2021.130155

Abreu, F.E.L., Batista, R.M., Castro, Í.B., Fillmann, G., 2021. Legacy and emerging antifouling biocide residues in a tropical estuarine system (Vitória state, SE, Brazil). Mar. Pollut. Bull. 166, 112255. https://doi.org/10.1016/j.marpolbul.2021.112255

Abreu, F.E.L., Natália, J., Braga, Í., Fillmann, G., 2020. Are antifouling residues a matter of concern in the largest South American port ? 398. https://doi.org/10.1016/j.jhazmat.2020.122937

Albanis, T.A., Lambropoulou, D.A., Sakkas, V.A., Konstantinou, I.K., 2002. Antifouling paint booster biocide contamination in greek marine sediments. Chemosphere 48, 475–485. https://doi.org/10.1016/S0045-6535(02)00134-0

ASTN, 1992. Standard guide for conducting static toxicity tests starting with embryos of four species of saltwater bivalve mollusks, in: Annual Book of ASTM Standards: Water and Environmental Technology. pp. 724–789.

Batista-andrade, J.A., Souza, S., Moço, R., Braga, I., Fillmann, G., Gilberto, E., 2018. From TBT to booster biocides : Levels and impacts of antifouling along coastal areas of Panama *. Environ. Pollut. 234, 243–252. https://doi.org/10.1016/j.envpol.2017.11.063

Bellas, J., 2007. Toxicity of the booster biocide Sea-Nine to the early developmental stages of the sea urchin Paracentrotus lividus. Aquat. Toxicol. 83, 52–61. https://doi.org/10.1016/j.aquatox.2007.03.011

Bellas, J., 2006. Comparative toxicity of alternative antifouling biocides on embryos and larvae of marine invertebrates. Sci. Total Environ. 367, 573–585. https://doi.org/10.1016/j.scitotenv.2006.01.028

Braithwaite, R.A., Fletcher, R.L., 2005. The toxicity of Irgarol 1051 and Sea-Nine 211 to the non-target macroalga Fucus serratus Linnaeus, with the aid of an image capture and analysis system. J. Exp. Mar. Bio. Ecol. 322, 111–121. https://doi.org/10.1016/j.jembe.2005.01.015

Campos, B.G., Cruz, A.C.F., Buruaem, L.M., Rodrigues, A.P.C., Machado, W.T. V, Abessa, D.M.S., 2016. Using a tiered approach based on ecotoxicological techniques to assess the ecological risks of contamination in a subtropical estuarine protected area. Sci. Total Environ. 544, 564–573. https://doi.org/10.1016/j.scitotenv.2015.11.124

Campos, B.G. de, Figueiredo, J., Perina, F., Abessa, D. de S., Loureiro, S., Martins, R., 2021. Occurrence, effects and environmental risk of antifouling biocides (EU PT21): Are marine ecosystems threatened? Crit. Rev. Environ. Sci. Technol. in press. https://doi.org/10.1080/10643389.2021.1910003

Chapman, P. M., Ho, K. T., Munns, W. R., Solomon, K., & Weinstein, M. P. (2002). Issues in sediment toxicity and ecological risk assessment. Marine Pollution Bulletin, 44(4), 271–278. https://doi.org/10.1016/S0025-326X(01)00329-0

Chen, L., Lam, J.C.W.W., 2017. SeaNine 211 as antifouling biocide: A coastal pollutant of emerging concern. J. Environ. Sci. 61, 68–79. https://doi.org/10.1016/j.jes.2017.03.040

Choueri, R.B., Cesar, A., Abessa, D.M.S., Torres, R.J., Morais, R.D., Riba, I., Pereira, C.D.S., Nascimento, M.R.L., Mozeto, A.A., DelValls, T.A., 2009. Development of site-specific sediment quality guidelines for North and South Atlantic littoral zones: Comparison against national and international sediment quality benchmarks. J. Hazard. Mater. 170, 320–331. https://doi.org/10.1016/j.jhazmat.2009.04.093

Cruz, A.C.F., Davanso, M.B., Araujo, G.S., Buruaem, L.M., Santaella, S.T., de Morais, R.D., Abessa, D.M.S.,

2014. Cumulative influences of a small city and former mining activities on the sediment quality of a subtropical estuarine protected area. Environ. Monit. Assess. 186, 7035–7046. https://doi.org/10.1007/s10661-014-3908-1

ECB, 2003. European Commission Bureau.Technical guidance document on risk assessment, part II. European Commission – Joint Research Centre, Institute for Health and Consumer protection, European Chemical Bureau;

Environmental Protection Agency [EPA], 1992. Pesticide Ecotoxicity Database [WWW Document]. URL https://cfpub.epa.gov/ecotox/search.cf (accessed 6.29.20).

Eom, H., Haque, N., Nam, S., Lee, D., Rhee, J., 2019. Effects of sublethal concentrations of the antifouling biocide Sea-Nine on biochemical parameters of the marine polychaete Perinereis aibuhitensis. Comp. Biochem. Physiol. Part C 222, 125–134. https://doi.org/10.1016/j.cbpc.2019.05.001

European Chemical Agency [ECHA], 2014. Assessment report, 4,5-Dichloro-2-octyl-2H-isothiazol-3-one (DCOIT), Regulation (EU) n°528/2012 concerning the making available on the market and use of biocidal products, evaluation of active substances.

Figueiredo, J., Loureiro, S., Martins, R., 2020. Hazard of novel anti-fouling nanomaterials and the biocides DCOIT and silver to marine organisms. Environ. Sci. Nano 7, 1670–1680. https://doi.org/10.1039/d0en00023j

Figueiredo, J., Oliveira, T., Ferreira, V., Sushkova, A., Silva, S., Carneiro, D., Cardoso, D.N., Gonçalves, S.F., Maia, F., Rocha, C., Tedim, J., Loureiro, S., Martins, R., 2019. Toxicity of innovative anti-fouling nano-based solutions in marine species. Environ. Sci. nano. https://doi.org/10.1039/C9EN00011A

Harino, H., Arifin, Z., Rumengan, I.F.M., Arai, T., Ohji, M., Miyazaki, N., 2012. Distribution of antifouling biocides and perfluoroalkyl compounds in sediments from selected locations in indonesian coastal waters. Arch. Environ. Contam. Toxicol. 63, 13–21. https://doi.org/10.1007/s00244-011-9747-y

Harino, H., Langston, W.J., 2009. Degradation of alternative biocides in the aquatic environment, in: Ecotoxicology of Antifouling Biocides. Springer Japan, pp. 397–412. https://doi.org/10.1007/978-4-431-85709-9_23

Harino, H., Midorikawa, S., Arai, T., Ohji, M., Cu, N.D., Miyazaki, N., 2006a. Concentrations of booster biocides in sediment and clams from Vietnam. J. Mar. Biol. Assoc. United Kingdom 86, 1163–1170. https://doi.org/10.1017/S0025315406014147

Harino, H., Mori, Y., Yamaguchi, Y., Shibata, K., Senda, T., 2005. Monitoring of antifouling booster biocides in water and sediment from the port of Osaka, Japan. Arch. Environ. Contam. Toxicol. 48, 303–310. https://doi.org/10.1007/s00244-004-0084-2

Harino, H., Ohji, M., Wattayakorn, G., Arai, T., Rungsupa, S., Miyazaki, N., 2006b. Occurrence of antifouling biocides in sediment and green mussels from Thailand. Arch. Environ. Contam. Toxicol. 51, 400–407. https://doi.org/10.1007/s00244-005-0246-x

Harino, H., Yamamoto, Y., Eguchi, S., Kawai, S., Kurokawa, Y., Arai, T., Ohji, M., Okamura, H., Miyazaki, N., 2007. Concentrations of antifouling biocides in sediment and mussel samples collected from Otsuchi Bay, Japan. Arch. Environ. Contam. Toxicol. 52, 179–188. https://doi.org/10.1007/s00244-006-0087-2

Jacobson, A.H., Willingham, G.L., 2000. Sea-nine antifoulant: An environmentally acceptable alternative to organotin antifoulants. Sci. Total Environ. 258, 103–110. https://doi.org/10.1016/S0048-9697(00)00511-8

Jesus, É.P.S. de, Figueirêdo, L.P. de, Maia, F., Martins, R., Nilin, J., 2021. Acute and chronic effects of innovative antifouling nanostructured biocides on a tropical marine microcrustacean. Mar. Pollut. Bull. 164, 111970. https://doi.org/10.1016/j.marpolbul.2021.111970

Jung, S.M., Bae, J.S., Kang, S.G., Son, J.S., Jeon, J.H., Lee, H.J., Jeon, J.Y., Sidharthan, M., Ryu, S.., Shin, H.W., 2017. Acute toxicity of organic antifouling biocides to phytoplankton Nitzschia pungens and zooplankton Artemia larvae. Mar. Pollut. Bull. https://doi.org/10.1016/j.marpolbul.2016.11.047

Kim, N.S., Shim, W.J., Yim, U.H., Hong, S.H., Ha, S.Y., Han, G.M., Shin, K.-H., 2014. Assessment of TBT and organic booster biocide contamination in seawater from coastal areas of South Korea. Mar. Pollut. Bull. 78, 201–208. https://doi.org/10.1016/j.marpolbul.2013.10.043

Lam, N.H., Jeong, H., Kang, S., Kim, D., Ju, M., Horiguchi, T., Cho, H., 2017. Organotins and new antifouling biocides in water and sediments from three Korean Special Management Sea Areas following ten years of tributyltin regulation : Contamination pro fi les and risk assessment 121, 302–312. https://doi.org/10.1016/j.marpolbul.2017.06.026

Lee, M.R.N., Kim, U.J., Lee, I.S., Choi, M., Oh, J.E., 2015. Assessment of organotin and tin-free antifouling paints contamination in the Korean coastal area. Mar. Pollut. Bull. 99, 157–165. https://doi.org/10.1016/j.marpolbul.2015.07.038

Lotufo, G.R., Abessa, D.M.S., 2002. Testes de toxicidade com sedimentos total e água intersticial estuarinos utilizando copépodos bentônicos. Métodos em Ecotoxicologia Mar. Apl. no Bras. 162.

Martínez, K., Barceló, D., 2001. Determination of antifouling pesticides and their degradation products in marine sediments by means of ultrasonic extraction and HPLC-APCI-MS. Fresenius. J. Anal. Chem. 370, 940–945. https://doi.org/10.1007/s002160100904

Martins, S.E., Fillmann, G., Lillicrap, A., Thomas, K. V., 2018a. Review: Ecotoxicity of organic and organo-metallic antifouling co-biocides and implications for environmental hazard and risk assessments in aquatic ecosystems. Biofouling 34, 34–52. https://doi.org/10.1080/08927014.2017.1404036

Martins, S.E., Fillmanna, G., Lillicrapb, A., Thomasb, K. V., 2018b. Review: ecotoxicity of organic and organo-metallic antifouling co-biocides and implications for environmental hazard and risk assessments in aquatic ecosystems. Biofouling 7014, 1–19. https://doi.org/10.1080/08927014.2017.1404036

Melo, S.L.R., Abessa, D.M.S., 2002. Testes de toxicidade com sedimentos marinhos utilizando anfípodos, in: Métodos Em Ecotoxicologia Marinha: Aplicações No Brasil. Artes Gráficas e Indústria, São Paulo, pp. 123–178.

Mochida, K., Hano, T., Onduka, T., Ichihashi, H., Amano, H., Ito, M., Ito, K., Tanaka, H., Fujii, K., 2015. Spatial analysis of 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (Sea-Nine 211) concentrations and probabilistic risk to marine organisms in Hiroshima Bay, Japan. Environ. Pollut. 204, 233–240. https://doi.org/10.1016/j.envpol.2015.05.012

Myers, J.H., Gunthorpe, L., Allinson, G., Duda, S., 2006. Effects of antifouling biocides to the germination and growth of the marine macroalga, Hormosira banksii (Turner) Desicaine. Mar. Pollut. Bull. 52, 1048–1055. https://doi.org/10.1016/j.marpolbul.2006.01.010

Onduka, T., Ojima, D., Ito, M., Ito, K., Mochida, K., Fujii, K., 2013. Toxicity of the antifouling biocide Sea-Nine 211 to marine algae, crustacea, and a polychaete. Fish. Sci. 79, 999–1006. https://doi.org/10.1007/s12562-013-0678-6

Pörtner, H.-O., 2012. Integrating climate-related stressor effects on marine organisms: unifying principles linking molecule to ecosystem-level changes. Mar. Ecol. Prog. Ser. 470, 273–290. https://doi.org/10.3354/MEPS10123

Power, M., & Lanno, R. P. (1997). Risk assessment of chemicals: an introduction. In Aquatic Toxicology (Vol. 38, Issues 1–3). https://doi.org/10.1016/s0166-445x(96)00829-6

Sakkas, V.A., Konstantinou, I.K., Albanis, T.A., 2002. Aquatic phototransformation study of the antifouling agent Sea-Nine 211: Identification of byproducts and the reaction pathway by gas chromatography-mass spectroscopy. J. Chromatogr. A 959, 215–227. https://doi.org/10.1016/S0021-9673(02)00430-2

Santos, J.V.N., Martins, R., Fontes, M.K., Galvao, B., Silva, M.B.M. do P., Maia, F., Abessa, D.M. de S., Perina, F.C., 2020. Can Encapsulation of the Biocide DCOIT Affect the Anti-Fouling Efficacy and Toxicity on Tropical Bivalves ? Appl. Sci. 10, 8579.

Shade, W.D., Hurt, S.S., Jacobson, A.H., Reinert, K.H., 1993. Ecological Risk Assessment of a Novel Marine Antifoulant, in: Gorsuch, J.W., Dwyer, F.J., Ingersoll, C.G., La, T.W.P. (Eds.), Environmental Toxicology and Risk Assessment: Second Volume. ASTM International, West Conshohocken, PA, pp. 381–408. https://doi.org/10.1520/STP13169S

Souza, I.S. de, Araujo, G.S. de, Cruz, A.C.F., Fonseca, T.G., Camargo, J.B.D.A. de, Medeiros, G.F., Abessa, D.M. de S., 2015. Using and integrated approach to assess the sediment quality of an estuary from the semi-arid coast of Brazil. Mar. Pollut. Bull. 104, 70–82. https://doi.org/10.1017/CBO9781107415324.004

Somero, G. N. (2011). The Physiology of Global Change: Linking Patterns to Mechanisms. Http://Dx.Doi.Org/10.1146/Annurev-Marine-120710-100935, 4, 39–61. https://doi.org/10.1146/ANNUREV-MARINE-120710-100935

Staples, C.A., Woodburn, K.B., Klecka, G.M., Mihaich, E.M., Hall, A.T., Ortego, L., Caspers, N., Hentges, S.G., 2008. Comparison of four species sensitivity distribution methods to calculate predicted no effect concentrations for bisphenol A. Hum. Ecol. Risk Assess. 14, 455–478. https://doi.org/10.1080/10807030802074170

Steen, R.J.C.A., Ariese, F., Hattum, B. Van, Jacobsen, J., Jacobson, A., 2004. Monitoring and evaluation of the environmental dissipation of the marine antifoulant (DCOIT) in a Danish Harbor 57, 513–521. https://doi.org/10.1016/j.chemosphere.2004.06.043

TGD, 2003. Technical Guidance Document on Risk Assessment in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances, Commission Regulation (EC) No 1488/94.

Thomas, K. V., McHugh, M., Hilton, M., Waldock, M., 2003. Increased persistence of antifouling paint biocides when associated with paint particles. Environ. Pollut. 123, 153–161. https://doi.org/10.1016/S0269-7491(02)00343-3

Tsunemasa, N., Hashimoto, K., Yamaoka, Y., Ueno, H., Okamura, H., 2006. Contamination of an Alternative Antifoulant in Coastal Waters of Hiroshima Bay. rnal Environ. Chem. 16, 201–211.

US EPA, 2001. Methods for Collection, Storage and Manipulation of Sediments for Chemical and Toxicological Analyses:Technical Manual (EPA-823-B-01-002). Washington.

Veiga, L.., Vital, N., 2002. Teste de toxicidade aguda com o microcrustáceo Artemia sp, in: Nascimento, I.A., Sousa, E.C.P.M., Nipper, M. (Eds.), Métodos Em Ecotoxicologia Marinha: Aplicações No Brasil. Editora Artes Gráficas e Indústria Ltda, São Paulo, pp. 111–122.

Wang, H., Li, Y., Huang, H., Xu, X., Wang, Y., 2011. Toxicity evaluation of single and mixed antifouling biocides using the Strongylocentrotus intermedius sea urchin embryo test. Environ. Toxicol. Chem. 30, 692–703. https://doi.org/10.1002/etc.440

Willingham, G.L., Jacobson, A.H., 1996. Designing an Environmentally Safe Marine Antifoulant. ACS Symp. Ser. 640, 224–233. https://doi.org/10.1021/bk-1996-0640.ch011

Xu, X., Wang, X., Li, Y., Wang, Yonghua, Wang, Yuan, 2011. Acute toxicity and synergism of binary mixtures of antifouling biocides with heavy metals to embryos of sea urchin Glyptocidaris crenularis. Hum. Exp. Toxicol. 30, 1009–21. https://doi.org/10.1177/0960327110385958

Zamboni, A., Costa, J.B., 2002. Teste de toxicidade com sedimentos marinhos utilizando tanaidáceos, in: Nascimento, I.A. (Ed.), Métodos Em Ecotoxicologia Marinha-Aplicações No Brasil. Artes Gráficas e Indústria Ltda, São Paulo, p. 262.

8. Supplementary Material

Table S1: Physicochemical properties during the *Artemia sp* toxicity test

	pH		Sal		OD		Temp	
Concentration ug/L	Beginning	End	Beginning	End	Beginning	End	Beginning	End
963	7.34	7.33	35	35	6.8	6.4	23.2	23.5
722	7.44	7.59	35	35	3.9	3.2	22.8	24.1
481	7.55	7.17	35	35	3.3	4.7	22.3	24.3
240	7.62	7.8	35	35	5.5	6.3	23.4	24
96	7.29	7.75	35	35	6.7	5.9	23.6	23.9
9	7.59	7.82	35	35	3.3	4.7	22.9	23.5
Water control	7.25	7.31	35	35	5.1	4.9	23.1	23.4
Solvent control	7.33	7.15	35	35	5.5	5.4	23.7	23.2

Table S2: Physicochemical properties during the *Echinometra lucunter* toxicity test

Concentration µg/L	pH		Sal		OD (mg/L)		Temp (C°)	
	Beginning	End	Beginning	End	Beginning	End	Beginning	End
96	7.11	7.68	35	35	5.3	4.4	23.1	23.7
72	7.06	7.32	35	35	5.4	5	23.4	24.6
48	7.52	7.6	35	35	4.3	5.3	23.2	22.5
24	7.2	7.26	35	35	5.9	5.9	23	23.9
12	7.45	7.16	35	35	5.2	5.1	23.1	23.3
4	7.72	7.45	35	35	4.7	4.3	23.8	24.8
0.09	7.67	7.2	35	35	5.1	4.8	24.7	23.9
0.009	7.52	7.52	35	35	5.9	5.9	23.8	23.8
Water control	7.01	7.55	35	35	5.4	5.2	23.4	24.4
Solvent control	7.75	7.31	35	35	5.9	5.3	24.6	22.3

Table S3: Physicochemical properties during the *Perna perna* toxicity test

Concentration ug/L	pH		Sal		OD		Temp	
	Beginning	End	Beginning	End	Beginning	End	Beginning	End
481	7.26	7.22	35	35	6.3	5.7	24.3	24.8
241	7.69	7.64	35	35	5.8	5.7	24.2	24.8
96	7.21	7.46	35	35	6.1	5.3	24	27.7
72	7.42	7.43	35	35	5.4	5.6	24.3	25
48	7.44	7.46	35	35	5.9	5.5	24.1	24.7
25	7.61	7.48	35	35	6.3	5.2	24.4	25.2
12	7.57	7.63	35	35	6.6	6	24	24.9
5	7.46	7.55	35	35	6.1	5	24.2	25.2
0.9	7.35	7.64	35	35	5.4	5.7	24.3	25.2
0.09	7.22	7.27	35	35	6.3	5.7	24.4	24.9
Water control	7.51	7.38	35	35	5.4	5.5	24	25.1
Solvent control	7.34	7.36	35	35	6	5.8	24.5	24.9

Table S4: Physicochemical properties during the *Tiburonella viscanna* toxicity test

Concentration ng/g	Sal		pH		OD (mg/L)		Temp. °C	
	Beginning	End	Beginning	End	Beginning	End	Beginning	End
65	34	34	8.2	8.0	4.3	6.1	24.3	25
107	34	35	8.0	7.9	6	6	24.3	25
409	35	35	7.9	7.7	3.4	3.4	24.3	25
15557	35	35	7.9	7.9	3.3	3.4	24.3	25
111402	34	35	8.2	8.1	4.8	3.8	24.3	25
Water control	34	34	8.1	8.0	5.6	5.4	24.3	25
Solvent control	34	35	7.7	8.1	3.8	5.6	24.3	25

Table S5: Physicochemical properties during the *Nitocra sp* toxicity test

Concentration ng/g	Sal		pH		OD (mg/L)		Temp. °C	
	Beginning	End	Beginning	End	Beginning	End	Beginning	End
65	21	20	7.8	7.82	4.2	4.3	24	24.8
107	20	22	7.43	7.92	4.8	5	24	24.7
409	23	21	7.72	8.23	4.5	4.6	24.4	24.4
15557	21	22	8.16	7.51	4.8	4	24.5	24.5
111402	23	20	7.84	8.18	4.3	4.9	24.9	24.3
Water control	23	23	8.3	7.9	4.3	4.3	24.1	24.1
Solvent control	23	23	7.2	7.7	4.1	4.5	24.1	24.0

Article 4 - Bioaccumulation and trophic transfer in mussels after short term exposure to DCOIT and SiNC-DCOIT

Bruno G. Campos*[a], Olga Kaczerewska[b], Frederico Maia[c], Denis M. S. Abessa[a], Susana Loureiro[d], Roberto Martins[d]

[a] *São Paulo State University (UNESP), Praça Infante Dom Henrique, s/n, Zipcode: 11330-900 São Vicente, São Paulo, Brazil*

[b] *CICECO – Aveiro Institute of Materials, Department of Materials and Ceramic Engineering, University of Aveiro, 3810-193 Aveiro, Portugal*

[c] *Smallmatek – Small Materials and Technologies, Lda, Rua Canhas, 3810-075 Aveiro, Portugal*

[d] *CESAM - Centre for Environmental and Marine Studies, Department of Biology, University of Aveiro 3810-193 Aveiro, Portugal*

Corresponding Author: *Bruno Galvão de Campos, São Paulo State University (UNESP), Praça Infante Dom Henrique, s/n, Zipcode: 11330-900 São Vicente, São Paulo, Brazil. E-mail: b.campos@unesp.br*

Status*:* Under consideration for submission as short communication (reporting of preliminary findings)

Abstract

After the ban of the tributyltin-based antifouling paints, DCOIT (4,5-Dichloro-2-octylisothiazol-3(2H)-one) has become one of the most frequently used antifouling biocide. Besides being considered a pseudo persistent contaminant in areas with intensive traffic of vessels and toxic to non-target species, the bioaccumulation and trophic transfer of DCOIT in marine organisms remains unknown. The present study aimed to investigate the bioaccumulation, trophic transfer and biomagnification 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. Our results indicated that the mussels rapidly internalized and metabolized both DCOIT and SiNC-DCOIT. The predator-prey biomagnification factors (BMF TL) suggested that both biocides can transfer up through a food chain, with higher concentrations of DCOIT and SiNC-DCOIT in the consumers (mussels) relative to the prey (microalgae) (BMF TL > 1). However, the bioaccumulation and bioconcentration factors indicated low bioaccumulation capability, suggesting that the depuration rate overcomes the uptake. Yet, we need to consider that in harbors and marinas where organisms are chronically exposed to DCOIT bioaccumulation may occur. The presented findings can contribute to the elaboration of more accurate environmental risk assessments for DCOIT and SiNC-DCOIT. In addition,

our results suggest that areas with a constant input of DCOIT bivalves may accumulate DCOIT once the uptake rate overcomes the depuration.

Keywords: Biomagnification, biocides, nanoparticles, antifouling, microalgae.

1. Introduction

After the ban of the use of tributyltin-based antifouling biocides by the International Marine Organization (IMO, 2001), DCOIT (4,5-Dichloro-2-octylisothiazol-3(2H)-one) has become one of the most frequently used antifouling biocides (Lam et al., 2017). Initially, DCOIT was proposed as a safe and environmentaly safe antifouling biocide due to its rapid degradation (Jacobson & Willingham, 2000). However, more than 20 years after its creation and usage DCOIT is now considered a pseudo persistent contaminant in areas with intensive traffic of vessels such as harbors and marinas (Chen & Lam, 2017), and has been detected worldwide, in both water and sediment (Campos et al., 2021) in concentrations up to 3.7 µg/L (Spain - Martínez & Barceló, 2001) and 0.28 µg/g (South-Korea - Lee et al., 2015), respectively. In addition, some studies have demonstrated that DCOIT causes extensive deleterious effects on primary producers and non-fouler marine organisms (Chen, Zhang, et al., 2014; Cima et al., 2008; Figueiredo et al., 2020; Jesus et al., 2021; Santos et al., 2020) with a PNEC (predicted non-effect concentration) calculated at 0.21 µg/L (Campos et al., 2021).

DCOIT is considered an effective antifouling biocide that may cause various negative effects on marine organisms. Chen et al. (2014) and Eom et al. (2019) observed, respectively, an increase of the levels of reactive oxygen species (ROS) in fish, and increased activity of the superoxide dismutase in polychaetes, both after exposure to environmentally relevant concentrations of DCOIT. These findings were corroborated by other studies that observed modulation of GPx (glutathione peroxidase), CAT (catalase), and LPO (lipid peroxidation) in bivalves (Gabe et al., 2021), corals (Cima et al., 2013), ascidians (Cima et al., 2008), and fish (Ito et al. 2013). In addition, Chen et al. (2014), and Do et al. (2018) observed that DCOIT can also cause neurotoxicity by inhibiting acetylcholinesterase, as demonstrated in mysids and fish.

Immunosuppression and imbalance of energy production can also be included in the list of DCOIT negative effects. Cima et al. (2008) and Bragadin et al. (2005) detected significant immunosuppressive effects on hemocyte cells from the ascidian *Botryllus schlosser*, and inhibition of the cytochrome-c oxidase resulting on reduction of ATP synthesis and alteration at the mitochondrial respiratory chain

Despite the available information on the DCOIT toxicity, to our knowledge very little is known about the bioaccumulation and trophic transfer of this compound in marine organisms. Nevertheless, accumulation on marine organisms may be expected due to the relatively low solubility of DCOIT in seawater (14 mg/L) and high log Kow (octanol-water partitioning coefficient) of 6.4 (Chen & Lam, 2017). According to Hilvarsson et al. (2009) the amount of bioaccumulated substance normally correlates with the Kow, thus a linear relationship between bioaccumulation and Kow is observed for most substances. However, according to the Technical Guidance Document on Risk Assessment Part II. of 2003 (EUR 20418 EN/2.), the Kow alone is not enough to classify a substance as bioaccumulative. Biomagnification, bioconcentration, and bioaccumulation factors (BMF, BCF and BFA, respectively) are traditionally used to assess the bioaccumulation and how much of a certain contaminant an organism can absorb from the environment or from their food (in the case of BMF). The information regarding bioaccumulation and trophic transfer is essential for an accurate hazard and risk assessment of a substance.

Recent nanotechnological advancements have demonstrated that encapsulating DCOIT in engineered nanomaterials (ENM) reduces the environmental effect and minimizes the unwanted leaching from marine coatings (Maia et al., 2015; Reybuck & Schwartz, 2008). Figueiredo et al. (2019, 2020) confirmed that DCOIT encapsulation on silica mesoporous nanocapsules (SiNC) significantly reduced the short-term toxicity and marine hazard of DCOIT on 12 marine species from bacteria to fish (reduction up to 214-fold for SiNC-DCOIT). However, despite the available knowledge regarding the SiNC-DCOIT, the bioaccumulation and trophic transfer capability of these nanostructured antifouling biocides are still unknown.

This study aimed to investigate the bioaccumulation, trophic transfer and biomagnification of DCOIT and SiNC-DCOIT from the marine microalgae *Tetraselmis chuii* to the mussel *Mytilus galloprovincialis* during a 2-phase experiment, including uptake (1h, 3h and 24h) and depuration (72h). The bioaccumulation for mussels was tested considering three different exposure scenarios: I) though food contamination; II) water contamination; and III) both water and food contamination. At the end of the experiment, the BCF for *T. chuii* and BCF, BAF, and BMF for *M. galloprovincialis* were calculated.

2. Material and Methods

2.1 Chemicals and exposure solutions

The stock solutions of the DCOIT and SiNC-DCOIT standards were prepared using Sigma-Aldrich™ reagent grade chemicals. Smallmatek, Small Materials and Technologies, Lda, generously created and supplied the tested nanomaterial SiNC-DCOIT (silica mesoporous nanocapsules filled with DCOIT). Details about the nanomaterials synthesis and characterization are fully described by Figueiredo et al. (2019).

2.2 Organisms acquisition and acclimation

Specimens of the mussel *Mitilus galloprovincialis* were collected at Costa Nova beach, Portugal, and acclimated in ASW (artificial seawater) for 7 days with continuous aeration, at 19±1ºC, 16:8 h light: dark photoperiod and feed with the microalgae *Tetraselmis chuii* every 48h (≈3 x 105 cells/ml). The microalgae *T.chuii* was cultivated in laboratory by using the culture medium Optimedium (purchased from Aqualgae) with a photoperiod of 16:8 h (light: dark)

2.3 DCOIT and SiNC-DCOIT bioaccumulation and trophic transfer test

Concentrations for both DCOIT and SiNC-DCOIT were expressed in µg of DCOIT/L. The experimental design can be seen in figure 1. For the bioaccumulation and biomagnification exposure, the uptake times of 1h, 3h, and 24h, and depuration of 72h were chosen based on previous experiments. The following treatments were assessed:

1. Contamination through seawater (waterborne exposure): The tested concentration was 80 µg of DCOIT/L, which is below the CL_{50} for *M. galloprovincialis* and above the detection limit for quantification. The bioconcentration factor (BCF) for M. *galloprovincialis* was assessed through this treatment.
2. Dietary exposure: 48h before the mussel exposure (previous experiments demonstrated that in 48h the DCOIT is incorporated into the microalgae) two cultures of the microalgae *T chuii* were contaminated with DCOIT and SiNC-DCOIT respectively, at 5 µg of DCOIT/L (10 times lower the CENO for *T.chuii*). The mussels were feed at the beginning of the experiment with 2.5 x 10^5 cells/mussel of *T. chuii*. Before feeding, the microalgae were rinsed with uncontaminated ASW through a 0.045 µm filter to remove the culture media.

Cell density was spectrophotometrically measured at fluorescence (λ_{exc} = 475 and λ_{emi} = 645 nm). This treatment allowed us to assess the biomagnification factor (BMF) of *M. galloprovincialis*, and by quantifying the DCOIT of the microalgae. The obtained data also allowed the calculation of the bioaccumulation factor (BAF) of DCOTI for *T.chuii*.

3. Contamination through both water and food: In this treatment, mussels were exposed to DCOIT and SiNC-DCOIT through water (80 µg of DCOIT/L) and food (5 µg of DCOIT/L) as described above (items 1, and 2). At the end of the experiment the BAFs were calculated for mussels.

Negative control with ASW was assembled for each treatment. For the control treatment in which the animals were feed, uncontaminated algae were provided. The experiment was made in triplicates with 4 organisms per test chamber (250ml glass flasks). After 24 h of exposure, the water was renewed, and no biocide was added for the depuration phase. The depuration phase lasts for 72 h and had the same aeration and photoperiod as for the uptake phase. Summing the uptake more depuration the experiment lasts for 96 h.

In each timestamp at the uptake and depuration, 3 organism and water aliquots (50ml) of each treatment were taken. The water and organisms were frozen at -80 C, then the organisms were lyophilized for chemical quantification. Three aliquots of contaminated microalgae were taken just after the feeding at the beginning of the exposure. The microalgae aliquots were centrifuged at 10000 rpm for 5 minutes to separate the water and microalgae, them both were frozen at -80 C until chemical quantification.

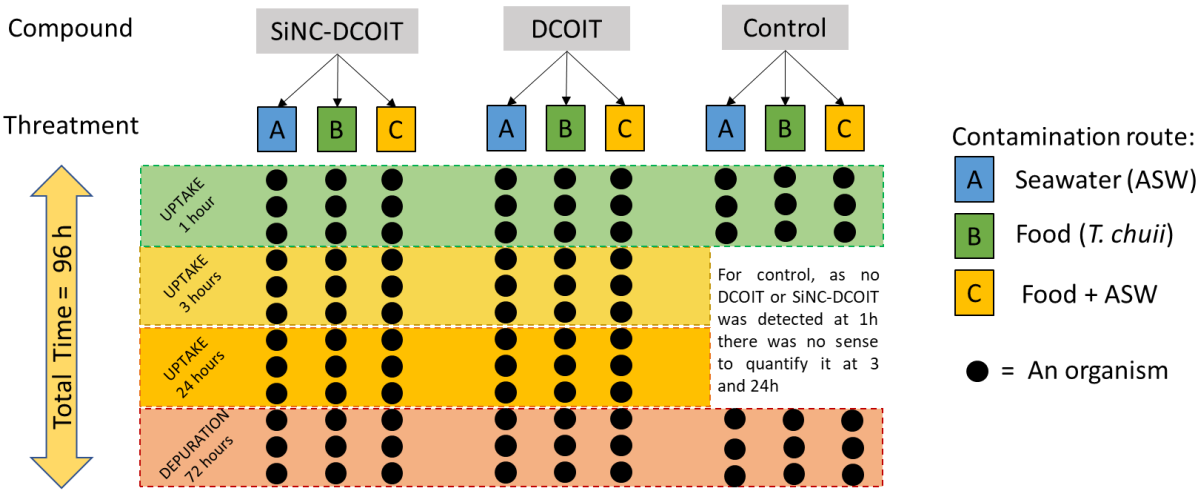


Figure 1: Experimental design of the bioaccumulation and biomagnification test with mussels of the species *mytilus galloprovincialis*. The microalgae *Tetraselmis chuii* was used as food.

2.4 Chemical quantification

2.4.1 Water Extraction

For DCOIT and SiNC-DCOIT extraction, 50ml of water media were passed through a solid-phase extraction system (SPE) using C-18 (500mg) cartridges previously activated with 4 ml of ethyl acetate (HPC-Grade) and 4 ml of ultrapure (UP) water, at 1 ml/min flux. Then the cartridges were washed with 4ml of UP water. Then the cartridges were eluted twice with 2 ml of ethyl acetate into 10ml flasks. The content of each flask was dried out and concentrated with N_2 until the volume of 1.5ml. Then the content was transferred to vials and refrigerates until the quantification at GC-ECD.

2.4.2 Mussel Extraction

The extraction of DCOIT from mussel soft tissues was using the vortex assisted matrix solid-phase dispersion method, adapted from Vieira et al. (2018). According to this method, 0.2g (lyophilized, dry weight) of mussel were macerated with 2 g of capped C-18 (extracted from new SPE C-18 cartridges) and 0.2 g of $NaSO_4$ until it was transformed into a powder. Next, this powder was transferred into 15 ml centrifuge tubes and 5 ml of ethyl acetate and 35 mmol/L of Acetic acid were added. Then the centrifuge tubes were vortexed for 5min and centrifuged at 9000 rpm for 5min. An aliquot of the supernatant was collected for chemical quantification at GC-CED.

2.4.3 Microalgae Extraction

For microalgae the extraction method was adapted from González-Barreiro et al. (2006). The samples of *T.chuii* were unfrozen at room temperature and resuspended with 5 ml of acetonitrile and sonicated with an Ultrasonic Probe Sonicator for 30 seconds (3 pulses of 10 seconds) then 35 mL of UP water was added and the content was centrifuged at 10000 rpm for 5 min (with an ultra-centrifuge). The supernatant was recovered, and the pellet was dried and weighted. The supernatant went to the already mentioned water extraction process.

2.4.4 DCOIT Quantification - Gas-chromatography analysis

After extraction, the samples were analyzed at GC-ECD (Gas-Chromatography/Electron Capture Detector) using ethyl acetate as mobile phase, with a C-18 column with a run time of 13.9 min (DCOIT elution time is 11.986 min), the oven temperature of 290°C. Between each sample, the column was washed 3 times with ethyl acetate. The DCOIT concentrations were

calculated based on a standard curve with a $R^2 > 0.98$. The retention time of DCOIT was 11.68 min.

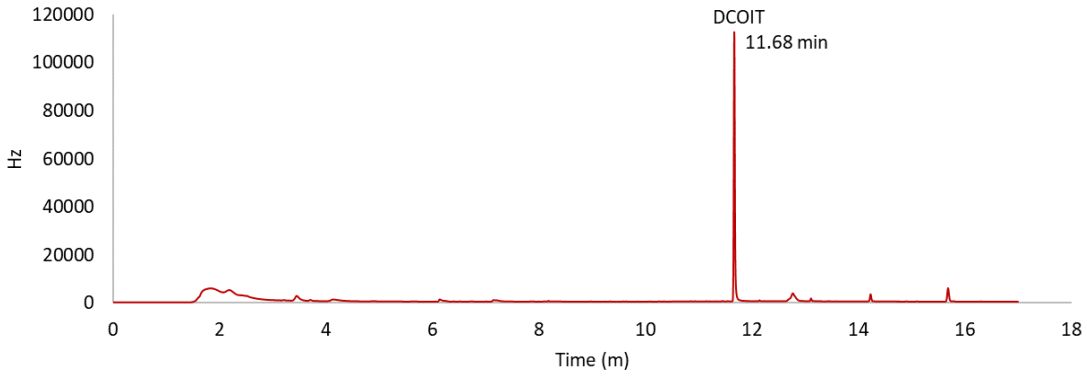


Figure 2: DCOIT standard (80 µg/L) chromatogram showing a retention time of 11.68 min.

2.5 Bioaccumulation, Bioconcentration and Biomagnification end-points calculation

The bioconcentration factor (BCF), bioaccumulation factor (BAF) and the predatory biomagnification factor (BMF_{TL}) were calculated for all timestamps during the uptake and depuration phase. BAF was calculated for the ASW treatment; BCF was calculated for the ASW + Food treatment; while BMF_{TL} was calculated for the food exposure treatment. The used formulas are presented below:

$$BCF = \frac{[Mussel]}{[ASW]}$$

$$BAF = \frac{[Mussel]}{[ASW + Microalgae]}$$

$$\text{BMFTL} = \frac{\left(\frac{[Mussel]}{[Microalgae]}\right)}{\left(\frac{Predatortrophiclevel}{Preytrophiclevel}\right)}$$

Brackets ([]) indicates concentration. Mussels and algae concentration were expressed in µg/Kg of dry weight, water concentration was expressed in µg/L.

3. Results and Discussion

Our data suggested that DCOIT and SiNC-DCOIT were not bioaccumulative, in less than 24 h mussels rapidly internalized and metabolized both forms of DCOIT. Yet, food exposure treatment indicated that besides biomagnification, DCOIT and SiNC-DCOIT can transfer up a food chain in a short-term.

Due to the variability mentioned above (with standard deviations greater than 15000 in some cases), the data were presented individually rather than using measures of central tendency (tables 1 to 6).

3.1 DCOIT bioaccumulation, biomagnification, and trophic transfer

According to Arnot & Gobas (2006) bioconcentration is the process by which a chemical substance is absorbed by an organism from the environment only through its respiratory and dermal surfaces. In the present study, the microalgae *T. chuii* presented a body burden of 139.27 ug of DCOIT/Kg (d.w.), corresponding to a BCF of 4.5. According to the criteria established by the European Chemicals Bureau for BCF and BAF, substances with a BCF or BAF value above 2000 are considered bioaccumulative (Technical Guidance Document on Risk Assessment Part II., 2003. EUR 20418 EN/2). Thus, for microalgae, DCOIT was not considered bioaccumulative.

For waterborne exposure, except for the replicate 3 at 24 h, DCOIT was detected in both ASW and mussel only at 1 h and 3 h, indicating that DCOIT was rapidly internalized and metabolized by the organisms. DCOIT was only considered bioaccumulative on replicate 1 at 3h with a BCF of 58119.3 as observed in table 1.

Table 1: Concentration of DCOIT on seawater (water media - ASW) at µg/L, on mussels at µg/kg and Bioconcentration Factor (BCF) during waterborne exposure. Asterisk (*) and bold indicates BCF >2000 (bioaccumulative)

Compound	Exposure	Replicate	Time (h)	Muss.Conc.	ASW.Conc.	BCF
DCOIT	Water	1	1	<LD	29.6	n.a
		2		19971.93	79.6	250.9
		3		20887.35	31.5	663.2
		1	3	29449.97	0.5	**58119.3***
		2		4231.77	57.6	73.5
		3		214.24	30.1	7.1
		1	24	<LD	<LD	n.a
		2		<LD	<LD	n.a
		3		<LD	29.7	n.a
		1	96	<LD	<LD	n.a
		2		<LD	<LD	n.a
		3		<LD	<LD	n.a

n.a = not applicable

Biomagnification is a process in which the concentration of a chemical in an organism exceeds that of its diet, and can be determined under field conditions and in laboratory feeding experiments (Hilvarsson et al. 2009). For the dietary uptake treatment, only replicate 2 presented DCOIT in both matrices during all time points as presented at Table 2 and Figures 2 and 3, with the respective tissue concentrations and BMFs of 19722 µg/Kg and 71 at 1 h, 78672 µg/Kg and 282 at 3h, 19724 µg/Kg and 71 at 24h, and 3259 and 11.7 at 96h (after 72 h of depuration). BMF values above 1 indicate trophic transfer and a potential for biomagnification. However, despite the obtained BMF values, the observed degradation and/or depuration of DCOIT indicated a lower probability of biomagnification. BAF and BCF, which classified DCOIT as non-bioaccumulative, also corroborated this hypothesis. Yet, we need to consider that in harbors and marinas organisms are chronically exposed to DCOIT. In this scenario, biomagnification and bioaccumulation still may occur since the uptake rate may overcome the depuration rate. Therefore, further studies to better understand the bioaccumulation and biomagnification of DCOIT on different exposure scenarios are needed.

Table 2: Concentration of DCOIT on seawater (water media - ASW) at µg/L, on mussels at µg/kg and predatory biomagnification factor (BMF_{TL}) during the DCOIT exposure through food (dietary uptake treatment).

Compound	Exposure	Replicate	Time (h)	Muss.Conc.	ASW.Conc	BMF_{TL}
DCOIT	Food	1	1	<LD	31.05	n.a
		2		19721.78	38.34	70.81
		3		<LD	34.88	n.a
		1	3	<LD	<LD	n.a
		2		78671.56	33.12	282.46
		3		<LD	<LD	n.a
		1	24	<LD	0	n.a
		2		19723.58	30.92	70.82
		3		<LD	<LD	n.a
		1	96	<LD	<LD	n.a
		2		3259.52	34.15	11.7
		3		<LD	<LD	n.a

n.a = not applicable

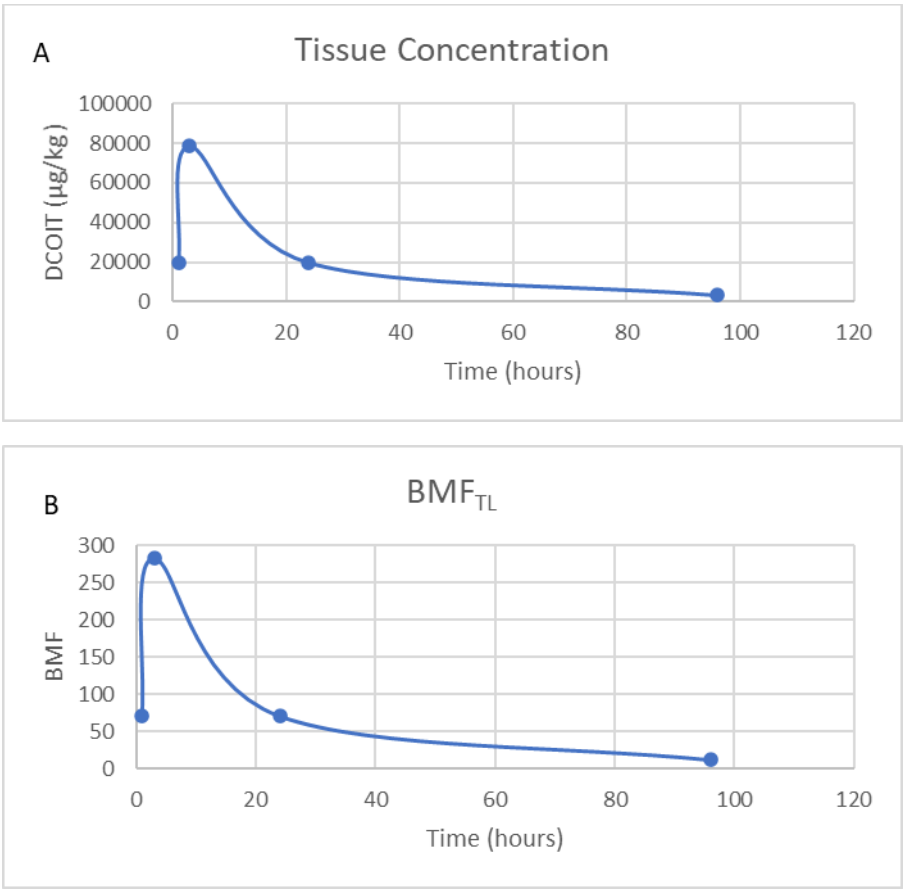


Figure 3: *Mytilus galloprovincialis* tissue concentration (µg/Kg) (A) and predatory biomagnification factor (BMF_{TL}) (B) for replica 2 organism at the food exposure treatment.

Considering that bioaccumulation is a process in which a chemical substance is absorbed in the tissues of an organism by all possible routes of exposure, i.e., dietary and direct uptake from the environment, in the present study we evaluated the bioaccumulation of DCOIT

by combining both uptakes from the dissolved phase and food in a single exposure treatment (water + food exposure). Our results showed that DCOIT was detected in the ASW during all timestamps. In mussel tissues, DCOIT was detected at 1 h and 3 h in the replicates 1 and 3, while in the replicate 2 DCOIT was detected at 1 h, 3 h, and 24 h (see table 3). In the replicate 2 the concentrations of DCOIT in water and mussels soft tissues were inversely correlated (R^2 = -0.99, see figure 4). These results suggest that the organisms instead of metabolizing the DCOIT, purged it during the depuration phase. In replicate 2, DCOIT concentration and BAF peaked at 24h with 39772 µg/Kg and 284 respectively. Nevertheless, no BAF values were superior to the 2000 threshold.

There is only one report regarding the bioaccumulation of DCOIT in fish that dates back to 1996 (Willingham & Jacobson, 1996), but subsequently, neither bioaccumulation nor trophic transfer was considered in further studies. Yet, Willingham & Jacobson (1996) showed *Lepomis macrochirus* fish rapidly internalized and degraded DCOIT for 49 days. Our results also indicated rapid uptake followed by biotransformation, however as demonstrated by Chen & Lam (2017) in his review, even short-term exposures of DCOIT can cause negative effects at various levels of biological organization on marine organisms. In addition, our data indicates that in regions with constant supply of DCOIT such as harbors and marinas, DCOIT can bioaccumulate in the biota (as the uptake rate would be greater than the depuration). Besides, DCOIT is already considered a pseudo-persistent contaminant in these areas (Chen & Lam, 2017). Thus, we encourage future *in-situ* studies on the bioaccumulation of DCOIT in marine organisms, specially for harbors, anchorage sites and marinas.

Table 3: Concentration of DCOIT on seawater (water media - ASW) at µg/L, on *Mytilus galloprovincialis* at µg/kg and predatory bioaccumulation factor (BAF) during the DCOIT exposure through ASW + Food.

Compound	Exposure	Replicate	Time (h)	Muss.Conc.	ASW.Conc.	BAF
DCOIT	ASW + Food	1	1	24743.29	30.79	145.51
		2		2223.93	29.73	13.16
		3		27134.94	30.41	159.93
		1	3	43323.68	0.86	309.18
		2		3332.59	30.4	19.64
		3		19832.22	31	116.51
		1	24	<LD	0.46	n.a
		2		39771.67	0.68	284.21
		3		<LD	29.9	n.a
		1	96	<LD	29.44	n.a
		2		<LD	30.05	n.a
		3		<LD	29.82	n.a

n.a = not applicable

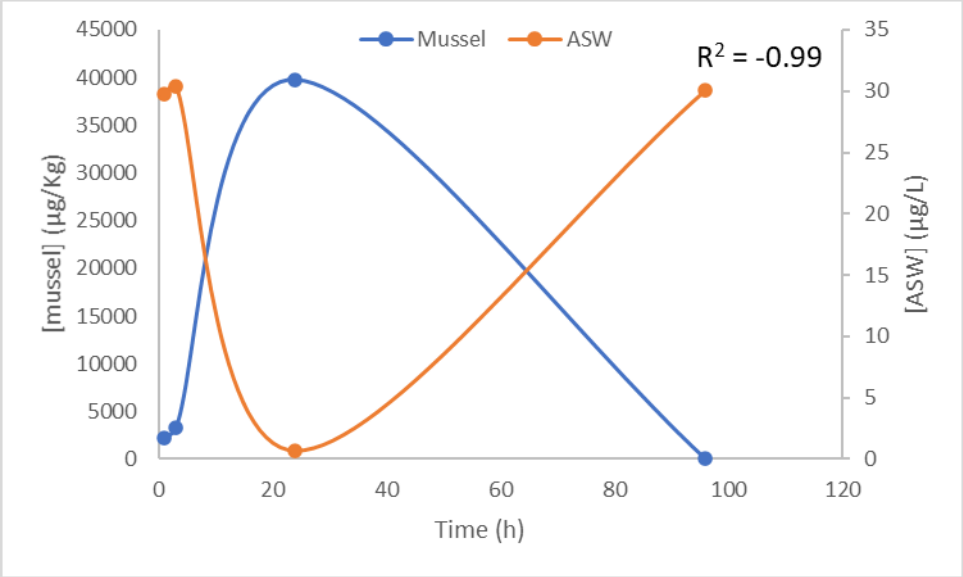


Figure 4: Replica 2, *Mytilus galloprovincialis* tissue concentration (µg/Kg) and ASW concentration (µg/Kg) of DCOIT, during the water + food exposure treatment. R^2 indicates the correlation between DCOIT in the media (ASW) and *M. galloprovincialis* tissue.

3.2 SiNC-DCOIT bioaccumulation, biomagnification, and trophic transfer

In the SiNC-DCOIT experiment, the concentration in the algae was 114.15 µg of DCOIT/Kg with a BCF of 3.9. These are the first data on the bioconcentration of SiNC-DCOIT in algae in the literature, and as for DCOIT, our data suggested that SiNC-DCOIT were not bioaccumulative for microalgae.

SiNC-DCOIT treatments showed that the concentrations of DCOIT in mussel soft tissues increased with time up to 3 h then declined on 24 h as presented in tables 4 to 6. For waterborne exposure treatment, DCOIT was detected at 1 h in mussel tissues from the replicate 2. At 3 h all replicates evidenced DCOIT internalization, indicating that different from free DCOIT, SiNC-DCOIT uptake might be slower. Yet, after 3 h no biocide was found in the mussels' soft tissues, suggesting that DCOIT was either metabolized or eliminated. Different from DCOIT, for SiNC-DCOIT no BCF value was highee than the threshold to be classified bioaccumulative, nevertheless, the replicate 1 at 3 h presented a BCF of 1827, which isclose to the threshold.

Table 4: Concentration of DCOIT on seawater (water media - ASW) at µg/L, on mussels at µg/kg and Bioconcentration Factor (BCF) during the DCOIT exposure through ASW.

Compound	Exposure	Replicate	Time (h)	Muss.Conc.	ASW.Conc	BCF
SiNC-DCOIT	water	1	1	<LD	47.06	n.a
		2		31116.47	163.26	190.59
		3		<LD	40.64	n.a
		1	3	1378.22	0.75	1826.61
		2		397.95	31.05	12.81
		3		1995.4	109.53	18.22
		1	24	<LD	0.46	n.a
		2		<LD	30.04	n.a
		3		<LD	<LD	n.a
		1	96	<LD	<LD	n.a
		2		<LD	30.68	n.a
		3		<LD	<LD	n.a

n.a = not applicable

Regarding the food exposure treatment, except for the replicate 3 at 1 h and replicate 2 at 24 h, no biocide was found in the mussels' soft tissues. The concentration of DCOIT in the ASW was above the detection limit only in the replicate 3 at 1 h and 3 h, and in the replicate 2 at all timestamps, with an average concentration of 30.2±0.3 µg/L (see table 5). The BMF_{TL} from replicates 2 and 4 at 1 h and 24 h suggest possible biomagnification, however, due to the limited observations and variance between the replicates, more studies are needed to confirm this observation.

Table 5: Concentration of DCOIT on seawater (water media - ASW) at µg/L, on mussels at µg/kg and Bioconcentration Factor (BCF) during the DCOIT exposure through ASW.

Compound	Exposure	Replicate	Time (h)	Muss.Conc.	ASW.Conc	BMF
SiNC-DCOIT	Food	1	1	<LD	<LD	n.a
		2		<LD	30.28	n.a
		3		19955.29	30.01	85.17
		1	3	<LD	<LD	n.a
		2		<LD	30.87	n.a
		3		<LD	29.95	n.a
		1	24	<LD	<LD	n.a
		2		28959.57	30.05	123.6
		3		<LD	<LD	n.a
		1	96	<LD	<LD	n.a
		2		<LD	30.45	n.a
		3		<LD	<LD	n.a

n.a = not applicable

In the Water + Food exposure treatment, DCOIT was detected in ASW at all timestamps. For mussels and ASW, DCOIT was detected at 1 h and 3 h in all replicates with an average concentration of 35506 ±22285 µg/kg and 49 ±15 µg/L for 1h and 9945 ±8543 µg/kg and 31 ±0.5 µg/L for 3h. At 3 h, concentrations in mussels' soft tissues were 3.5 fold lower than the 1h, and at 24 h all concentrations were below the LD. At 96 h the mussels from replicate 1 presented 2834 µg/kg of DCOIT, suggesting that this organism did not metabolize the biocide as the others did.

Table 6: Concentration of DCOIT on seawater (water media - ASW) at µg/L, on mussels at µg/kg and Bioconcentration Factor (BCF) during the DCOIT exposure through ASW.

Compound	Exposure	Replicate	Time (h)	Muss.Conc.	ASW.Conc	BAF
SiNC-DCOIT	ASW + Food	1	1	21033.58	<LD	179.54
		2		61169.79	38.15	393.88
		3		24316.79	59.79	137.43
		1	3	6122.18	31.64	41.15
		2		19733.75	31.37	132.87
		3		3981.79	30.57	26.95
		1	24	<LD	<LD	n.a
		2		<LD	41.38	n.a
		3		<LD	30.28	n.a
		1	96	2834.96	<LD	24.2
		2		<LD	31.76	n.a
		3		<LD	30.18	n.a

n.a = not applicable

Fonseca et al. (2020) and Gabe et al. (2021) corroborate our hypothesis that DCOIT is rapidly internalized and metabolized by bivalves as they observed negative effects on *Perna perna* mussels after short-term exposures to DCOIT. After 96 h Gabe et al. (2021) observed oxidative stress and damage through the modulation of important enzymes related to the antioxidant defense systems (e.g. CAT, GPx, GST). In its turn Fonseca et al. (2020) observed a reduction of hemocyte viability after 24 h of exposure. Despite the low BCF and BAF observed in the present work, according to Kookana et al. (2013) there is a strong relationship between log K_{ow} and BCF in mussels, under laboratory conditions. By studding 19 different organic compounds of varying hydrophobicities, Geyer et al. (1982) found the following relationship:

$$\text{Log BCF} = 0.858 \times \text{logKow} - 0.808.$$

Based on this relationship, the predicted log BCF for DCOIT (logKow = 6.2) would be 4.51 (worst scenario, considering the highest value of logKow available in the literature), which is higher than the measured values, and greater than the thresholds to be considered bioaccumulative. The presented data suggest that in environments under constant input of DCOIT bioaccumulation potential may become more severe, thus environmental risks could emerge. Yet, the LogKow for DCOIT may change based on the environment and the condition where it is obtained, according to the literature, the LogKow for DCOIT ranges from 2.8 (Jacobson, 1993) to 6.4 (Harino, 2004), indicating that the bioaccumulation of DCOIT may vary based on the environmental condition and physic-chemical properties of the media (water or sediment).

This is a preliminary study that for the first time assessed the bioaccumulation, biomagnification, and trophic transfer in the free and encapsulated forms of DCOIT. Both the nanoengineered and free form of DCOIT showed a similar pattern, being rapidly internalized and metabolized. In both cases we also observed higher concentrations in the consumers (mussels) relative to the prey (microalgae). However, due to the uncertainty caused by the small sample size and large variability among replicates, we encourage further studies to corroborate our findings. Nevertheless, the present study can be used as a proxy for future studies.

4. Conclusion

Our results showed that DCOIT and SiNC-DCOIT presented BCF and BAF < 2000, thus they can be considered non-bioaccumulative. Both biocides presented a rapid uptake (<24h) and depuration (<72h); however, they can be transferred along the trophic chain, and then biomagnification may become possible, especially under constant inputs of DCOIT, such

as observed in harbor areas and marinas. The presented data should be used as a proxy for future studies that aim to detail how bioaccumulation and trophic transfer of DCOIT and SiNC-DCOIT occur. Finally, we encourage in-situ bioaccumulation studies in areas with constant input of DCOIT.

5. References

Arnot, J. A., & Gobas, F. A. P. C. (2006). A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. 297, 257–297. https://doi.org/10.1139/A06-005

Bragadin, M. A. B., Avoni, B. R. P., Cutari, G. U. S., & Anente, S. A. M. (2005). AN IN VITRO STUDY OF THE INTERACTION OF SEA-NINE WITH RAT LIVER MITOCHONDRIA. Environmental Toxicology and Chemistry, 24(5), 1074–1078.

Campos, B. G. de, Figueiredo, J., Perina, F., Abessa, D. de S., Loureiro, S., & Martins, R. (2021). Occurrence, effects and environmental risk of antifouling biocides (EU PT21): Are marine ecosystems threatened? Critical Reviews in Environmental Science and Technology, in press. https://doi.org/10.1080/10643389.2021.1910003

Chen, L., & Lam, J. C. W. W. (2017). SeaNine 211 as antifouling biocide: A coastal pollutant of emerging concern. Journal of Environmental Sciences, 61, 68–79. https://doi.org/10.1016/j.jes.2017.03.040

Chen, L., Ye, R., Xu, Y., Gao, Z., Au, D. W. T., & Qian, P. Y. (2014). Comparative safety of the antifouling compound butenolide and 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT) to the marine medaka (Oryzias melastigma). Aquatic Toxicology, 149, 116–125. https://doi.org/10.1016/j.aquatox.2014.01.023

Chen, L., Zhang, H., Sun, J., Wong, Y. H., Han, Z., Au, D. W. T., Bajic, V. B., & Qian, P. Y. (2014). Proteomic changes in brain tissues of marine medaka (Oryzias melastigma) after chronic exposure to two antifouling compounds: Butenolide and 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT). Aquatic Toxicology, 157, 47–56. https://doi.org/10.1016/j.aquatox.2014.09.010

Cima, F., Bragadin, M., & Ballarin, L. (2008). Toxic effects of new antifouling compounds on tunicate haemocytes. I. Sea-Nine 211TM and chlorothalonil. Aquatic Toxicology, 86(2), 299–312. https://doi.org/10.1016/j.aquatox.2007.11.010

Cima, F., Ferrari, G., Ferreira, N. G. C., Rocha, R. J. M., Serôdio, J., Loureiro, S., & Calado, R. (2013). Preliminary evaluation of the toxic effects of the antifouling biocide Sea-Nine 211 in the soft coral Sarcophyton cf. glaucum (Octocorallia, Alcyonacea) based on PAM fluorometry and biomarkers. Marine Environmental Research, 83, 16–22. https://doi.org/10.1016/j.marenvres.2012.10.004

Do, J. W., Haque, N., Lim, H., Hwa, B., & Lee, D. (2018). Constant exposure to environmental concentrations of the antifouling biocide Sea-Nine retards growth and reduces acetylcholinesterase activity in a marine mysid. Aquatic Toxicology, 205(August), 165–173. https://doi.org/10.1016/j.aquatox.2018.10.019

Eom, H., Haque, N., Nam, S., Lee, D., & Rhee, J. (2019). Effects of sublethal concentrations of the antifouling biocide Sea-Nine on biochemical parameters of the marine polychaete Perinereis aibuhitensis. Comparative Biochemistry and Physiology, Part C, 222(May), 125–134. https://doi.org/10.1016/j.cbpc.2019.05.001

Figueiredo, J., Loureiro, S., & Martins, R. (2020). Hazard of novel anti-fouling nanomaterials and biocides DCOIT and silver to marine organisms. Environmental Science: Nano, 7(6), 1670–1680. https://doi.org/10.1039/d0en00023j

Figueiredo, J., Oliveira, T., Ferreira, V., Sushkova, A., Silva, S., Carneiro, D., Cardoso, D. N., Gonçalves, S. F., Maia, F., Rocha, C., Tedim, J., Loureiro, S., & Martins, R. (2019). Toxicity of innovative anti-fouling nano-based solutions in marine species. Environmental Science Nano, 5. https://doi.org/10.1039/C9EN00011A

Fonseca, V. B., Guerreiro, A. da S., Vargas, M. A., & Sandrini, J. Z. (2020). ’. Comparative Biochemistry and Physiology Part - C: Toxicology and Pharmacology, 232(March), 108737. https://doi.org/10.1016/j.cbpc.2020.108737

Gabe, H. B., Guerreiro, A. da S., & Sandrini, J. Z. (2021). Molecular and biochemical effects of the antifouling DCOIT in the mussel Perna perna. Comparative Biochemistry and Physiology Part - C: Toxicology and Pharmacology, 239(August 2020), 108870. https://doi.org/10.1016/j.cbpc.2020.108870

Geyer, H., Sheehan, P., Kotzias, D., Freitag, D., & Korte, F. (1982). Prediction of ecotoxicological behaviour of chemicals: Relationship between physico-chemical properties and bioaccumulation of organic chemicals in the mussel Mytilus edulis. Chemosphere, 11(11), 1121–1134. https://doi.org/10.1016/0045-6535(82)90122-9

González-Barreiro, O., Rioboo, C., Herrero, C., & Cid, A. (2006). Removal of triazine herbicides from freshwater systems using photosynthetic microorganisms. Environmental Pollution, 144(1), 266–271. https://doi.org/10.1016/j.envpol.2005.12.014

Hilvarsson, A., Ohlauson, C., Blanck, H., & Granmo, Å. (2009). Bioaccumulation of the new antifoulant medetomidine in marine organisms. Marine Environmental Research, 68(1), 19–24. https://doi.org/10.1016/j.marenvres.2009.03.007

International Marine Organization. (2001). International Convention on the Control of Harmful Antifouling Systems on Ships.

Ito, M., Mochida, K., Ito, K., Onduka, T., & Fujii, K. (2013). Induction of apoptosis in testis of the marine teleost mummichog Fundulus heteroclitus after in vivo exposure to the antifouling biocide 4,5-dichloro-2-n-octyl-3(2H)-isothiazolone (Sea-Nine 211). Chemosphere, 90(3), 1053–1060. https://doi.org/10.1016/j.chemosphere.2012.08.052

Jacobson, A. H., & Willingham, G. L. (2000). Sea-nine antifoulant: An environmentally acceptable alternative to organotin antifoulants. Science of the Total Environment, 258(1–2), 103–110. https://doi.org/10.1016/S0048-9697(00)00511-8

Jesus, É. P. S. de, Figueirêdo, L. P. de, Maia, F., Martins, R., & Nilin, J. (2021). Acute and chronic effects of innovative antifouling nanostructured biocides on a tropical marine microcrustacean. Marine Pollution Bulletin, 164, 111970. https://doi.org/10.1016/j.marpolbul.2021.111970

Kookana, R. S., Shareef, A., Fernandes, M. B., Hoare, S., Gaylard, S., & Kumar, A. (2013). Bioconcentration of triclosan and methyl-triclosan in marine mussels (Mytilus galloprovincialis) under laboratory conditions and in metropolitan waters of Gulf St Vincent, South Australia. Marine Pollution Bulletin, 74(1), 66–72. https://doi.org/10.1016/j.marpolbul.2013.07.030

Lam, N. H., Jeong, H. ho, Kang, S. dong, Kim, D. J., Ju, M. J., Horiguchi, T., & Cho, H. S. (2017). Organotins and new antifouling biocides in water and sediments from three Korean Special Management Sea Areas following ten years of tributyltin regulation: Contamination profiles and risk assessment. Marine Pollution Bulletin, 121(1–2), 302–312. https://doi.org/10.1016/j.marpolbul.2017.06.026

Lee, M. R. N., Kim, U. J., Lee, I. S., Choi, M., & Oh, J. E. (2015). Assessment of organotin and tin-free antifouling paints contamination in the Korean coastal area. Marine Pollution Bulletin, 99(1–2), 157–165. https://doi.org/10.1016/j.marpolbul.2015.07.038

Maia, F., Silva, A. P., Fernandes, S., Cunha, A., Almeida, A., Tedim, J., Zheludkevich, M. L., & Ferreira, M. G. S. (2015). Incorporation of biocides in nanocapsules for protective coatings used in maritime applications. CHEMICAL ENGINEERING JOURNAL, 270, 150–157. https://doi.org/10.1016/j.cej.2015.01.076

Martínez, K., & Barceló, D. (2001). Determination of antifouling pesticides and their degradation products in marine sediments by means of ultrasonic extraction and HPLC-APCI-MS. Fresenius' Journal of Analytical Chemistry, 370(7), 940–945. https://doi.org/10.1007/s002160100904

Reybuck, S. E., & Schwartz, C. (2008). Blends of encapsulated biocides (Patent No. US7377968B2).

Santos, J. V. N., Martins, R., Fontes, M. K., Galvao, B., Silva, M. B. M. do P., Maia, F., Abessa, D. M. de S., & Perina, F. C. (2020). Can Encapsulation of the Biocide DCOIT Affect the Anti-Fouling Efficacy and Toxicity on Tropical Bivalves ? Applied Sciences (Switzerland), 10(23), 8579.

Technical Guidance Document on Risk Assessment Part II., 2003. EUR 20418 EN/2. Institute for Health and Consumer Protection, European Chemicals Bureau, E. C. (n.d.). Technical Guidance Document on Risk Assessment Part II., 2003. EUR 20418 EN/2. Institute for Health and Consumer Protection, European

Chemicals Bureau.

Vieira, A. A., Caldas, S. S., Kupski, L., Tavella, R. A., & Primel, E. G. (2018). Extraction of chlorothalonil , dichlofluanid , DCOIT , and TCMTB from fish tissues employing the vortex assisted matrix solid-phase dispersion. Microchemical Journal, 143(March), 92–98. https://doi.org/10.1016/j.microc.2018.07.034

Willingham, G. L., & Jacobson, A. H. (1996). Designing an Environmentally Safe Marine Antifoulant. ACS Symposium Series, 640, 224–233. https://doi.org/10.1021/bk-1996-0640.ch011

Chapter 3

Toxicity of innovative antifouling additives on an early life stage of the oyster *Crassostrea gigas*: short and long-term exposure effects

Bruno Galvão de Campos[a,b]*, Mariana Bruni Marques do Prado e Silva[a], Francisco Avelelas[c], Frederico Maia[d], Susana Loureiro[e], Fernando Perina[a], Denis Moledo de Souza Abessa[a], Roberto Martins[e]

[a]*São Paulo State University (UNESP), Praça Infante Dom Henrique, s/n, Zipcode: 11330-900 São Vicente, São Paulo, Brazil*

[b]*Department of Biology, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal*

[c] *MARE, School of Tourism and Maritime Technology, Polytechnic of Leiria*

[d]*Smallmatek – Small Materials and Technologies, Lda, Rua Canhas, 3810-075 Aveiro, Portugal*

[e]*CESAM - Centre for Environmental and Marine Studies and Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal*

***Corresponding Author**: Bruno Galvão de Campos, São Paulo State University (UNESP), Praça Infante Dom Henrique, s/n, Zipcode: 11330-900 São Vicente, São Paulo, Brazil. E-mail: b.campos@unesp.br*

Status: Published at: *Environmental Science and Pollution Research*

DOI: 10.1007/s11356-021-17842-3

Abstract

Recent advances in nanotechnology have allowed the encapsulation of hazardous anti-fouling (AF) biocides in silica mesoporous nanocapsules (SiNC) reducing their short-term toxicity. However, the chronic effects of such novel nanoadditives remain understudied. The present study aimed to assess short- and long-term sub-lethal effects of soluble forms (DCOIT and Ag) and nanostructured forms (SiNC-DCOIT and SiNC-DCOIT-Ag) of AF biocides and the "empty" nanocapsule (SiNC) on juveniles of *Crassostrea gigas* after 96 h and 14 days of exposure. Juvenile oysters exposed for a short period to free DCOIT and $AgNO_3$ presented worse physiological status comparing with those exposed to the nanostructured forms. The long-term exposure to DCOIT and Ag^+ caused an extensive biochemical impairment comparing with the tested nanomaterials, which included oxidative damage, activation of the antioxidant defense system, and neurotransmission impairment. Despite the negative effects mostly

observed on the health condition index and AChE, the encapsulation of the abovementioned AF biocides into SiNC seems to be a technological advantage towards the development of AF nanoadditives with lower long-term toxicity comparing with the soluble forms of such biocides.

Keywords: engineered nanomaterials, anti-fouling biocide, biofouling, chronic ecotoxicity, sub-lethal toxicity, DCOIT

1. Introduction

Marine biofouling in human-made immersed structures is an unsolved problem that is associated with major ecological and socio-economic impacts worldwide (Silva et al. 2019). Historically, the application of maritime antifouling (AF) coatings has been considered the most efficient way to prevent the adhesion of organisms to the structures' surface. More than 90% of the AF coatings available in the market are based on the use of antifouling compounds in their composition (Readman 2006; Lagerström et al. 2020), despite the recent development of non-biocidal, amphiphilic, self-cleaning, and/or fouling-release coatings (Ruiz-Sanchez et al. 2020; Selim et al. 2020). The self-polishing copolymers (SPC) are amongst the most used due to the long-lasting protection - 60 months of estimated service life on commercial vessels - comparatively to the ablative or insoluble matrices containing other biocides that can guarantee up to 36-48 months of AF protection (Tait and Inglis 2016). SPC coatings usually contain both cuprous compounds and booster biocides, namely DCOIT and Zn pyrithiones (Tait and Inglis 2016). In particular, DCOIT (4,5-dichloro-2-octylisothiazol-3(2H)-one), marketed as Kathlon™ 910 SB, Sea-Nine™ 211N or Parmetol® S15, has been proposed as a safe alternative to the already banned organotin-based antifouling compounds, due to its remarkable physical-chemical properties (Jacobson and Willingham 2000), being recognized through the US "Presidential Green Chemistry Challenge Award" in 1996, in the category of "design of chemicals which are less toxic than current alternatives". However, some studies have already demonstrated that DCOIT causes extensive deleterious effects on non-fouler marine organisms, such as severe growth inhibition in bacteria and photosynthetic species, acute toxicity on micro- and macroinvertebrates (Cima et al. 2008; Chen et al. 2014b; Figueiredo et al. 2020; Santos et al. 2020; Jesus et al. 2021) and embryotoxicity on molluscs, echinoderms and fish. Median lethal or effect concentration values span 4 orders of magnitude, ranging from 0.0004 mg/L for the coccolithophore *Emiliania huxleyi* (Devilla et al. 2005) to 3.25 mg/L for the polychaete

Hediste diversicolor (Figueiredo et al. 2019). Thus, the use of DCOIT as a booster biocide in antifouling paints may be potentially harmful to marine organisms (Campos et al. 2021).

Nanotechnology focuses on the development of materials at the nanoscale with a wide range of specific beneficial properties, to optimize their effectiveness and/or reduce costs and adverse impacts. The use of silica mesoporous nanocapsules as nano-containers loaded with active compounds is considered a cutting-edge technology that has already been successfully applied in catalysis, drug delivery, and biomedicine (Yu et al. 2014; Lei et al. 2020; Ribeiro et al. 2020), and more recently for antifouling purposes (Maia et al. 2015; Avelelas et al. 2017; Gutner-Hoch et al. 2018, 2019; Figueiredo et al. 2020). Recent nanotechnological developments have proposed the encapsulation of DCOIT in engineered nanomaterials (ENM) aiming at reducing the environmental impact and minimizing the undesired leaching of DCOIT from maritime coatings, due to their capacity of controlling the biocidal release from the nanostructures (Reybuck and Schwartz 2008; Maia et al. 2015). The AF biocide DCOIT has been immobilized in amino-urea-formaldehyde shell systems (Reybuck and Schwartz 2008), silica mesoporous nanocapsules (SiNC) (Maia et al. 2015; Michailidis et al. 2017), silicon dioxide nanoparticles (Aidarova et al. 2018), halloysite nanotubes (Fu et al. 2019), and polyurea shell nanocapsules (Aidarova et al. 2019). The promising antibacterial performance of DCOIT immobilized in SiNC (SiNC-DCOIT) (Maia et al., 2015) stimulated the development of a new version of this ENM, including now silver nitrate as a bactericide (SiNC-DCOIT-Ag), to successfully tackle the formation of biofilm, on which depends the conditioning of substratum that favors the subsequent biofouling settlement and growth (Figueiredo et al. 2019). For instance, in the case of SiNC-DCOIT, Figueiredo et. al (2019, 2020) showed that encapsulation caused a reduction of up to 214-fold on the short-term toxicity and up to 25-fold on the marine hazard of DCOIT on 12 marine species, from bacteria to fish. Despite the extensive knowledge acquired in both studies, the long-term effects of these novel nanoadditives are still unknown. Taking into account the fate and behavior of both ENM, as observed by Figueiredo et al. (2019), important questions remain unanswered: Can the slow but continuous release of DCOIT and/or Ag from the nanocapsules exert worst effects than the free biocides during a long-term exposure? Can these effects disturb the physiological and biochemical performance of invertebrates during more sensitive life stages, like their early growth stage?

The edible oyster *Crassostrea gigas*, which represents an important source of protein for humans, is widely farmed worldwide in oyster cultures and has a high socioeconomic relevance. However, many oyster farms are located close to marinas and/or ports where DCOIT

has been detected in both sediments (up to 0.28 µg DCOIT/g; Lee et al. 2015) and waters (up to 3.7 µg DCOIT/L; Martínez and Barceló 2001). Thus, farmed oysters are expected to be affected by AF biocidal release. In this context, the present study aims at assessing for the first time the short- and long-term effects of the novel AF nanoadditives (SiNC-DCOIT; SiNC-DCOIT-Ag) on juveniles of *C. gigas*, after 96-hour and 14-day exposure, and comparing the effects with the respective counterparts, namely the unloaded nanostructured shell (SiNC) and the soluble forms of both biocides, DCOIT and Ag.

2. Material and Methods

2.1 Chemicals

In the present study, Sigma-Aldrich™ reagent grade chemicals were used to prepare the stock solutions of the standards of DCOIT and $AgNO_3$, as well as in analyzes of biochemical biomarkers. The tested nanomaterials, SiNC (unloaded silica mesoporous nanocapsules), SiNC-DCOIT (SiNC loaded with DCOIT), and SiNC-DCOIT-Ag (SiNC loaded with DCOIT and Ag) were kindly developed and supplied by Smallmatek, Small Materials and Technologies, Lda. Details about the nanomaterials synthesis and characterization are fully described by Figueiredo et al. (2019).

2.2 Tested organisms

Juveniles of *Crassostrea gigas* (length: 1 ± 0.5 cm; 3-month-old oysters) used in this study were collected from an oyster culture located in Gafanha da Encarnação, in the Mira Channel of the Ria de Aveiro (Portugal). The oysters were acclimated for 7 days in a 20 L tank filled with artificial saltwater (ASW) under constant aeration and controlled conditions (temperature 19 ± 1ºC, salinity 35, photoperiod 16:8 h - light:dark) and fed *ad libitum* with live microalgae *Tetraselmis chuii*, every 72 h (~1.5×10^5 cells/organism), cultured at the facilities of the University of Aveiro (Department of Biology, Portugal).

2.3 Tested concentrations

For the 96 h toxicity test, exposure concentrations of 1, 10, 100, 1000, and 10000 µg/L were prepared for each compound from a stock solution of 10 mg/L prepared with filtered (0.45 µm) artificial saltwater (ASW), salinity 35. Also, 30 min sonication with a visual inspection for precipitates or suspended particles was used to ensure the homogeneity of the stock solution immediately before each experiment. Similarly, for the 14-day exposure test, the following

concentrations were prepared: 0.1, 1, 10, 100, and 1000 µg/L. To properly compare the effects of the main biocide, DCOIT, in both free and nanostructured forms, the tested concentrations were expressed in function on the DCOIT content of the nanocapsules. The tested concentrations were chosen based on the NOEC (no observed effect concentration) data gathered from Figueiredo et al. (2020).

2.4 Short-term toxicity testing

2.4.1 Toxicity test

The short-term effects of SiNC, SiNC-DCOIT, SiNC-DCOIT-Ag, DCOIT, and $AgNO_3$ were assessed on juveniles of *C. gigas* after 96 h. Five replicates (*n*=5) containing 3 organisms each were prepared per treatment in 50 mL glass containers, with constant aeration and room temperature, photoperiod of 16:8 h (light:dark). The physicochemical parameters throughout the test were: salinity 35 ± 0, temperature 19 ± 1 ℃; pH 7.80 ± 0.07 and dissolved oxygen 84.4 ± 4.4%. After the exposure, 5 organisms randomly selected from each treatment were used to measure the following endpoints.

2.4.2 Endpoints

2.4.2.1 Condition Index (CI)

The health status of the oysters at the end of the exposure period was measured through the health condition index (CI). It corresponds to the ratio between the dry weights of the oyster soft tissue (Tdw) and its respective shell (Sdw), following the method proposed by Lucas and Beninger (1985). Both shell and soft tissues (n=5) were dried for 48 h at 60 ºC. The CI was expressed as:

$$CI(\%) = (Tdw/Sdw) \times 100$$

2.4.2.2 Air survival

The capacity of bivalves to survive air exposure after the toxicity test can be considered a good indicator of the fitness of the organism at the end of the exposure (Santos et al. 2020). Organisms (n=5) were individually exposed to air in 24-well microplates and the mortality was daily checked as described by Martins et al. (2017). Oysters were considered dead when the valves were open and/or there was not any reaction to external stimulus.

2.5 Chronic exposure toxicity tests

2.5.1 Toxicity test

Juvenile organisms of *C. gigas* were exposed for 14 days to five exposure concentrations of SiNC, SiNC-DCOIT, SiNC-DCOIT-Ag, DCOIT, and $AgNO_3$, plus a negative control (only ASW), as previously described. Each replicate (n=5) was composed of 3 individuals, placed in glass chambers containing 50 mL of the test solution with continuous aeration, at room temperature and photoperiod of 16:8 h (light:dark). The solutions were changed every 72 h to ensure the effectiveness of the compounds; immediately after the media renewal, the organisms were fed with a solution of microalgae *Tetraselmis chuii* (1500 cells/mL). Measured physicochemical parameters in all replicates, during the entire test, are summarized as follows: salinity 35 ± 0, temperature 19 ± 1 °C, pH: 7.7 ± 0.2 and dissolved oxygen: 75.1 ± 13.6%.

2.5.2 Endpoints

2.5.2.1 Adhesion as a physiological endpoint

Oysters produce cement with ≈90% of calcium carbonate in its composition, to adhere and form reefs in their natural habitat (Burkett et al. 2010). At the end of the exposure, oysters' adhesion was evaluated through a visual and physical inspection of organisms attached to the glass chambers, which included a mechanical stimulation to confirm organisms' survival after the long-term exposure.

2.5.2.2 Biochemical biomarkers

Oysters were weighed and eviscerated, and the entire soft tissue was collected (n=3, each replicate a pool of 3 oysters). Soft-tissue samples were stored at -80°C until the biochemical biomarkers' analyses. Samples were homogenized with phosphate buffer (0.1M, pH 7.4, apart from AChE with a pH 7.2) at 0.5% w/v. Then, an aliquot was separated for lipid peroxidation (LPO), which was spectrophotometrically (535 nm) evaluated through the thiobarbituric acid reactive substances determination (TBARS), following the method proposed by Ohkawa et al. (1979) and Bird and Draper (1984), LPO results were expressed as nmol of TBARS equivalents per g of wet weight.

The remaining homogenate was centrifuged at 10000 g for 20 min at 4 °C, and the supernatant was then used for the quantification of total protein content and the activity of antioxidant (glutathione peroxidase - GPx, glutathione reductase - GR, catalase - CAT), detoxification (glutathione-S-transferase - GST), and neurotransmission (acetylcholinesterase -

AChE) related enzymes. The protein quantification was spectrophotometrically performed at 595 nm using the Bradford (1976) method. Before each enzymatic determination, the protein content of each sample was normalized to around 0.5 mg mL−1, apart from the AChE samples, which were normalized to 0.2–0.3 mg mL−1. GST, GPx, and GR activities were determined spectrophotometrically at 340 nm following the protocols developed by Habig et al. (1974), Mohandas et al. (1984), Cribb et al. (1989), and the adaptations for microplate proposed by (Frasco and Guilhermino 2002; Martins et al. 2017). CAT activity was spectrophotometrically determined at 240 nm through the method described by Clairborne (1985). AChE activity was spectrophotometrically measured at 414 nm through the colorimetric method described by Ellman et al. (1961), and adapted by Guilhermino et al. (1996) for 96-well microplates. Results were expressed as μmol of substrate hydrolyzed per min per mg protein in the case of CAT, GPx, GR, and GST and nmol of substrate hydrolyzed per min per mg protein for AChE activity.

2.6 Statistical analyses

Data were first checked for normality and homoscedasticity using Shapiro-Wilk and Brown-Forsythe test, respectively. One-way analysis of variance (ANOVA) followed by the Dunnett multiple comparison test was used to check significant differences between treatments and the negative control. The comparison among chemicals within a given concentration was tested using a two-way ANOVA (factor 1 = tested chemical; factor 2 = tested exposure concentration) followed by the Tukey's multiple comparison test.

Then, a dual approach was used to integrate the acquired data. The first tier (Tier-1) aimed at exploring the relationships between the biochemical and physiological effects and understanding some possible toxicity pathways of each tested compound. The results obtained for each substance were analyzed separately, being integrated using principal component analyses (PCA), which resulted in an ordination diagram for each tested compound. In the second tier (Tier-2), all toxicity data for all treatments and substances tested were analyzed in a single principal coordinate analysis (PCO), considering all measured biochemical and physiological endpoints and the Euclidean distance matrix calculation. Prior to this analysis, data were standardized by the Z-score method. The most correlated endpoints (Spearman correlation > 0.5) were presented as vectors. The Tier-1 and Tier-2 analyses were performed using the STATISTICA v.10 software and PRIMER v.6, respectively.

3 Results

Table 1 reports the NOEC and LOEC values of the tested compounds measured for all endpoints in both short-term and long-term toxicity tests.

3.1 Short-term sublethal toxicity tests

3.1.1 Condition Index (CI)

SiNC-DCOIT-Ag and DCOIT presented the lowest NOEC (<1 µg/L), in terms of health condition index. No significant differences were found in terms of CI of oysters exposed to all AF nanomaterials (Fig 1). SiNC and Ag results suggested an inverted (concentration is inversely proportional to response) and biphasic response relationship respectively, with a LOEC of 1 µg/L for SiNC and 100 µg/L for Ag, followed by a NOEC of 10000 µg/L. DCOIT caused detrimental physiological effects at much lower concentrations than the nanostructured form demonstrated by the significant reduction of CI ($p<0.05$) compared to SINC-DCOIT (-63%) at 100 µg/L. The exposure to the nanostructured form containing both biocides (SiNC-DCOIT-Ag) was significantly more deleterious than the ionic form of Ag at 1 µg/L (reduction of 64% in the CI). No CI significant differences were observed between SiNC-DCOIT and SiNC-DCOIT-Ag (Figure 1).

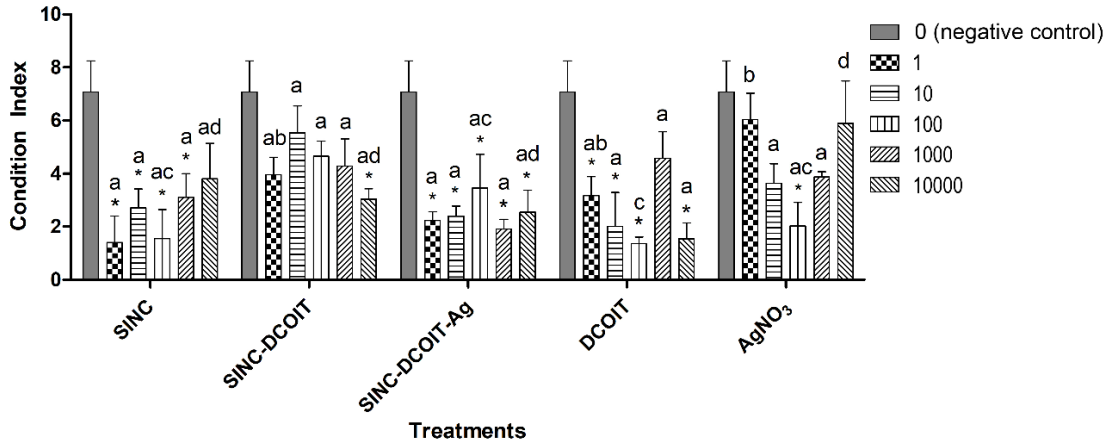


Fig. 1 Health condition index (mean with Standard Error (SEM) of *Crassostrea gigas* after a 96 h exposure to engineered nanomaterials with antifouling properties (SiNC-DCOIT; SiNC-DCOIT-Ag) and respective counterparts, namely the free biocides (DCOIT and AgNO3) and empty silica mesoporous nanocapsules (SiNC). Asterisks (*) indicate significant differences between the treatment and the control (ANOVA followed by Dunnett test, $p < 0.05$), while "a", "b", "c" or "d" indicate significant differences between chemicals within the same exposure concentration (2-way ANOVA followed by the Bonferroni test, $p < 0.05$). Exposure concentrations are given in µg/L.

Table 1: Lowest observed effect concentration (LOEC) and no observed effect concentration (NOEC) of sublethal endpoints evaluated under chronic exposure (biochemical endpoints) and short-term tests (CI, and Air survival). Bold represent the lowest NOECs for each chemical, in the respective exposure test.

Toxicity tests	Endpoints	SiNC		SiNC-DCOIT		SiNC-DCOIT-Ag		DCOIT		$AgNO_3$	
		NOEC	LOEC	NOEC	LOEC	NOEC	LOEC	NOEC	LOEC	NOEC	LOEC
		µg SiNC/L		µg DCOIT/L		µg DCOIT/L		µg DCOIT/L		µg Ag^{+}/L	
Short-term exposure test	CI	n.a.	1	1000	10000	**<1**	1	**<1**	1	n.a.	100
	AS	10000	>10000	10000	>10000	10000	>10000	10000	>10000	1000	10000
Chronic exposure test	GR	1000	>1000	1000	>1000	1000	>1000	1000	>1000	1000	>1000
	GST	1000	>1000	100	1000	1000	>1000	10	100	10	100
	GPx	1000	>1000	1000	>1000	1000	>1000	**< 0.1**	0.1	1000	>1000
	CAT	**10**	100	**0.1**	1	10	100	10	100	**<0.1**	0.1
	AChE	1000	>1000	1000	>1000	1000	>1000	0.1	1	100	1000
	LPO	1000	>1000	1000	>1000	**0.1**	1	**0.1**	1	**0.1**	1

CI = condition index; AS = air survival; GR = glutathione reductase; GST = glutathione S transferase; GPx = glutathione peroxidase; CAT = catalase; AChE = acetylcholinesterase; LPO = lipid peroxidation. n.a. = not applicable

3.1.2 Air survival (AS) (after the exposure to the tested chemicals)

The oysters from the control group survived 15 days at air exposure, similarly to most treatments surviving 12 ± 2.2 days, on average (Figure 2). The AS capacity of the oysters submitted to 10000 µg/L of $AgNO_3$ (AS = 5.8 ± 4.2 days; Figure 2) was significantly lower than the AS capacity of control organisms (AS > 15 days) and organisms exposed to 10000 µg DCOIT/L (AS = 12 days).

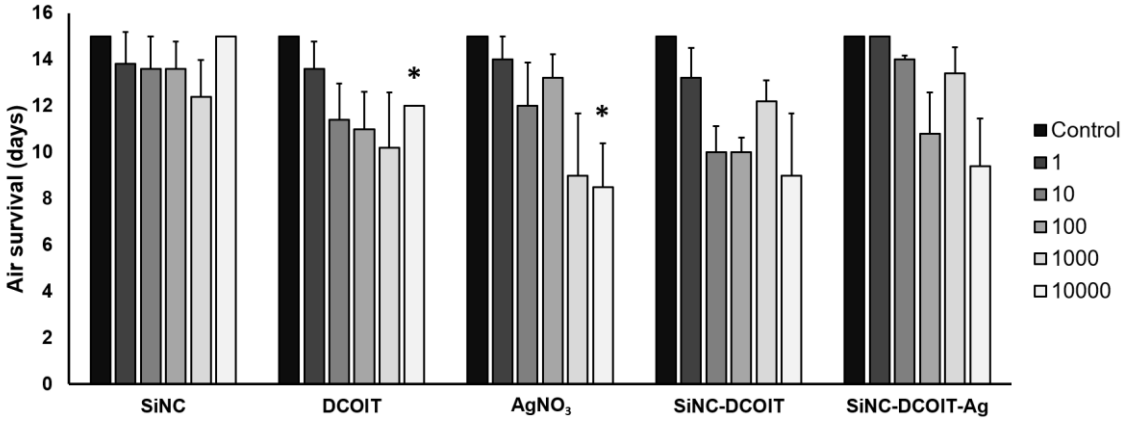


Fig. 2*:* Air survival (mean ± Standard Error (SE) in days) of *Crassostrea gigas* during a 15 day period evaluated after a 96 h exposure to engineered nanomaterials with antifouling properties (SiNC-DCOIT; SiNC-DCOIT-Ag) and respective counterparts, namely the free biocides (DCOIT and $AgNO_3$) and empty silica mesoporous nanocapsules (SiNC). Asterisks (*) indicate significant differences between the treatment and the control. Exposure concentrations are given in µg/L.

3.2 Chronic toxicity test

3.2.1 Lethality

This test was designed to be sub-lethal. However, the long-term exposure of juvenile oysters of *C. gigas* to 1000 µg DCOIT/L of free DCOIT and SiNC-DCOIT-Ag caused 100% and 66% of mortality, respectively. Therefore, all the sublethal endpoints are presented excluding the highest exposure concentration of such compounds.

3.2.2 Adhesion

A total of 60% of the control organisms were adhered in the end of the test. The adhesion rate by compound can be summarized in the following order: $AgNO_3$ (65%) > SiNC (55%) > SINC-DCOIT (50%) > SINC-DCOIT-Ag (45%) > DCOIT (24%). The adhesion data did not follow a dose-response pattern (the higher the concentration, the lower the adhesion) after 14 days of exposure to the tested compounds, demonstrated by the no/low correlation between the exposure concentration of each chemical and the adhesion rate (Pearson's $R<0.33$ for SiNC, SINC-DCOIT, SINC-DCOIT-Ag or DCOIT; R=0.57 for AgNO3).

3.2.3 Biochemical biomarkers

Data of biochemical biomarkers are plotted in Figure 3, and NOEC/LOEC values are summarized in Table 1. Oyster's chronic exposure to DCOIT, $AgNO_3$ and SiNC-DCOIT-Ag exhibited significant oxidative stress, demonstrated by the high levels of lipid peroxidation (LPO), at 1 µg/L of such chemicals. Treatments caused no significant differences in the GR activity (Table 1). CAT activity was significantly increased at 1 µg/L of SiNC-DCOIT and 100 µg/L of SiNC, DCOIT and SiNC-DCOIT-Ag, while low exposure concentrations of $AgNO_3$ caused significant inhibition of this antioxidant enzyme (LOEC=0.1 µg/L). GPx modulation was only observed for DCOIT with a significant increase at 0.1 and 1 µg DCOIT/L. The activity of GST was significantly reduced for both DCOIT and $AgNO_3$ at 100 µg/L and SiNC-DCOIT at 1000 µg/L. DCOIT and $AgNO_3$ exposure caused neurotransmission impairment on oysters shown by the significant inhibition of the AChE activity at the exposure concentration of 1 and 1000 µg/L, respectively.

Effects caused by the free DCOIT differed from those induced by ENM SiNC-DCOIT, by causing a GPx increase (0.1 and 1 µg/L) and AChE inhibition (at 1 µg/L), and from those of SiNC-DCOIT-Ag by inducing a GPx increase (0.1 µg/L) and inhibiting the GST activity (100 µg/L; Fig.3). $AgNO_3$ treatments caused an increase of LPO (10 µg/L) and a reduction of CAT activity (0.1, 1, 10 and 100 µg/L) in comparison with SiNC-DCOIT-Ag (Fig.3). Comparing the free biocides, DCOIT induced an increase of CAT (at all concentrations) and GST (1 µg/L) activities (Fig.3).

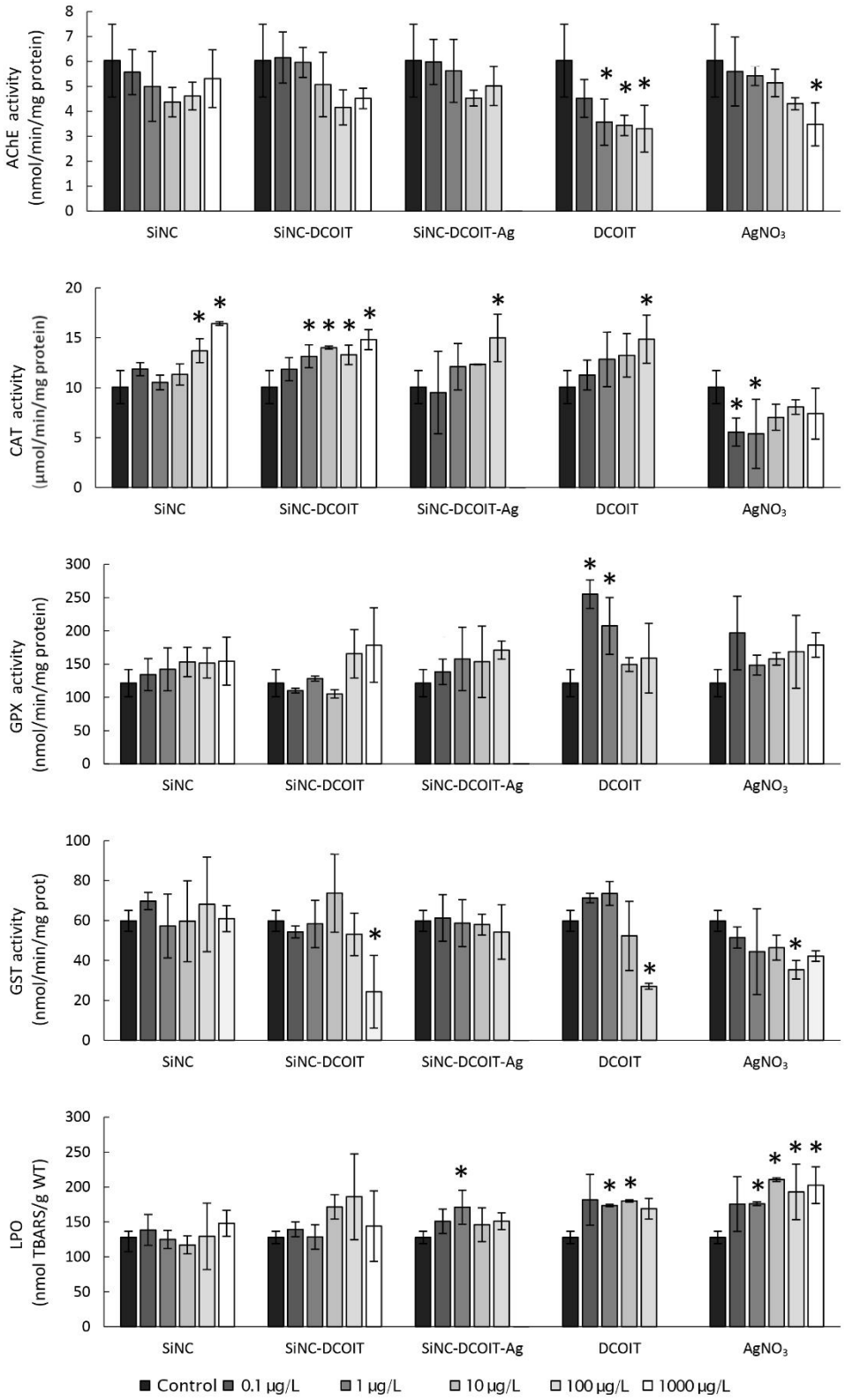


Fig. 3 Biochemical effects of Crassostrea gigas (mean with SD) after a 14 days exposure to engineered nanomaterials with antifouling properties (SiNC-DCOIT; SiNC-DCOIT-Ag) and respective counterparts, namely the free biocides (DCOIT and AgNO3) and empty silica mesoporous nanocapsules (SiNC). Asterisks (*) indicate significant differences between the treatment and the control (ANOVA followed by Dunnett test, $p < 0.05$). LPO

= Lipid Peroxidation; GST = Glutathione S-Transferase; GPX = Glutathione Peroxidase; CAT = Catalase; AChE = Acetylcholinesterase.

3.3 Data integration and comprehensive multivariate analyses

Figure 4 shows the integration of the biochemical and physiological responses induced by each separate compound, through a principal component analysis (PCA) (complete table of PCA results available as supplementary material). The first two principal components (PC) of the PCAs explained 76, 78, 79, 79, and 89% of the total variance of data of organisms exposed to SiNC, $AgNO_3$, SiNC-DCOIT, SiNC-DCOIT-Ag and DCOIT, respectively. The PC1 explained 46% of the total variance in the case of SiNC, 52% for $AgNO_3$, 51% for SINC-DCOIT, 47% for SINC-DCOIT-Ag, and 90% for DCOIT. Globally, by looking at the factor loadings (>0.45), this PC grouped the biomarkers AChE and GR (in most cases) and the physiological endpoints (air survival, adhesion and condition index) in the positive part of the axis, and oxidative stress biomarkers (CAT, GPx, and LPO) in the negative part of the axis. Regarding $AgNO_3$, the PC 1 split LPO and GPx responses from all the other biomarkers. The PC2 results were more scattered among the compounds and no patterns were clearly defined.

Figure 5 shows the integration based on a comprehensive ordination analysis for all measured endpoints in all treatments. In general, there is a split between the effects caused by the free and the nanostructured forms of both biocides (Figure 5). The PCO axis 1 explains 27.5% of the total variance, isolating the control, SiNC, SiNC-DCOIT at low concentrations (1 and 10 µg DCOIT/L) and SiNC-DCOIT-Ag treatments, in the positive part of the axis, from DCOIT treatments and high tested concentrations of Ag and SiNC-DCOIT (0.1 and 1 mg DCOIT/L) in the negative side of the axis. The most correlated variables with the PCO axis 1 are AChE and GPx activities, LPO levels, GST activity and air survival (Spearman *rho*=0.76, -0.75, -0.68, 0.58 and 0.53, respectively). The PCO axis 2 accounts for 17.7% of the total variance and splits the control, Ag and SiNC-DCOIT-Ag treatments in the positive part of the axis from DCOIT, SiNC-DCOIT and SiNC treatments.

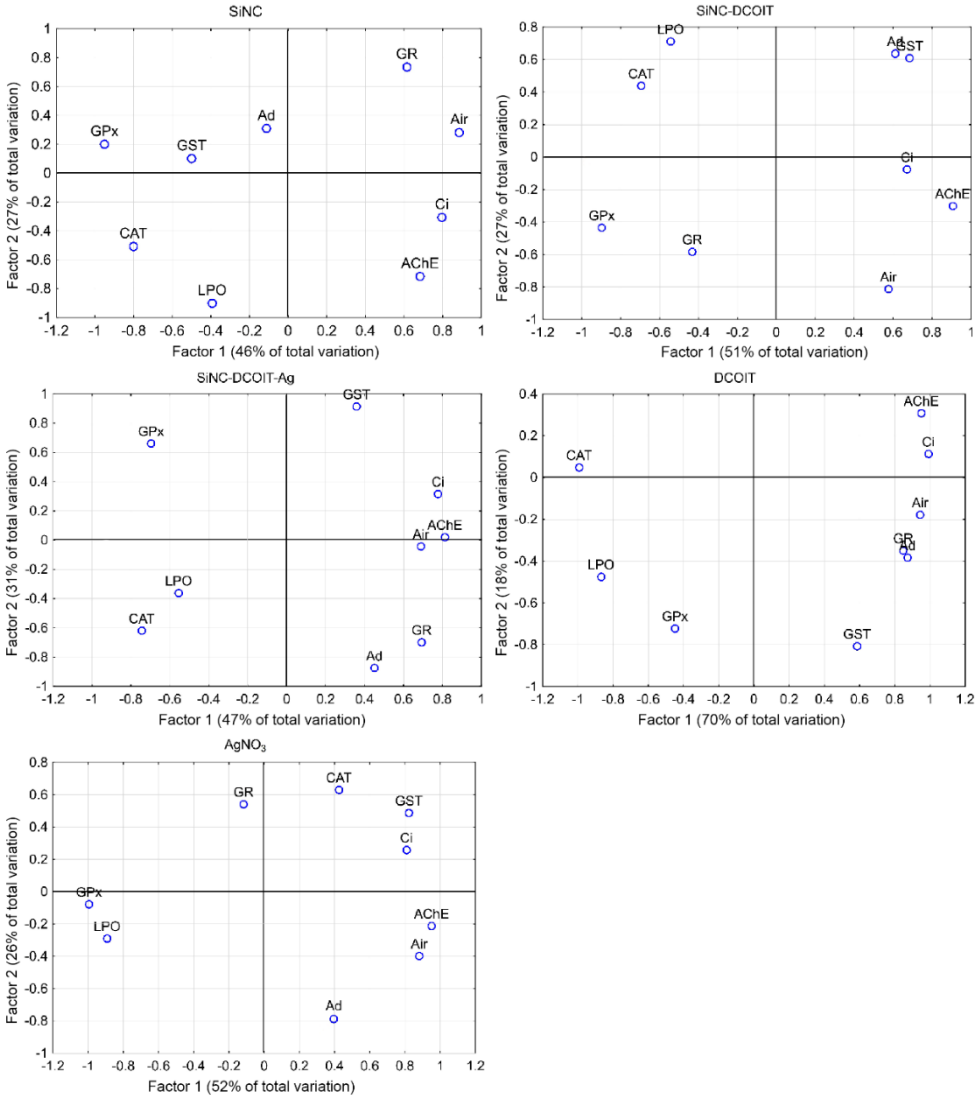


Fig4. Principal components analysis (PCA) based on biochemical and physiological endpoints for tested compound individually. Ad = Adhesion, Air = Air Survival, Ci = Condition Index, LPO = Lipid Peroxidation, GPx = Glutathione Peroxidase, GR = Glutathione Reductase, CAT = Catalase, GST = Glutathione-S-Transferase, AChE = Acetylcholinesterase.

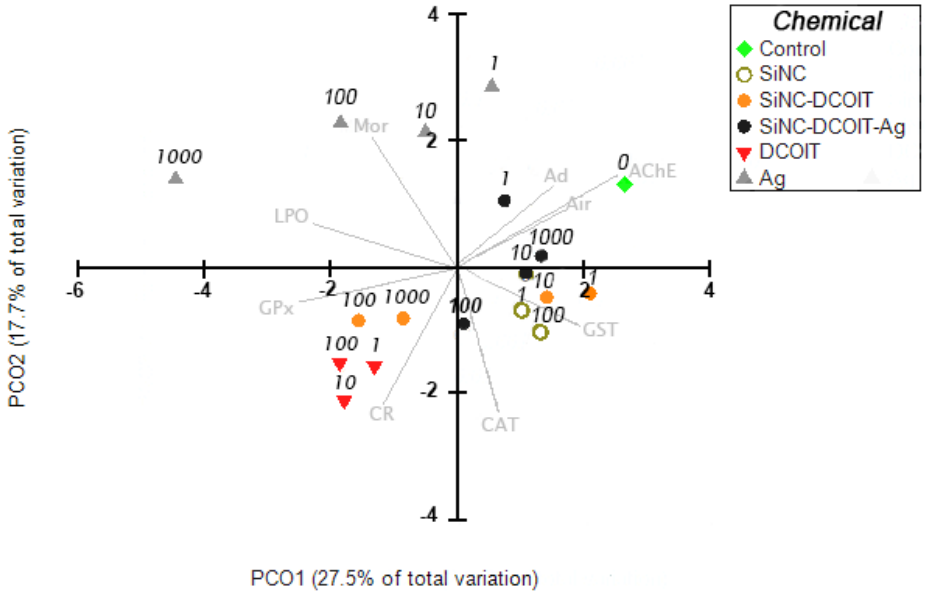


Fig. 5 Principal coordinates analysis (PCO) based on all measured endpoints in all treatments. Values above each symbol correspond to the exposure concentrations (µg/L) of a given tested chemical (identified by symbols). Ad = adhesion, Air = air survival capacity, CR = clearance rate, Mor = Mortality LPO = lipid peroxidation, GPx = glutathione peroxidase, GR = glutathione reductase, CAT = catalase, GST = glutathione-S-transferase, AChE = acetylcholinesterase.¬

4. Discussion

To our knowledge, this is the first study demonstrating a generalized impairment of ecologically relevant physiological and biochemical endpoints in bivalves exposed to DCOIT and Ag, ENMs in both soluble and SiNC-based nanostructured forms. Effects induced by free biocides were particularly severe if compared with their respective recently developed SiNC-based ENMs nanostructured forms (SINC-DCOIT and SINC-DCOIT-Ag), as evidenced by the individual analyses and the integrative approaches. The present findings show that short- and long-term exposure scenarios to SiNC, SINC-DCOIT, SINC-DCOIT-Ag, DCOIT and $AgNO_3$ cause negative effects at different scales on juveniles of the oyster species *C. gigas*. By integrating the data, the biomarkers were correlated with the physiological endpoints, signalizing a possible translation of the biochemical effects of oxidative stress and neurotoxicity to changes on ecologically relevant levels of biological organization, demonstrated as an overall impairment of organisms' physiological performance. As new information regarding the toxicity and half-life of third-generation AF biocides is produced, becomes clear the necessity to evaluate the toxicity of such chemicals, to find and select those capable of affecting target organisms at low concentrations but not remaining in the environment for long periods and causing harmful effects on biota.

In the present study, the unloaded SiNC caused physiological changes and activated the antioxidant defense system at low exposure concentrations. The absence of lipoperoxidation and other enzymatic changes in the long-term exposure may indicate that organisms were able to successfully neutralize the produced ROS and prevent oxidative damage, however, the observed oxidative stress and the impact on the health condition index may represent an environmental risk in a real scenario. The present study sets the lowest LOEC (1 µg/L) of all previous studies aimed at assessing the toxicity of SiNC in several marine temperate and tropical species (e.g., Avelelas et al., 2017; Gutner-Hoch et al., 2018, 2019; Figueiredo et al., 2019; Santos et al., 2020; Jesus et al., 2021). The toxic effects of SiNC have been attributed to residues of the surfactant CTAB (cetyl trimethylammonium bromide) (Figueiredo et al. 2019), used during the SiNC synthesis, which was recently proposed to be replaced by greener alternatives (Kaczerewska et al. 2020a) to develop less toxic SiNC (Kaczerewska et al. 2020b). However, all tested SiNC exposure concentrations caused a significant decrease of the health condition index, apart from the highest tested concentration that might be associated with the environmental behavior of SiNC on saltwater. This leads to a not often observed contradiction of the Paracelsus concept (the dose makes the venom), with a NOEC value higher than the LOEC value. In fact, Figueiredo et al. (2019) demonstrated that in artificial saltwater, the hydrodynamic particle size of SiNC tends to increase with exposure time and concentration, forming larger aggregates/agglomerates (704±104 nm in dispersions of 10 mg/L) which may explain the reduction of SiNC bioavailability and toxicity (CI) at 10 mg SiNC/L, contrasting with the low exposure concentrations that caused a severe CI decrease (average size of aggregates/agglomerates of 514±71 nm in dispersions of 0.001 mg/L; Figueiredo et al., 2019).

DCOIT exposure caused extreme toxicity on *C. gigas* juveniles, impairing several biochemical and physiological endpoints at concentrations as low as 0.1 or 1 µg/L, below or very close to the maximum measured environmental concentration (0.28 µg DCOIT/L; (Steen et al. 2004) in seawater, representing therefore a threat for this species in a real scenario. DCOIT reduced the adhesion capacity and the CI of *C. gigas* juveniles, indicating a shift in the energetic budget from growth to detoxification. According to Gagné (2014) organisms exposed to xenobiotics increase their metabolism and energy consumption to detoxify. On early life stages of the tropical mussel *Perna perna*, DCOIT was also extremely toxic, namely on the fertilization rates (40 min-EC_{50}=0.063 µg.L^{-1}) and byssus thread production (72 h-EC_{50}=96.1 µg.L^{-1}) (Santos et al. 2020). During the larval settlement stage, marine invertebrates experience relevant changes of the protein glycosylation pattern, becoming more sensitive to chemicals

and more prone to oxidative stress, energetic unbalances (Chandramouli et al. 2012) and neurotoxicity. The decrease in AChE observed in the present study, which may signalize a neurotoxicity impairment due to a constant supply of acetylcholine (Ricciardi et al. 2006), can be linked to the oysters reduction of the adhesion capacity since ChEs contributes to the neuronal differentiation and development, adhesion and signaling (Pezzementi and Chatonnet 2010) . The long-term exposure to DCOIT also caused the activation of CAT and GPx, both responsible to neutralize reactive oxygen species (ROS), thus indicating oxidative stress. Isothiazolinone-based biocides, such as DCOIT, have shown to increase intracellular ROS levels on human keratinocytes (Ettorre et al. 2003) and fish (Chen et al. 2014), explaining the activation of the antioxidant system. However, differently from SiNC, the antioxidant defense system did not prevent damages on cell membranes (LPO increase). In addition, the observed GST inhibition at the highest tested concentration can occur through an indirect or direct interaction with ROS (Borković-Mitić et al. 2013). Oxidative stress and damage caused by DCOIT were previously reported in the literature for bivalves (Gabe et al. 2021), corals (Cima et al. 2013), ascidians (Cima et al. 2008), polychaetes (Eom et al. 2019), and fish (Ito et al. 2013; Chen et al. 2014a). In particular, the chronic exposure (14 d) of polychaetes *Perinereis aibuhitensis* to 1 µg DCOIT/L caused an increase of CAT and GPx activities, lipoperoxidation, and decrease of AChE, similarly to the present study. The authors also observed an increase in the superoxide dismutase activity, an enzyme that is essential for cellular defense against oxidative stress (Eom et al. 2019). Chen et al. (2014) found an increase of the CAT activity and AChE inhibition in liver tissues of fish exposed to 3 µg DCOIT/L for 28 days. Cima et al. (2013) also demonstrated a reduction of GSTs activity in the soft coral *Sarcophyton* cf. *glaucum* exposed to 10 µg DCOIT/L for 72 hours. GST is involved in the phase II of xenobiotics biotransformation and depuration (Fontes et al. 2017), thus, such reduction possibly indicates the failure of GST-mediated detoxification mechanisms (Chikezie 2015). In fact, the downregulation of GST genes was recently documented by Gabe et al. (2021) in MGST-like genes in *Perna perna* mussels exposed to 10 µg DCOIT/L for 96 h. Gabe et al. (2021) also observed that DCOIT inhibited the CAT activity (in the gills) and increased the GST activity (in the digestive gland) at 0.1 and 10 µg DCOIT/L, respectively. These findings are not in agreement with our results for *C. gigas*, possibly due to differences of model species, particularly, the exposure period, which may influence the chemical transformations and processes of depuration inside the body. DCOIT generally degrades fast (half-life below one day), both biologically and photochemically in natural seawater (Silva et al. 2020), however,

there is little information on the intracellular transformation or degradation pathways of DCOIT (Zhu et al. 2017).

$AgNO_3$ was also amongst the most toxic compound. Silver is a biocide commonly used against a wide range of gram-positive and negative bacteria (Fewtrell 2014) to prevent the biofilm formation (Fewtrell 2014). Consequently, it has a high potential to be used as an additive in antifouling biocides. However, silver is generally considered one of the most toxic metals, particularly for marine biota (Rementeria et al. 2017), with a deterministic PNEC of 0.12 µg/L (Figueiredo et al. 2020). Silver inhibits the ATPase activity inducing impairment of the marine invertebrates metabolism (Bianchini et al. 2005), thus resulting in a general mass loss (Thomas et al. 2020). Rementeria et al. (2016) detected a progressive decrease of the CI in adult *C. gigas* chronically exposed to 0.5 µg/L of Ag for 28 days; nevertheless, in the present study, a statistically relevant reduction of the CI was only observed at 100 µg/L, yet the significant decrease in the air survival capacity (in a dose-dependent manner) corroborated to the low energy budget state. The CI for $AgNO_3$ resulted in a U-shape dose-response pattern, which could be an overcompensation to disruption in homeostasis, also known as hormesis (Calabrese and Baldwin 2001). Studies have shown that heavy metal can cause hormetic effects in mussels (Lefcort et al. 2008; Tyne et al. 2015; Agathokleous et al. 2019), nevertheless, further studies are recommended to validate the present findings. Another plausible explanation is the reduction of Ag bioavailability at 1000 and 10000 µg/L due to speciation. In seawater, salinity and dissolved organic matter is involved in Ag speciation (Herce-Sesa et al. 2019). Several studies demonstrated that such complexes have reduced the Ag toxicity (Leblanc et al. 1984; Janes and Playle 1995; Hogstrand et al. 1996; Rose-Janes and Playle 2000; Zhang et al. 2012; Kim et al. 2013). In the present study, oysters exposed to $AgNO_3$ produced a high quantity of mucus (visual inspection). According to Fernández-Boo et al. (2019) this mucus protects the organism from chemical and physical injuries, acting as a defense mechanism. In addition, this mucus is rich in amino acids and other organic elements, which contribute to the formation of Ag stable organic complexes (Mousavi et al. 2015). It is known that Ag toxicity is closely related to its interaction with biological macromolecules leading to the production of ROS, oxidative stress, membrane damage, and genotoxicity (McShan et al. 2014), as corroborated by the present findings of the long-term exposure experiments. In fact, metal ions, such as Ag^{+}, Cu^{2+} and Zn^{2+}, act as a competitive inhibitors for CAT (Atli et al. 2006; Ma et al. 2017), which impairment can contribute to oxidative damage, here evidenced by inhibition of CAT and increase of LPO. Similarly, Gomes et al. (2014) observed inhibition of CAT and AChE activity

in mussels *Mytilus galloprovincialis* exposed to 10 µg and 10000 µg Ag^{+}/L, respectively, for 15 days. Authors highlighted an irreversible impairment of the antioxidant and nervous systems due to the overwhelming levels of free ROS originated by Ag^{+}.

Both antifouling nanomaterials, SiNC-DCOIT and SiNC-DCOIT-Ag, caused negative physiological effects in the oysters, as demonstrated by the CI. The long-term exposure to SINC-DCOIT caused a significant modulation of CAT indicating oxidative stress, however, neither oxidative damage nor neurotoxicity was evidenced. As observed for DCOIT, SINC-DCOIT significantly decreased GST at the highest concentration, which may be expected considering the abovementioned downregulation of GTS-like genes promoted by DCOIT (Gabe et al. 2021). The induction of CAT activity signalizes the presence of ROS, which can also directly or indirectly impair GSTs. The SiNC-DCOIT-Ag long-term exposure caused both oxidative stress and damage, as CAT and LPO were increased. Mussels *Mytilus galloprovincialis* exposed to 10 mg of Ag nanoparticles for 15 days also presented oxidative stress and damage, as evidenced by the activation of antioxidant enzymes (SOD, CAT, and GPx) and occurrence of lipid peroxidation (Gomes et al. 2014). In addition, Ale et al. (2019) found significant lipoperoxidation, increased GST activity and, a dose-dependent reduction of the neutral red retention time, in the same mussel species, but at 3 orders of magnitude lower, at 10 µg AgNPs/L. Despite the sublethal toxicity of the nanostructured biocides, our data indicate that the free forms of DCOIT and Ag were more toxic (LOECs = 0.1 µg/L; below MEC of DCOIT and Ag) than the nanoforms (LOECs = 1 µg/L), in agreement with recent studies from chronic (Jesus et al., 2021) and short-term exposure tests (Figueiredo et al. 2019, 2020; Santos et al. 2020). Figueiredo et al. (2019, 2020) also demonstrated that DCOIT and $AgNO_3$ are more toxic and hazardous compounds compared with SINC-DCOIT-Ag and SINC-DCOIT. In the tropical mussel species *Perna perna*, exposure to DCOIT (EC_{50} lower =0.063 µg/L) reduced the fertilization success by three orders of magnitude higher than the SiNC-DCOIT (EC_{50}=8.6 µg/L), as well as affected the byssus thread formation and air survival. The toxicity reduction in the nanostructured forms of DCOIT and Ag is associated with the controlled release of biocides from the silica nanocapsules (Michailidis et al. 2017; Figueiredo et al. 2019). Similar results were found for other encapsulated AF biocides as zosteric acid (Geiger et al. 2004), zinc pyrithione (ZnPT) and copper pyrithione (CuPT) (Avelelas et al. 2017; Gutner-Hoch et al. 2018, 2019).

5. Conclusion

The present study shows that the encapsulation of AF biocides can reduce the long-term toxic effects on mollusk bivalves of commercially available biocides when immobilized in

silica mesoporous nanocapsules, and also corroborate the existing literature on the reduction of their short-term effects (Avelelas et al. 2017; Gutner-Hoch et al. 2018, 2019; Figueiredo et al. 2019, 2020; Santos et al. 2020). Nevertheless, hollow SiNC caused oxidative stress on the tested juvenile oysters even at a very low concentrations being therefore encouraged the development of less toxic SiNC alternatives. Finally, foreground data will contribute to refine existing PNECs as *C. gigas* juveniles showed a high sensitivity to the tested compounds compared to the literature.

The short- and long-term effects on juveniles of *C. gigas* indicate that the biocides DCOIT and Ag present higher toxicity compared to the novel AF nanoadditives (SiNC-DCOIT; SiNC-DCOIT-Ag), despite the toxicity of the SiNC. The gradient of toxicity was: DCOIT (lower LOECs for GST, GPx, AChE, LPO and CI) > $AgNO_3$ (GST, CAT and survival in air) > SINC-DCOIT-Ag (CI) > SiNC (CI) > SINC-DCOIT. Both AF nanoadditives proved to be less toxic alternatives than the free biocides that caused detrimental sub-lethal effects at environmental relevant concentrations on the tested species.

6 Declarations

6.1 Ethics approval and consent to participate

Not applicable

6.2 Consent for publication

Not applicable

6.3 Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author

6.4 Competing interests

The authors declare that they have no competing interests

6.5 Funding

- Bruno Galvão de Campos: São Paulo Research Foundation (FAPESP) (FAPESP: 2019/19898-3 and FAPESP: 2017/10211-0);
- Mariana B. M. P Silva: São Paulo Research Foundation (FAPESP) (FAPESP: 2019/24945-0 and FAPESP: 2018/25379-6)
- Dr. Roberto Martins: FCT – Fundação para a Ciência e a Tecnologia, I.P., (CEECIND/01329/2017).
- Dr. Denis Abessa: National Council for Scientific and Technological Development (CNPq #308533/2018-6).

- CESAM: FCT/MCTES (UIDP/50017/2020+UIDB/50017/2020), through national funds.
- This project was also carried out in the framework of the bilateral project "Exposure and bioaccumulation assessment of antifouling nanomaterials in marine organisms from temperate and tropical waters" funded by FCT and CAPES (ref. 4265 DRI/FCT and #88881.156405/2017-01, respectively) and the project NANOGREEN (CIRCNA/BRB/0291/2019) funded by national funds (OE), through FCT.

6.6 Authors' contributions

BGC, DMSA, FCP, and RM contributed to the study conception and design. RM, BGC, DMSA, and SL contributed to the funding. FA and FM prepared the material, BGC, MBMPS, and RM collected and analyzed the data. All authors read and reviewed the manuscript.

7. References

Agathokleous E, Feng ZZ, Iavicoli I, Calabrese EJ (2019) The two faces of nanomaterials: A quantification of hormesis in algae and plants. Environ Int 131:. https://doi.org/10.1016/j.envint.2019.105044

Aidarova S, Sharipova A, Issayeva A, et al (2018) Synthesis of Submicrocontainers with "Green" Biocide and Study of Their Antimicrobial Activity. Colloids and Interfaces 2:67. https://doi.org/10.3390/colloids2040067

Aidarova SB, Issayeva AB, Sharipova AA, et al (2019) Synthesis and study of the properties of containers of DCOIT with the polyurea shell. Int J Nanotechnol 16:. https://doi.org/10.1504/IJNT.2019.102386

Ale A, Liberatori G, Vannuccini ML, et al (2019) Exposure to a nanosilver-enabled consumer product results in similar accumulation and toxicity of silver nanoparticles in the marine mussel Mytilus galloprovincialis. Aquat Toxicol 211:46–56. https://doi.org/10.1016/j.aquatox.2019.03.018

Atli G, Alptekin Ö, Tükel S, Canli M (2006) Response of catalase activity to Ag+, Cd2+, Cr6+, Cu2+ and Zn2+ in five tissues of freshwater fish Oreochromis niloticus. Comp Biochem Physiol - C Toxicol Pharmacol 143:218–224. https://doi.org/10.1016/j.cbpc.2006.02.003

Avelelas F, Martins R, Oliveira T, et al (2017) Efficacy and Ecotoxicity of Novel Anti-Fouling Nanomaterials in Target and Non-Target Marine Species. Mar Biotechnol 19:164–174. https://doi.org/10.1007/s10126-017-9740-1

Bianchini A, Playle RC, Wood CM, Walsh PJ (2005) Mechanism of acute silver toxicity in marine invertebrates. Aquat Toxicol 72:67–82. https://doi.org/10.1016/j.aquatox.2004.11.012

Bird RP, Draper HH (1984) Comparative studies on different methods of malonaldehyde determination. Methods Enzymol 105:299–305

Borković-Mitić S, Pavlović S, Perendija B, et al (2013) Influence of some metal concentrations on the activity of antioxidant enzymes and concentrations of vitamin e and SH-groups in the digestive gland and gills of the freshwater bivalve Unio tumidus from the Serbian part of Sava River. Ecol Indic 32:212–221. https://doi.org/10.1016/j.ecolind.2013.03.024

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein

utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Burkett JR, Hight LM, Kenny P, Wilker JJ (2010) Oysters produce an organic-Inorganic adhesive for intertidal reef construction. J Am Chem Soc 132:12531–12533. https://doi.org/10.1021/ja104996y

Calabrese EJ, Baldwin LA (2001) U-Shaped Dose-Responses in Biology, Toxicology, and Public Health. Annu Rev Public Health 22:15–33. https://doi.org/10.1146/annurev.publhealth.22.1.15

Campos BG de, Figueiredo J, Perina F, et al (2021) Occurrence, effects and environmental risk of antifouling biocides (EU PT21): Are marine ecosystems threatened? Crit Rev Environ Sci Technol in press: https://doi.org/10.1080/10643389.2021.1910003

Chandramouli KH, Zhang Y, Wong YH, Qian PY (2012) Comparative glycoproteome analysis: Dynamics of protein glycosylation during metamorphic transition from pelagic to benthic life stages in three invertebrates. J Proteome Res 11:1330–1340. https://doi.org/10.1021/pr200982k

Chen L, Ye R, Xu Y, et al (2014a) Comparative safety of the antifouling compound butenolide and 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT) to the marine medaka (Oryzias melastigma). Aquat Toxicol 149:116–125. https://doi.org/10.1016/j.aquatox.2014.01.023

Chen L, Zhang H, Sun J, et al (2014b) Proteomic changes in brain tissues of marine medaka (Oryzias melastigma) after chronic exposure to two antifouling compounds: Butenolide and 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT). Aquat Toxicol 157:47–56. https://doi.org/10.1016/j.aquatox.2014.09.010

Chikezie PC (2015) Glutathione S-transferase Activity in Diagnostic Pathology. Metabolomics 05: https://doi.org/10.4172/2153-0769.1000153

Cima F, Bragadin M, Ballarin L (2008) Toxic effects of new antifouling compounds on tunicate haemocytes. I. Sea-Nine 211TM and chlorothalonil. Aquat Toxicol 86:299–312. https://doi.org/10.1016/j.aquatox.2007.11.010

Cima F, Ferrari G, Ferreira NGC, et al (2013) Preliminary evaluation of the toxic effects of the antifouling biocide Sea-Nine 211 in the soft coral Sarcophyton cf. glaucum (Octocorallia, Alcyonacea) based on PAM fluorometry and biomarkers. Mar Environ Res 83:16–22. https://doi.org/10.1016/j.marenvres.2012.10.004

Clairborne A (1985) Catalase activity. In: Greenwald RA (ed) CRC Handbook of Methods in Oxygen Radical Research. CRC Press, Boca Raton, FL, pp 283–284

Cribb AE, Leeder JS, Spielberg SP (1989) Use of a microplate reader in an assay of glutathione reductase using 5, 5′-dithiobis (2-nitrobenzoic acid). Anal Biochem 183:195–196

Devilla R, Brown M, Donkin M, et al (2005) Impact of antifouling booster biocides on single microalgal species and on a natural marine phytoplankton community. Mar Ecol Prog Ser 286:1–12. https://doi.org/10.3354/meps286001

Ellman GL, Courtney KD, Andres V, Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7:88–95. https://doi.org/10.1016/0006-2952(61)90145-9

Eom H, Haque N, Nam S, et al (2019) Effects of sublethal concentrations of the antifouling biocide Sea-Nine on biochemical parameters of the marine polychaete Perinereis aibuhitensis. Comp Biochem Physiol Part C 222:125–134. https://doi.org/10.1016/j.cbpc.2019.05.001

Ettorre A, Andreassi M, Anselmi C, et al (2003) Involvement of oxidative stress in apoptosis induced by a mixture of isothiazolinones in normal human keratinocytes. J Invest Dermatol 121:328–336. https://doi.org/10.1046/j.1523-1747.2003.12360.x

Fernández-Boo S, Gervais O, Prado-Alvarez M, et al (2019) Is pallial mucus involved in oyster defense against the parasite Bonamia ostreae? Fish Shellfish Immunol 91:443. https://doi.org/10.1016/j.fsi.2019.04.215

Fewtrell L (2014) Silver: water disinfection and toxicity. Aberystwyth Univ Aberystwyth

Figueiredo J, Loureiro S, Martins R (2020) Hazard of novel anti-fouling nanomaterials and biocides DCOIT and silver to marine organisms. Environ Sci Nano 7:1670–1680. https://doi.org/10.1039/d0en00023j

Figueiredo J, Oliveira T, Ferreira V, et al (2019) Toxicity of innovative anti-fouling nano-based solutions in

marine species. Environ Sci nano. https://doi.org/10.1039/C9EN00011A

Fontes MK, Gusso-choueri PK, Maranho LA, et al (2017) A tiered approach to assess effects of diclofenac on the brown mussel Perna perna: A contribution to characterize the hazard. Water Res 132:361–370. https://doi.org/10.1016/j.watres.2017.12.077

Frasco MF, Guilhermino L (2002) Effects of dimethoate and beta-naphthoflavone on selected biomarkers of Poecilia reticulata. Fish Physiol Biochem 26:149–156

Fu Y, Wang W, Zhang L, et al (2019) Development of marine antifouling epoxy coating enhanced with clay nanotubes. Materials (Basel) 12:1–12. https://doi.org/10.3390/ma1224195

Gabe HB, Guerreiro A da S, Sandrini JZ (2021) Molecular and biochemical effects of the antifouling DCOIT in the mussel Perna perna. Comp Biochem Physiol Part - C Toxicol Pharmacol 239:108870. https://doi.org/10.1016/j.cbpc.2020.108870

Gagné F (2014) Cellular Energy Allocation. Biochem Ecotoxicol Princ Methods 131–144. https://doi.org/10.1016/B978-0-12-411604-7.00008-8

Geiger T, Delavy P, Hany R, et al (2004) Encapsulated zosteric acid embedded in poly[3-hydroxyalkanoate] coatings - Protection against biofouling. Polym Bull 52:65–72. https://doi.org/10.1007/s00289-004-0253-5

Gomes T, Pereira CG, Cardoso Ć, et al (2014) Effects of silver nanoparticles exposure in the mussel Mytilus galloprovincialis. Mar Environ Res 101:208–214. https://doi.org/10.1016/j.marenvres.2014.07.004

Gutner-Hoch E, Id RM, Oliveira T, et al (2018) Antimacrofouling Efficacy of Innovative Inorganic Nanomaterials Loaded with Booster Biocides. 1–12. https://doi.org/10.3390/jmse6010006

Gutner-Hoch E, Martins R, Maia F, et al (2019) Toxicity of engineered micro- and nanomaterials with antifouling properties to the brine shrimp Artemia salina and embryonic stages of the sea urchin Paracentrotus lividus. Environ Pollut 251:530–537. https://doi.org/10.1016/j.envpol.2019.05.031

Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione S-transferases the first enzymatic step in mercapturic acid formation. J Biol Chem 249:7130–7139

Hartmann JT, Beggel S, Auerswald K, et al (2016) Establishing mussel behavior as a biomarker in ecotoxicology. Aquat Toxicol 170:279–288. https://doi.org/10.1016/j.aquatox.2015.06.014

Herce-Sesa B, López-López JA, Moreno C (2019) Selective ionic liquid solvent bar micro-extraction for estimation of ultra-trace silver fractions in marine waters. Sci Total Environ 650:27–33. https://doi.org/10.1016/j.scitotenv.2018.08.438

Hogstrand C, Galvez F, Wood CM (1996) Toxicity, silver accumulation and metallothionein induction in freshwater rainbow trout during exposure to different silver salts. Environ Toxicol Chem An Int J 15:1102–1108

Ito M, Mochida K, Ito K, et al (2013) Induction of apoptosis in testis of the marine teleost mummichog Fundulus heteroclitus after in vivo exposure to the antifouling biocide 4,5-dichloro-2-n-octyl-3(2H)-isothiazolone (Sea-Nine 211). Chemosphere 90:1053–1060. https://doi.org/10.1016/j.chemosphere.2012.08.052

Jacobson AH, Willingham GL (2000) Sea-nine antifoulant: An environmentally acceptable alternative to organotin antifoulants. Sci Total Environ 258:103–110. https://doi.org/10.1016/S0048-9697(00)00511-8

Janes N, Playle RC (1995) Modeling silver binding to gills of rainbow trout (Oncorhynchus mykiss). Environ Toxicol Chem An Int J 14:1847–1858

Jesus ÉPS de, Figueirêdo LP de, Maia F, et al (2021) Acute and chronic effects of innovative antifouling nanostructured biocides on a tropical marine microcrustacean. Mar Pollut Bull 164:111970. https://doi.org/10.1016/j.marpolbul.2021.111970

Kaczerewska O, Martins R, Figueiredo J, et al (2020a) Environmental behaviour and ecotoxicity of cationic surfactants towards marine organisms. J Hazard Mater 392:122299. https://doi.org/10.1016/j.jhazmat.2020.122299

Kaczerewska O, Sousa I, Martins R, et al (2020b) Gemini surfactant as a template agent for the synthesis of more eco-friendly silica nanocapsules. Appl Sci 10:1–13. https://doi.org/10.3390/app10228085

Kim JY, Kim K-T, Lee BG, et al (2013) Developmental toxicity of Japanese medaka embryos by silver nanoparticles and released ions in the presence of humic acid. Ecotoxicol Environ Saf 92:57–63

La Peyre MK, Bernasconi SK, Lavaud R, et al (2020) Eastern oyster clearance and respiration rates in response to acute and chronic exposure to suspended sediment loads. J Sea Res 157:101831. https://doi.org/10.1016/j.seares.2019.101831

Lagerström M, Ferreira J, Ytreberg E, Eriksson-Wiklund AK (2020) Flawed risk assessment of antifouling paints leads to exceedance of guideline values in Baltic Sea marinas. Environ Sci Pollut Res 27:27674–27687. https://doi.org/10.1007/s11356-020-08973-0

Leblanc GA, Mastone JD, Paradice AP, et al (1984) The influence of speciation on the toxicity of silver to fathead minnow (Pimephales promelas). Environ Toxicol Chem 3:37–46. https://doi.org/10.1002/etc.5620030106

Lee MRN, Kim UJ, Lee IS, et al (2015) Assessment of organotin and tin-free antifouling paints contamination in the Korean coastal area. Mar Pollut Bull 99:157–165. https://doi.org/10.1016/j.marpolbul.2015.07.038

Lefcort H, Freedman Z, House S, Pendleton M (2008) Hormetic effects of heavy metals in aquatic snails: Is a little bit of pollution good? Ecohealth 5:10–17. https://doi.org/10.1007/s10393-008-0158-0

Lei Q, Guo J, Noureddine A, et al (2020) Sol–Gel-Based Advanced Porous Silica Materials for Biomedical Applications. Adv Funct Mater 1909539:1–28. https://doi.org/10.1002/adfm.201909539

Lucas A, Beninger PG (1985) The use of physiological condition indices in marine bivalve aquaculture. Aquaculture 44:187–200. https://doi.org/10.1016/0044-8486(85)90243-1

Ma X, Deng D, Chen W (2017) Inhibitors and Activators of SOD, GSH-Px, and CAT. In: Enzyme Inhibitors and Activators. InTech

Maia F, Silva AP, Fernandes S, et al (2015) Incorporation of biocides in nanocapsules for protective coatings used in maritime applications. Chem Eng J 270:150–157. https://doi.org/10.1016/j.cej.2015.01.076

Martínez K, Barceló D (2001) Determination of antifouling pesticides and their degradation products in marine sediments by means of ultrasonic extraction and HPLC-APCI-MS. Fresenius J Anal Chem 370:940–945. https://doi.org/10.1007/s002160100904

Martins R, Oliveira T, Santos C, et al (2017) Effects of a novel anticorrosion engineered nanomaterial on the bivalve Ruditapes. Environ Sci Nano 4:1064–1076. https://doi.org/10.1039/c6en00630b

McShan D, Ray PC, Yu H (2014) Molecular toxicity mechanism of nanosilver. J Food Drug Anal 22:116–127. https://doi.org/10.1016/j.jfda.2014.01.010

Michailidis M, Sorzabal-Bellido I, Adamidou EA, et al (2017) Modified Mesoporous Silica Nanoparticles with a Dual Synergetic Antibacterial Effect. ACS Appl Mater Interfaces 9:38364–38372. https://doi.org/10.1021/acsami.7b14642

Mohandas J, Marshall JJ, Duggin GG, et al (1984) Differential distribution of glutathione and glutathione-related enzymes in rabbit kidney: possible implications in analgesic nephropathy. Biochem Pharmacol 33:1801–1807

Mousavi MPS, Gunsolus IL, Pérez De Jesús CE, et al (2015) Dynamic silver speciation as studied with fluorous-phase ion-selective electrodes: Effect of natural organic matter on the toxicity and speciation of silver. Sci Total Environ 537:453–461. https://doi.org/10.1016/j.scitotenv.2015.07.151

Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351–358. https://doi.org/10.1016/0003-2697(79)90738-3

Pack KE, Rius M, Mieszkowska N (2021) Long-term environmental tolerance of the non-indigenous Pacific oyster to expected contemporary climate change conditions. Mar Environ Res 164:105226. https://doi.org/10.1016/j.marenvres.2020.105226

Pezzementi L, Chatonnet A (2010) Evolution of cholinesterases in the animal kingdom. Chem Biol Interact 187:27–33. https://doi.org/10.1016/j.cbi.2010.03.043

Readman JW (2006) Development, Occurrence and Regulationof Antifouling Paint Biocides: Historical Review

and Future Trends. Antifouling Paint Biocides 1–15. https://doi.org/10.1007/698_5_047

Rementeria A, Mikolaczyk M, Lanceleur L, et al (2016) Assessment of the effects of Cu and Ag in oysters Crassostrea gigas (Thunberg, 1793) using a battery of cell and tissue level biomarkers. Mar Environ Res 122:11–22. https://doi.org/10.1016/j.marenvres.2016.09.002

Rementeria A, Mikolaczyk M, Peña A, et al (2017) Assessment of health status of oysters (Crassostrea gigas) exposed to environmentally relevant concentrations of Ag and Cu in brackish waters. J Sea Res 130:229–238. https://doi.org/10.1016/j.seares.2017.04.013

Reybuck SE, Schwartz C (2008) Blends of encapsulated biocides. 2

Ribeiro T, Rodrigues AS, Calderon S, et al (2020) Silica nanocarriers with user-defined precise diameters by controlled template self-assembly. J Colloid Interface Sci 561:609–619. https://doi.org/10.1016/j.jcis.2019.11.036

Ricciardi F, Binelli A, Provini A (2006) Use of two biomarkers (CYP450 and acetylcholinesterase) in zebra mussel for the biomonitoring of Lake Maggiore (northern Italy). Ecotoxicol Environ Saf 63:406–412. https://doi.org/10.1016/j.ecoenv.2005.02.007

Rose-Janes NG, Playle RC (2000) Protection by two complexing agents, thiosulphate and dissolved organic matter, against the physiological effects of silver nitrate to rainbow trout (Oncorhynchus mykiss) in ion-poor water. Aquat Toxicol 51:1–18

Ruiz-Sanchez AJ, Guerin AJ, El-Zubir O, et al (2020) Preparation and evaluation of fouling-release properties of amphiphilic perfluoropolyether-zwitterion cross-linked polymer films. Prog Org Coatings 140:105524. https://doi.org/10.1016/j.porgcoat.2019.105524

Santos JVN, Martins R, Fontes MK, et al (2020) Can Encapsulation of the Biocide DCOIT Affect the Anti-Fouling Efficacy and Toxicity on Tropical Bivalves ? Appl Sci 10:8579

Selim MS, El-Safty SA, Shenashen MA, et al (2020) Progress in biomimetic leverages for marine antifouling using nanocomposite coatings. J Mater Chem B 8:3701–3732. https://doi.org/10.1039/c9tb02119a

Silva ER, Ferreira O, Ramalho PA, et al (2019) Eco-friendly non-biocide-release coatings for marine biofouling prevention. Sci Total Environ 650:2499–2511. https://doi.org/10.1016/j.scitotenv.2018.10.010

Silva V, Silva C, Soares P, et al (2020) Isothiazolinone biocides: Chemistry, biological, and toxicity profiles. Molecules 25:. https://doi.org/10.3390/molecules25040991

Steen RJCA, Ariese F, Hattum B Van, et al (2004) Monitoring and evaluation of the environmental dissipation of the marine antifoulant (DCOIT) in a Danish Harbor. 57:513–521. https://doi.org/10.1016/j.chemosphere.2004.06.043

Tait L, Inglis G (2016) Optimising settlement arrays for surveillance of non-indigenous biofouling species

Thomas M, Jon B, Craig S, et al (2020) The world is your oyster: low-dose, long-term microplastic exposure of juvenile oysters. Heliyon 6:e03103. https://doi.org/10.1016/j.heliyon.2019.e03103

Tyne W, Little S, Spurgeon DJ, Svendsen C (2015) Hormesis depends upon the life-stage and duration of exposure: Examples for a pesticide and a nanomaterial. Ecotoxicol Environ Saf 120:117–123. https://doi.org/10.1016/j.ecoenv.2015.05.024

Yu YJ, Xing JL, Pang JL, et al (2014) Facile synthesis of size controllable dendritic mesoporous silica nanoparticles. ACS Appl Mater Interfaces 6:22655–22665. https://doi.org/10.1021/am506653n

Zhang H, Smith JA, Oyanedel-Craver V (2012) The effect of natural water conditions on the anti-bacterial performance and stability of silver nanoparticles capped with different polymers. Water Res 46:691–699

Zhu Y, Xue J, Cao J, Xiao H (2017) A potential mechanism for degradation of 4,5-dichloro-2-(n-octyl)-3[2H]-isothiazolone (DCOIT) by brown-rot fungus Gloeophyllum trabeum. J Hazard Mater 337:72–79. https://doi.org/10.1016/j.jhazmat.2017.04.072

8. Supplementary Material

Table S1: Eigenvalues, Factor Loadings, and Factor Scores for the PCA for each tested compound.

SiNC

Eigenvalues				
	Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative %
1	4.88	48.79	4.88	48.79
2	2.76	27.62	7.64	76.41
3	1.55	15.53	9.19	91.94

Factor Loadings (Marked loadings are >,450000)			
	F1	F2	F3
GR	0.61	0.74	-0.19
AChE	0.68	-0.71	-0.14
CAT	-0.80	-0.51	-0.31
GPx	-0.95	0.20	0.03
LPO	-0.39	-0.90	-0.18
GST	-0.50	0.10	-0.56
Air	0.89	0.28	-0.16
Ad	-0.11	0.31	-0.88
Ci	0.80	-0.31	-0.50
Expl.Var	4.88	2.76	1.55
Prp.Totl	0.49	0.28	0.16

Factor Scores			
	F1	F2	F3
Control	1.60	-0.41	-0.65
SINC 0.001	0.19	0.00	1.77
SINC 0.01	-0.11	1.35	-0.30
SINC 0.1	-0.82	0.41	-0.54
SINC 1	-0.85	-1.35	-0.27

SiNC-DCOIT

Eigenvalues				
	Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative %
1	5.15	51.48	5.15	51.48
2	2.76	27.60	7.91	79.08
3	1.63	16.27	9.53	95.35

Factor Loadings (Marked loadings are >,450000)			
	F1	F2	F3
GR	-0.43	-0.58	0.69
AChE	0.91	-0.30	-0.27
CAT	-0.69	0.44	-0.50
GPx	-0.90	-0.43	0.04
LPO	-0.54	0.71	0.42
GST	0.68	0.61	0.16
Air	0.58	-0.81	-0.07
Ad	0.61	0.64	0.26
Ci	0.67	-0.08	0.70
Expl.Var	5.15	2.76	1.63
Prp.Totl	0.51	0.28	0.16

Factor Scores			
	F1	F2	F3
Control	1.17	-0.92	0.98
SD 0.001	0.42	-0.28	-1.42
SD 0.01	0.51	1.60	-0.02
SD 0.1	-1.08	0.27	0.91
SD 1	-1.02	-0.66	-0.44

DCOIT

Eigenvalues				
	Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative %
1	7.09	70.86	7.09	70.86
2	1.87	18.68	8.95	89.54
3	1.05	10.46	10.00	100.00

Factor Loadings (Marked loadings are >,450000)			
	F1	F2	F3
GR	0.85	-0.35	0.40
AChE	0.95	0.31	0.01
CAT	-0.99	0.05	-0.11
GPx	-0.45	-0.72	-0.53
LPO	-0.87	-0.47	0.14
GST	0.59	-0.81	-0.07
Air	0.94	-0.18	-0.28
Ad	0.87	-0.38	0.30
Ci	0.99	0.11	-0.06
Expl.Var	7.09	1.87	1.05
Prp.Totl	0.71	0.19	0.10

Factor Scores			
	F1	F2	F3
Control	1.38	0.58	0.03
D 0.001	-0.01	-1.23	-0.87
D 0.01	-0.42	-0.37	1.39
D 0.1	-0.96	1.01	-0.56

SiNC-DCOIT-Ag

Eigenvalues				
	Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative %
1	4.80	48.00	4.80	48.00
2	3.16	31.55	7.95	79.55
3	1.26	12.64	9.22	92.19

Factor Loadings (Marked loadings are >,450000)			
	F1	F2	F3
GR	0.69	-0.70	-0.05
AChE	0.81	0.02	-0.02
CAT	-0.74	-0.62	0.20
GPx	-0.70	0.66	-0.20
LPO	-0.56	-0.36	-0.66
GST	0.36	0.91	-0.19
Air	0.69	-0.04	-0.60
Ad	0.45	-0.87	0.01
Ci	0.78	0.31	0.53
Expl.Var	4.80	3.16	1.26
Prp.Totl	0.48	0.32	0.13

Factor Scores			
	F1	F2	F3
Control	1.62	0.24	0.71
SDA 0.001	0.12	0.27	-1.54
SDA 0.01	-0.39	0.54	-0.32
SDA 0.1	-1.05	0.72	0.99
SDA 1	-0.31	-1.75	0.17

$AgNO_3$

Eigenvalues				
	Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative %
1	5.26	52.63	5.26	52.63
2	2.62	26.24	7.89	78.87
3	1.58	15.77	9.46	94.64

Factor Loadings (Marked loadings are >,450000)			
	F1	F2	F3
GR	-0.12	0.54	0.81
AChE	0.95	-0.21	-0.01
CAT	0.43	0.63	0.61
GPx	-1.00	-0.08	0.01
LPO	-0.89	-0.29	0.06
GST	0.82	0.49	-0.08
Air	0.88	-0.40	0.15
Ad	0.40	-0.78	0.36
Ci	0.81	0.26	-0.52
Expl.Var	5.26	2.62	1.58
Prp.Totl	0.53	0.26	0.16

Factor Scores			
	F1	F2	F3
Control	1.48	0.94	0.31
A 0.001	0.38	-1.06	-1.38
A 0.01	-0.19	-0.36	0.57
A 0.1	-0.49	-0.70	1.13
A 1	-1.18	1.18	-0.63

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