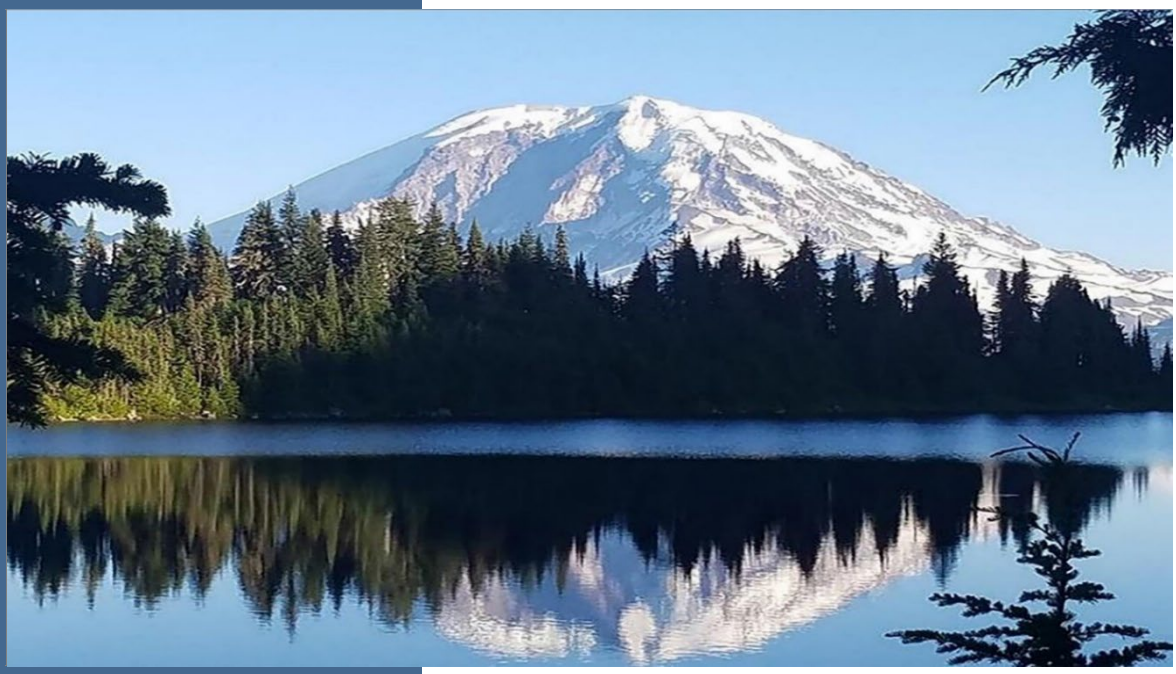


# Toxicological Effects of Antifouling Agents on Non-target Marine Species

By Ali Mahmoodi and Xianming Shi

For the **Hazardous Waste and Toxics Reduction Program**

Washington State Department of Ecology

Olympia, Washington

November 2023, Publication 23-04-066

DEPARTMENT OF ECOLOGY
State of Washington

# Publication Information

This document is available on the Department of Ecology's website at:
https://apps.ecology.wa.gov/publications/summarypages/2304066.html

**Cover photo credit**

- Standard Ecology image, 2019

### Related Information

Publication 23-04-024: Quality Assurance Project Plan: Performance Test of Green Alternatives to Copper Antifouling Paints[1]

# Contact Information


### Hazardous Waste and Toxics Program

P.O. Box 47600
Olympia, WA 98504-7600
Phone: 360-407-6700
**Website:** Washington State Department of Ecology[2]


# ADA Accessibility

The Department of Ecology is committed to providing people with disabilities access to information and services by meeting or exceeding the requirements of the Americans with Disabilities Act (ADA), Section 504 and 508 of the Rehabilitation Act, and Washington State Policy #188.

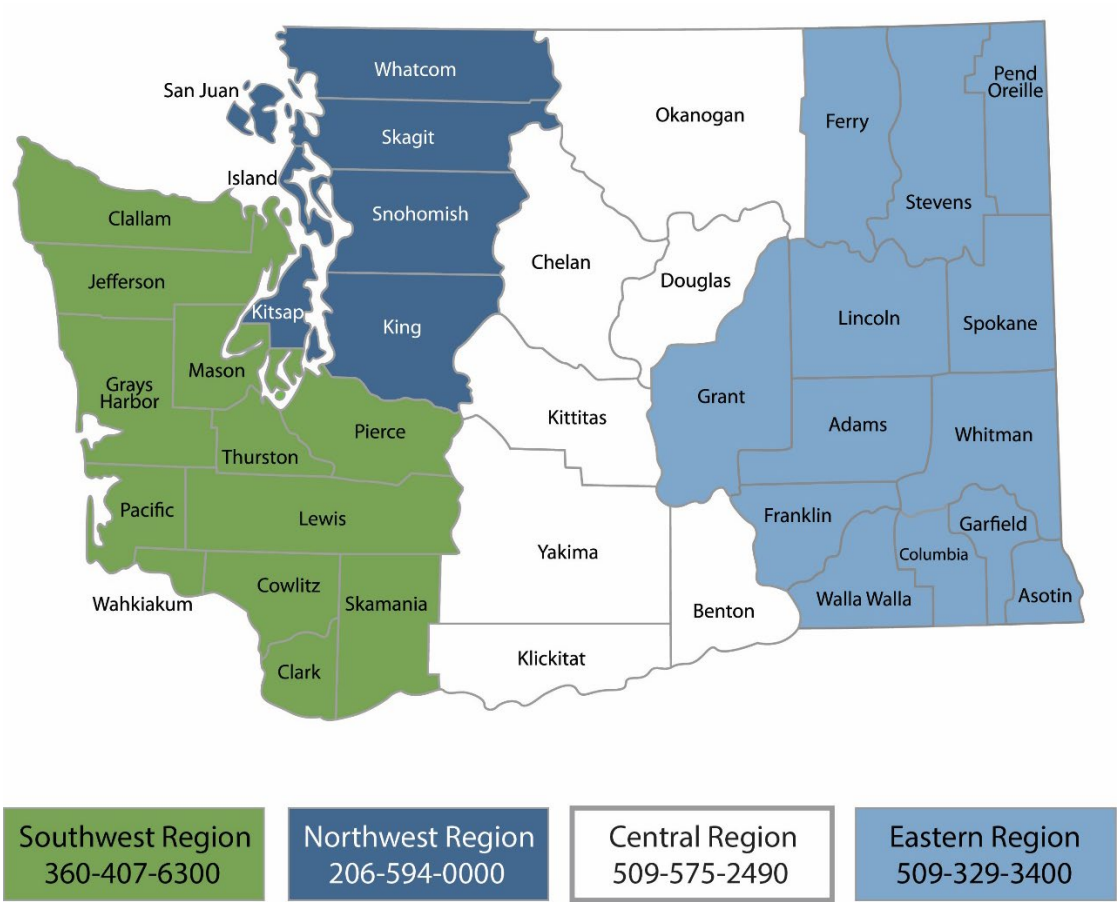
To request an ADA accommodation, contact Ecology by phone at 360-407-6700 or email at hwtrpubs@ecy.wa.gov. For Washington Relay Service or TTY call 711 or 877-833-6341. Visit **Ecology's accessibility webpage**[3] for more information.

---

[1] https://apps.ecology.wa.gov/publications/SummaryPages/2304024.html
[2] ecology.wa.gov/contact
[3] ecology.wa.gov/Accessibility

# Department of Ecology's Regional Offices

## Map of Counties Served




| Southwest Region<br>360-407-6300 | Northwest Region<br>206-594-0000 | Central Region<br>509-575-2490 | Eastern Region<br>509-329-3400 |
|---|---|---|---|

| Region | Counties served | Mailing Address | Phone |
|---|---|---|---|
| **Southwest** | Clallam, Clark, Cowlitz, Grays Harbor, Jefferson, Mason, Lewis, Pacific, Pierce, Skamania, Thurston, Wahkiakum | PO Box 47775<br>Olympia, WA 98504 | 360-407-6300 |
| **Northwest** | Island, King, Kitsap, San Juan, Skagit, Snohomish, Whatcom | PO Box 330316<br>Shoreline, WA 98133 | 206-594-0000 |
| **Central** | Benton, Chelan, Douglas, Kittitas, Klickitat, Okanogan, Yakima | 1250 W Alder St<br>Union Gap, WA 98903 | 509-575-2490 |
| **Eastern** | Adams, Asotin, Columbia, Ferry, Franklin, Garfield, Grant, Lincoln, Pend Oreille, Spokane, Stevens, Walla Walla, Whitman | 4601 N Monroe<br>Spokane, WA 99205 | 509-329-3400 |
| **Headquarters** | Across Washington | PO Box 46700<br>Olympia, WA 98504 | 360-407-6000 |

# Toxicological Effects of Antifouling Agents on Non-target Marine Species

**By**

**Ali Mahmoodi**
Graduate Student
Washington State University

**Dr. Xianming Shi**
Professor and Chair of Civil & Environmental Engineering
Washington State University

**Prepared for the**
**Hazardous Waste and Toxics Reduction Program**
Washington State Department of Ecology
Olympia, Washington

**November 2023 | Publication 23-04-066**

# Table of Contents

**List of Figures and Tables** ..... **5**

Figures ..... 5

Tables ..... 6

**Abstract** ..... **7**

**Introduction** ..... **8**

**Methodology** ..... **11**

**Types of Antifouling Agents** ..... **12**

Biocidal ..... 12

Non-Biocidal ..... 15

**Toxicity and Safety of Antifouling Paint Biocides** ..... **17**

DCOIT/Sea-Nine 211 ..... 17

Tralopyril/Econea ..... 22

Zinc Pyrithione ..... 26

Copper Pyrithione ..... 33

Non-Biocidal Antifouling Coatings ..... 41

**Comparative Studies** ..... **43**

**Conclusion** ..... **46**

**References** ..... **48**

**Appendix A. Acronyms and Abbreviations** ..... **57**

# List of Figures and Tables

**Figures**

Figure 1. Chemical structure of (A) DCOIT/Sea-Nine 211, (B) Tralopyril/Econea, (C) zinc pyrithione (ZnPT), and (D) copper pyrithione (CuPT). ..... 15

Figure 2. Mortality rates of embryonic flounder at 48 hours after exposure to different concentrations of Diuron, Irgarol 1051, and DCOIT/Sea-Nine 211. ..... 20

Figure 3. (A) The figure shows typical examples of apoptotic germ cells (indicated by arrows) in the testis of mummichog, *Fundulus heteroclitus,* from the solvent-only control group (b) and the 1.0-µg/L group (c). (B) The graph shows the incidence of apoptotic germ cells in the testis after exposure to various concentrations of DCOIT/Sea-Nine 211. ..... 21

Figure 4. The effects of exposure to Tralopyril on the levels of (A) dopamine (DA), (B) acetylcholine (ACh), as well as (C) acetylcholinesterase (AchE) activity in 168-hour post-fertilization (hpf) larvae. ..... 25

Figure 5. The influence of waterborne zinc pyrithione (ZnPT) exposure at various concentrations (0, 1, 10, and 50 µg/L) on biochemical parameters, A) total protein, B) glucose, and C) albumin in the serum of olive flounder *P. olivaceus*. ..... 30

Figure 6. Impact of zinc pyrithione (ZnPT) exposure at different concentrations (0, 1, 10, and 50 µg/L) on the antioxidant defense system of olive flounder (*P. olivaceus*) over 30 days..... 31

Figure 7. Copper (Cu) concentration in the gill tissues of trout that were treated with copper pyrithione (CuPT) and control groups.. ..... 35

Figure 8. Light micrographs of the gill tissues of trout (A) untreated (control) or (B-D) treated with copper pyrithione (CuPT) at different concentrations of 16, 32, and 64 µg/L..... 36

Figure 9. Transmission electron micrographs of trout gill chloride cells under control conditions and following treatment with 64 µg/L copper pyrithione (CuPT). ..... 37

Figure 10. The mean mortality rate of *L. vannamei* after a 48-hour exposure to various concentrations of copper pyrithione (CuPT). ..... 39

Figure 11. The results of reactive oxygen species (ROS) production (A) and hemocyte apoptotic ratio (B) in *L. vannamei* in response to varying copper pyrithione (CuPT) exposures. ..... 40

**Tables**

Table 1. Half-life of DCOIT/Sea-Nine 211 at different environmental conditions..... 18

Table 2. The toxicological effect of DCOIT on commercial marine species..... 22

Table 3. Half-life of Tralopyril at different environmental conditions. ..... 23

Table 4. The toxicological effect of Tralopyril on commercial sea species..... 26

Table 5. Half-life of zinc pyrithione (ZnPT) at different environmental conditions..... 27

Table 6. The effect of zinc pyrithione (ZnPT) and copper pyrithione (CuPT) on survival rates of embryonic flounder. ..... 32

Table 7. The toxicological effect of zinc pyrithione (ZnPT) on commercial sea species..... 33

Table 8. Half-life of copper pyrithione (CuPT) at different environmental conditions. ..... 35

Table 9. The toxicological effect of copper (Cu) and copper pyrithione (CuPT) on commercial sea species..... 41

Table 10. The toxicological effect of different antifouling biocides on commercial sea species. 45

Table 11. Acronyms, abbreviations, and their meanings. ..... 57

# Abstract

Antifouling boat paints that contain toxic biocides are commonly used to prevent the growth of marine organisms on the hull of boats. However, the release of these biocides into surrounding waters can have adverse environmental impacts on non-target species such as salmon, trout, and shrimp.

This review provides a comprehensive analysis of the toxicological impact of commonly used biocidal agents on marine wildlife, with a focus on commercial marine species. Biocidal agents covered in the review include DCOIT/Sea-Nine 211, Tralopyril/Econea, zinc, and copper-based agents.

This work addresses knowledge gaps by synthesizing the current understanding of long-term effects of biocidal exposure, the mechanisms of toxicity, and the comparative toxicity of different compounds. Understanding the toxicity and safety of biocidal agents and their impact on non-target species is crucial for mitigating their potential environmental impacts and developing more effective and less toxic alternatives.

# Introduction

Antifouling boat paints contain biocides, including organic and metal-based biocides, which prevent the growth of organisms such as barnacles, algae, and marine life on boat hulls, improving performance and reducing maintenance [1]. The European Chemicals Agency estimates the global production of marine antifouling paints to be more than 900 million liters yearly. They project a compound annual growth rate of 5.5% [2].

The release of these biocides into surrounding waters has raised concerns about environmental impacts on marine wildlife, particularly non-target species such as fish, crustaceans, mollusks, zooplankton, and other marine invertebrates [3]. The persistence, bioaccumulation, and biomagnification of these chemicals can lead to long-term effects on marine ecosystems and their inhabitants. Therefore, understanding the toxicity and safety of antifouling paint biocides and their impact on non-target species is crucial for mitigating their potential environmental impacts [4].

There are several types of antifouling agents, including biocidal and non-biocidal options. Copper is the most used biocide in paint. Alternatively, biocidal agents, such as 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT, commercially known as Sea-Nine 211), 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile (Tralopyril, commercially known as Econea), zinc pyrithione (ZnPT), and copper pyrithione (CuPT), are used in antifouling paints to kill or deter marine organisms [5]. Non-biocidal agents, on the other hand, work by creating a slippery surface that makes it difficult for marine organisms to adhere to the surface of the treated structure [6].

In general, biocidal agents have the potential to cause toxicological impacts on marine wildlife, including reduced growth, mortality, developmental abnormalities, and reproductive toxicity [7]. The toxicity of biocidal agents depends on various factors, including the species of the organism, the concentration and duration of exposure, and the mode of action of the biocidal agent [8]. Copper-based biocides, in particular, are highly toxic to a wide range of aquatic organisms and can cause significant damage to marine ecosystems in high concentrations [9]. Therefore, it is important to use biocidal agents responsibly and ensure that their use is properly regulated to minimize their impact on marine wildlife.

Non-target species, such as salmon, are important commercial and recreational fish species widely distributed in both freshwater and marine environments. Nonetheless, the growing

exposure of non-target species to biocidal agents found in antifouling paints, such as copper (cuprous oxide, cupric oxide, copper pyrithione) and zinc pyrithione, has been shown through studies to have detrimental effects on these species, leading to compromised growth, reproduction, survival, altered behavior and physiological processes, reduced growth, increased mortality, as well as the potential for bioaccumulation and biomagnification in the food chain [10, 11]. These effects can have significant ecological consequences and pose risks to human health if contaminated fish are consumed.

Despite growing concerns about the environmental impacts of antifouling paint biocides on marine wildlife, there is a noticeable lack of studies that have investigated the full extent of these impacts. Most toxicity studies of biocidal agents have been conducted over short-term periods, and there is limited information on the long-term effects of exposure to these compounds [12]. Long-term exposure can lead to bioaccumulation and biomagnification of these compounds in the food chain, which can have significant ecological impacts [13–16].

Although the toxic effects of biocidal agents have been well documented, the underlying mechanisms of toxicity are not fully understood. A better understanding of these mechanisms could help in the development of more effective and less toxic biocidal agents. Moreover, the majority of toxicity studies conducted on biocidal agents have primarily emphasized their impacts on target organisms rather than non-target organisms. Additionally, there are very few studies that directly compare the toxicity of different compounds, while there is a significant body of literature on the toxic effects of individual biocidal agents. Comparative studies could provide valuable insights into the mechanisms of toxicity of different compounds and help in the development of more effective and less toxic biocidal agents.

The objective of this review is to provide a comprehensive and up-to-date analysis of the current state of knowledge regarding the toxicological impact of most common biocidal agents, including DCOIT/Sea-Nine 211, Tralopyril/Econea, zinc, and copper-based agents, on marine wildlife, with a focus on valuable species such as salmon. Specifically, this work aims to address the existing knowledge gaps by synthesizing the available literature on the long-term effects of exposure to biocidal agents, the underlying mechanisms of toxicity, the effects of biocidal agents on non-target organisms, and the comparative toxicity of different biocidal compounds. By doing so, this review seeks to provide insights into the ecological impacts of biocidal agents on marine ecosystems and to identify potential strategies for the development of more

effective and less toxic biocidal agents, with a particular emphasis on the protection and conservation of valuable species.

# Methodology

The purpose of this literature review is to investigate the potential ecological impacts of antifouling boat paints that contain toxic chemicals, known as antifouling paint biocides, on marine wildlife, particularly on non-target species. We used the following methodology:

(1) **Selection of review topics:** We selected the review topics based on the research question, which is to investigate the potential ecological impacts of antifouling boat paints on marine wildlife, particularly on non-target species.

(2) **Systematic literature search**: We conducted a systematic search in several databases, including Web of Science, PubMed, and Google Scholar, using the following keywords: antifouling paint, antifouling biocide, marine wildlife, salmon, toxicity, ecological impacts, DCOIT/Sea-Nine, Tralopyril/Econea, zinc pyrithione, copper pyrithione, persistence, bioaccumulation, and biomagnification. We limited the search to articles published after 2019. However, we used earlier publications in some cases.

(3) **Inclusion and exclusion criteria**: We screened the articles based on the relevance of their content to the research question. We only included articles that focused on the ecological impacts of antifouling boat paints on marine wildlife. Articles that did not meet these criteria were excluded.

(4) **Quality assessment**: We assessed the quality of the articles based on the credibility of the source and the methods used in the research. We only included articles that were published in peer-reviewed journals and had a high credibility score.

(5) **Data analysis and synthesis**: We analyzed and synthesized the articles to identify the potential ecological impacts of antifouling boat paints on marine wildlife, particularly on non-target species such as salmon. We categorized the findings based on the type of impact, such as toxicity, bioaccumulation, and ecological disturbance.

# Types of Antifouling Agents

Antifouling agents can be classified into two broad categories:

(1) Biocidal agents; and

(2) Non-biocidal agents.

Biocidal agents are toxic chemicals that kill or inhibit the growth of fouling organisms. These agents are divided into two subcategories: organic biocides and inorganic biocides [17]. Organic biocides refer to carbon-based compounds that exhibit high toxicity to marine organisms, with notable examples including tributyltin (TBT) and organotin compounds like triphenyltin. In contrast, inorganic biocides primarily encompass heavy metals such as copper, which possess toxic properties affecting various marine organisms [18].

Non-biocidal agents are substances that do not kill fouling organisms, but instead prevent them from attaching to the surface. These agents can be divided into two subcategories: foul-release agents and foul-resistant agents [19]. They work by creating a slippery surface or a hydration layer that prevents fouling organisms from attaching. Examples include silicone-based coatings and fluoropolymer coatings.

In our literature review, we have chosen to focus specifically on the biocidal agents that could be used as alternatives to copper oxides. These agents include DCOIT/Sea-Nine 211, Tralopyril/Econea, zinc pyrithione (ZnPT), and copper pyrithione (CuPT). Other less common biocidal agents, such as Irgarol, Diuron, and Dichlofluanid, have been excluded from our review.

## Biocidal

Biocidal agents are compounds that are added to boat paints to prevent the growth of marine organisms, such as algae, barnacles, and mussels, on the hulls of boats. In terms of chemistry, the active ingredient of Sea-Nine 211 is the biocide 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT). Econea, a technical grade of Tralopyril, exhibits a wide range of effectiveness against hard fouling marine organisms due to its broad spectrum of activity. On the other hand, ZnPT and CuPT are organic compounds that incorporate a metal ion (either zinc or copper) bound to a ligand molecule.

## DCOIT/Sea-Nine 211

DCOIT/Sea-Nine 211 is an organic compound that belongs to the family of isothiazolone compounds. In this publication, we will refer to this biocide as DCOIT, as it is the active ingredient of Sea-Nine 211. DCOIT contains a chlorine atom and a n-octyl group attached to a heterocyclic ring (see Figure 1A). It works by inhibiting the enzymes involved in the energy metabolism of microorganisms. Specifically, it targets the electron transport chain and the oxidative phosphorylation process, which are essential for generating adenosine triphosphate (ATP), the main source of energy for cells [20]. By disrupting these processes, DCOIT can kill a wide range of marine organisms, including bacteria, fungi, algae, and invertebrates. However, it can also be toxic to non-target organisms, such as fish and crustaceans, if they are exposed to high concentrations of the biocide [21]. Therefore, it is important to examine it in a controlled manner and to follow appropriate safety and environmental guidelines.

DCOIT is generally considered less toxic than some other biocidal agents, such as copper-based compounds [22]. However, it can still have toxic effects on aquatic organisms if it is released in high concentrations [23]. The toxicity of DCOIT depends on factors such as the concentration, duration, and frequency of exposure, as well as the sensitivity of the organism.

## Tralopyril/Econea

Tralopyril (4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile) is a biocidal agent commonly used in antifouling paints for marine vessels (see Figure 1B). It is commercially known as Econea. In this publication, we will refer to the biocide as Tralopyril.

The compound was developed as an alternative to toxic organotin compounds and other biocides that have been found to have negative environmental impacts [24]. Tralopyril has been found to be effective against a wide range of fouling organisms, including barnacles, algae, and diatoms, while having lower toxicity to non-target species [25].

According to the manufacturer's description, Tralopyril is claimed to have a relatively short hydrolytic half-life of three hours in seawater [7, 25]. However, it is important to approach this information with caution, as no specific experimental details are provided to substantiate this claim.

Tralopyril has been approved for use in antifouling paints in some countries, subject to restrictions and regulations on its use and release into the environment. However, concerns

remain about its potential impacts on non-target organisms and the environment, particularly regarding its persistence and potential for bioaccumulation [26].

## Zinc Pyrithione

Zinc pyrithione (ZnPT) is a coordination complex of zinc and pyrithione, a commonly used biocide in antifouling paints for boats and ships (See Figure 1C). In antifouling paints, ZnPT is typically used as a source of zinc ions, which are released slowly over time to provide long-lasting protection against fouling organisms. The zinc ions act as a biocide by disrupting the metabolic processes of the target organisms [3]. The compound is highly effective against a range of microorganisms, including bacteria and fungi, and is known for its ability to control the growth of fouling organisms on submerged surfaces. ZnPT works by disrupting the cell membranes of the target organisms, leading to cell death [27].

The compound has been extensively studied for its antimicrobial properties, as well as its potential toxicity to non-target organisms and the environment. While ZnPT has been approved for use in antifouling paints, there have been concerns about its potential to bioaccumulate and its persistence in the marine environment [28].

## Copper Pyrithione

Copper pyrithione (CuPT) is an organic compound that consists of a copper ion ($Cu^{2+}$) bonded to a pyrithione ligand (See Figure 1D) [29]. It is commonly used as a biocide in antifouling paints to prevent the growth of marine organisms on boat hulls and submerged surfaces. Copper-based biocides work by releasing copper ions into the surrounding water, which disrupt the metabolic processes of the target organisms and prevent them from attaching to submerged surfaces. While copper-based biocides have been effective in reducing fouling and improving the performance of boats and ships, there have been concerns about their potential toxicity to non-target organisms, particularly in coastal and estuarine environments where copper can accumulate in sediments and impact benthic communities [29–31].

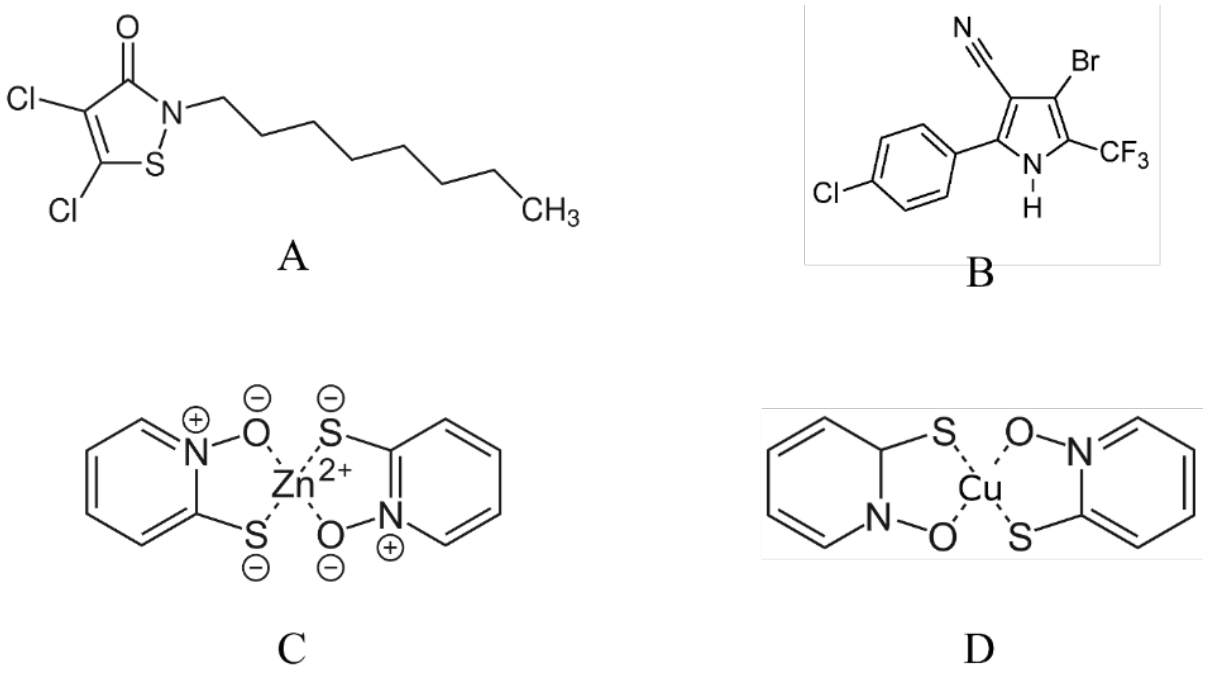



Figure 1. Chemical structure of (A) DCOIT/Sea-Nine 211, (B) Tralopyril/Econea, (C) zinc pyrithione (ZnPT), and (D) copper pyrithione (CuPT).

## Non-Biocidal

Physical antifouling coatings can be classified into fouling release coatings and fouling resistant coatings [18]. Foul-release coatings are designed to facilitate the easy removal of fouling organisms from the coated surface. These coatings work by allowing for weak adhesion between the foulants and the surface. When fouling organisms come into contact with the coated surface, they can still adhere, but the bond is intentionally made weak. As a result, the fouling organisms do not firmly attach to the surface, and they can be easily dislodged with the application of a limited mechanical force. They are typically made of silicone or fluoropolymer materials to prevent fouling attachment. Silicone-based coatings, such as polydimethylsiloxane (PDMS), have good desorption ability, while fluoropolymers offer high chemical stability and water repellency.

On the other hand, foul-resistant coatings are designed to prevent the adhesion of proteins, algae, and/or bacteria to the coated surface altogether. These coatings create a strongly hydrated surface, which forms a physical and free energy barrier that makes it difficult for foulants to adhere. The presence of hydrated layer on the surface acts as a repellant, providing a barrier that discourages fouling organisms from attaching to the surface. Examples include coatings made of polyethylene glycol (PEG), hydrogels, and zwitterionic polymers. PEG coatings

reduce cell adhesion and protein adsorption, while hydrogels feature softness and elasticity that deter organism attachment. Zwitterionic polymers bind tightly to water and exhibit strong resistance to marine bacteria and diatoms. It is important to note that fouling resistance coatings may have limited broad-spectrum antifouling efficacy and susceptibility to coverage by marine silt. Combining multiple antifouling methods is a promising approach to formulating an effective marine antifouling coating [32].

# Toxicity and Safety of Antifouling Paint Biocides

## DCOIT/Sea-Nine 211

### Persistence, bioaccumulation, and biomagnification

Based on the European Chemicals Agency, an antifouling compound is classified as persistent if its half-life ($DT_{50}$) in marine water is more than 60 days [33, 34]. The degradation kinetics of DCOIT vary widely in different seawater samples and depend on environmental factors such as temperature, sunlight, and pH [35]. As shown in Table 1, the half-life of DCOIT has been reported to range from less than 1 to 80 days, depending on various environmental factors [36].

DCOIT has a moderate potential to bioaccumulate in aquatic organisms. The bioconcentration factor (BCF) and the biomagnification factor (BMF) has not extensively studied, but in one study, BCF of DCOIT for fish has been reported to be 14 [37]. It was found that bluegill sunfish exposed to 1.2 µg/L of radiolabeled DCOIT for 28 days showed bioaccumulation of DCOIT in fillet and viscera, with bioconcentration factors ranging from 7 to 200 for fillet and 110 to 1200 for viscera. However, its relatively high sediment-specific equilibrium sorption constants ($K_d$=253) and sediment independent (related to organic carbon) sorption constant ($K_{OC}$=4.2) suggests that this compound has potential to bioaccumulate in animal tissues [36, 37].

The bioaccumulation potential of antifouling compounds is commonly evaluated using the theoretical $LogK_{ow}$ value as an indicator [38, 39]. It is a measure of the lipophilicity or hydrophobicity of a substance, which is a significant factor in its potential for bioaccumulation. It is the logarithm of the octanol-water partition coefficient ($K_{ow}$), which represents the ratio of a compound's concentration in octanol to its concentration in water at equilibrium. Higher $LogK_{ow}$ values indicate that a substance is more hydrophobic and has a higher potential for bioaccumulation. Based on this, a substance with a $LogK_{ow}$ of 3 is considered to be moderately hydrophobic and values higher than 3 means it is potentially bioaccumulative in seawater. The $LogK_{ow}$ value has been reported as 3.59 for DCOIT/Sea-Nine 211 [40].

Table 1. Half-life of DCOIT/Sea-Nine 211 at different environmental conditions.

| Factor | $t_{1/2}$ | Environmental condition | Reference |
|---|---|---|---|
| pH 4 | 6.8 days | DO* 0.5 mg/L; no sunlight; sterile buffer; 25°C | [35] |
| pH 4 | 9 days (pH 5) | sterile buffer | [41] |
| pH 7 | 1.2 days | DO 0.5 mg/L; no sunlight; sterile buffer; 25°C | [35] |
| pH 7 | > 30 days | sterile buffer | [41] |
| pH 9 | 3.7 days | DO 0.5 mg/L; no sunlight; sterile buffer; 25°C | [35] |
| pH 9 | 12 days | sterile buffer | [41] |
| 4°C | > 64 days | DO 0.5 mg/L; no sunlight; sterile seawater | [35] |
| 25°C | 27.9 days | DO 0.5 mg/L; no sunlight; sterile seawater | [35] |
| 25°C | 24.8 days | DO 8.1 mg/L; no sunlight; sterile seawater | [35] |
| 40°C | 4.5 days | DO 0.5 mg/L; no sunlight; sterile seawater | [35] |
| Photolysis | 6.8 days | DO 0.5 mg/L; sterile seawater | [35] |
| Photolysis | 13.4 days | aqueous solution (pH 7.0) | [41] |
| Dark | 14.4 days | DO 0.5 mg/L; sterile seawater | [35] |
| Dark | 79.7 days | aqueous solution (pH 7.0) | [41] |
| Biodegradation | > 4 days | natural seawater without sunlight at 25°C | [35] |
| Biodegradation | 10 days | natural seawater without sunlight at 30°C | [42] |
| Biodegradation | < 1 day | natural seawater | [43] |
| Biodegradation | 1.9 days | natural seawater with light at 15°C | [44] |
| Biodegradation | 13.1 days | natural seawater under sunlight | [45] |
| Biodegradation | 6.4 days | natural river water under sunlight | [45] |
| Biodegradation | 5.5 days | natural lake water under sunlight | [45] |

* Note: DO stands for dissolved oxygen.

## Toxicity

DCOIT is known to have toxic effects on marine organisms, including fish, invertebrates, and algae. The toxic effects of DCOIT depend on various factors, such as exposure concentration, exposure duration, and organism sensitivity. It has been reported that chronic exposure of marine medaka to 3.3 µg/L DCOIT leads to oxidative stress in the liver, inhibition of neurotransmitters in the brain, and impaired reproductive function [46]. DCOIT also acts as an

estrogen mimicker and interferes with the endocrine system of adult medaka. In fish brain, DCOIT can competitively bind to Gα proteins and inhibit protein activation by substrates, and mitogen-activated protein kinase signaling pathways play an important role in DCOIT toxicity [47].

In crustaceans, DCOIT has been shown to induce death and inhibit egg production in species such as *Acartia tonsa, Balanus 19mphitrite, Tigriopus japonicas, Mysidopsis bahia,* shrimps*, Uca pugilator,* and *Portunus trituberculatus* [37, 48, 49]. Specifically, DCOIT induced the death of *A. tonsa* at an $LC_{50}$ of 57 nmol/L (16.1 µg/L) and inhibited egg production at an $EC_{50}$ of 72 nmol/L (20.3 µg/L) within 48 hours [50].

The toxicological effects of antifouling agents on commercial species such as fish and shrimp are particularly important due to the potential impact on human health and the economy. Fish and shrimp are important sources of protein for human consumption. They are also important for the global economy, providing significant revenues through fishing and aquaculture industries.

DCOIT has chronic toxicity to Pacific white shrimp, and studies have shown that exposure to 30 µg/L of DCOIT significantly reduces the survival and weight gain of shrimp [51]. It also induced changes in hepatopancreatic morphology and metabolism, including high anaerobic respiration and the accumulation of triglycerides. Exposure to 15 µg/L or 30 µg/L DCOIT led to high Na+/K+-ATPase activity and melanin deposition in the gills. Shrimp exposed to 15 µg/L DCOIT showed more differentially expressed genes than those in the control, and these genes were involved in biological processes such as starch and sucrose metabolism and choline metabolism in cancer. The findings suggested that DCOIT can interfere with shrimp metabolism, growth, and survival at concentrations as low as 30 µg/L and can induce altered gene expression at a concentration of 15 µg/L.

Another study investigated the adverse effects of three commercial alternative biocides, Diuron, Irgarol 1051, and DCOIT/Sea-Nine 211, on non-target pelagic fish (flounder) embryos [52]. As shown in Figure 2, the mortality rate was highest in embryos exposed to DCOIT/Sea-Nine 211, ranging from 18% to 100%. However, there were no significant differences in mortality rates between the exposure and control groups for embryos exposed to Irgarol 1051 and Diuron.

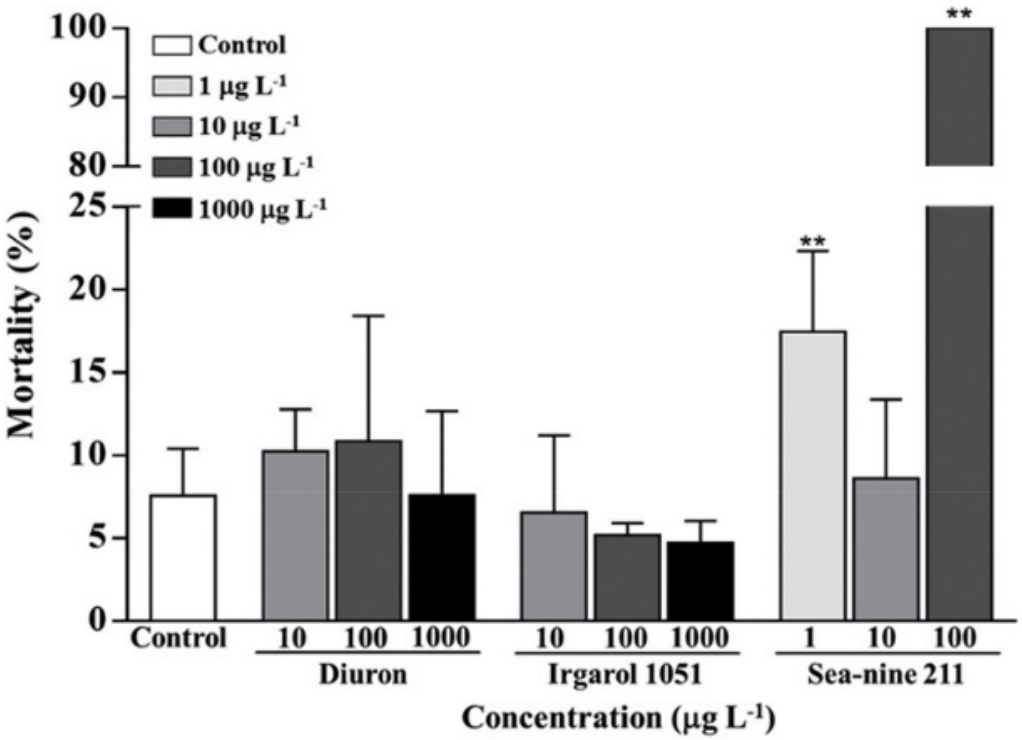



Figure 2. Mortality rates of embryonic flounder at 48 hours after exposure to different concentrations of Diuron, Irgarol 1051, and DCOIT/Sea-Nine 211. The control group consisted of embryonic flounder exposed to dimethyl sulfoxide (DMSO) [52]. Figure permissions by Elsevier.

Additionally, the effects of these biocides on developmental malformation and transcriptional changes were analyzed. It was found that all three biocides produced similar developmental malformations, such as tail-fin fold defects and dorsal body axis curvature. However, the potencies of the biocides differed, with DCOIT/Sea-Nine 211 being the most toxic followed by Irgarol 1051 and then Diuron. Consistent with this trend, genes related to heart formation were more highly expressed in embryos exposed to DCOIT/Sea-Nine 211, while genes related to fin malformation were more highly expressed in embryos exposed to Irgarol 1051.

A recent study investigated the testicular toxicity of DCOIT/Sea-Nine 211 in the mummichog (*Fundulus heteroclitus*) after a 28-day exposure period [53]. As can be seen in Figure 3, the incidence of apoptotic cells was found to be higher in the groups exposed to 1.0 and 3.0 μg/L of DCOIT/Sea-Nine 211 compared to the control groups. Also, the results showed a reduction in the signal intensity of anti-apoptotic protein Bcl-xL in a dose-dependent manner.

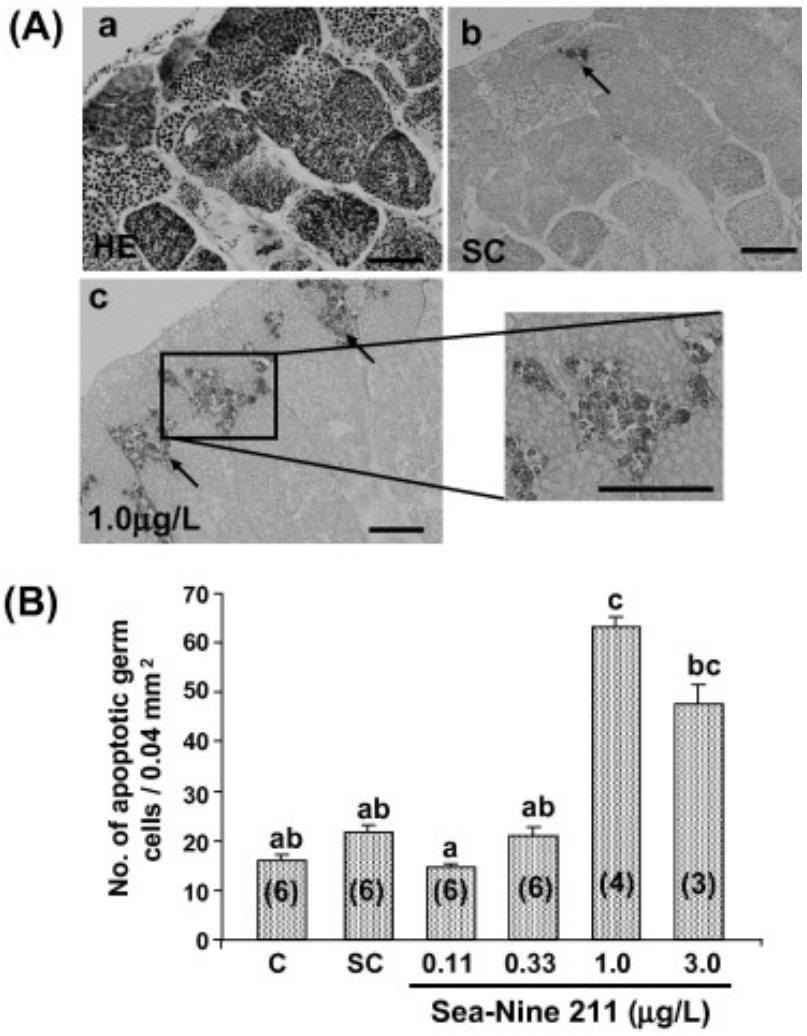



Figure 3.
(A) The figure shows typical examples of apoptotic germ cells (indicated by arrows) in the testis of mummichog, *Fundulus heteroclitus,* from the solvent-only control group (b) and the 1.0-µg/L group (c). A semi-adjacent section of b stained with hematoxylin-eosin is shown in (a). The control group shows no apoptotic germ cells, while the 1.0-µg/L group has a higher number of apoptotic germ cells. The scale bar is 50 µm. Figure permissions by Elsevier.
(B) The graph shows the incidence of apoptotic germ cells in the testis after exposure to various concentrations of DCOIT/Sea-Nine 211. The number of apoptotic germ cells increased in a dose-dependent manner, with significant differences between the 1.0- and 3.0-µg/L groups and the control group [53]. Figure permissions by Elsevier.

According to Shade et al., an $LC_{50}$ value of 14 µg/L was observed after seven days exposure of juvenile rainbow trout to DCOIT/Sea-Nine 211, indicating adverse effects on this species [54]. In another study, the effects of 2.5 µg/L DCOIT on marine medaka (*Oryzias melastigma*) were investigated over a 28-day exposure period [55]. Results indicated alterations in hepatic oxidative stress, neurotransmission, and sex hormone homeostasis in the exposed fish. The

toxic effect of DCOIT/Sea-Nine 211 on other commercial species such as Bivalvia, tropical oyster, and brown mussel has been also reported (see Table 2) [56–58].

Table 2. The toxicological effect of DCOIT on commercial marine species.

| Species | Exposure time | Concentration | Toxicological effect | Reference |
|---|---|---|---|---|
| Pacific white shrimp | 28 days | 30 µg/L | $LC_{50}$ | [51] |
| Flounder | 48 h | 1 to 100 µg/L | 18 to 100% of mortality | [52] |
| Killifish | 28 days | 0.1 to 3 µg/L | increased the number of apoptotic spermatocytes, a reduction in the signal intensity of anti-apoptotic protein Bcl-xL | [53] |
| Rainbow trout | 7 days | 0.014 µg/L | $LC_{50}$ | [54] |
| Medaka | 28 days | 2.5 µg/L | alterations in hepatic oxidative stress, neurotransmission, and sex hormone homeostasis | [55] |
| Bivalvia | 96 h | 130 µg/L | decreased the GST, SOD, CAT activity | [56] |
| Tropical oyster | 120 h | 0.2 to 151 µg/L | triggered oxidative stress, damage of membrane lipids | [57] |
| Brown mussel | 24 h and 96 h | 10 µg/L | reduced the activity of the antioxidant defense system | [58] |

Note: CAT stands for catalase, GST stands for glutathione-S-transferase, and SOD stands for superoxide dismutase. They are all enzymes that play important roles in protecting cells from oxidative stress caused by reactive oxygen species (ROS).

# Tralopyril/Econea

## Persistence, bioaccumulation and biomagnification

The available information on assessing the half-life for Tralopyril is limited. This means that there is a lack of comprehensive data or studies specifically focused on determining the exact duration of Tralopyril's half-life in environmental conditions, particularly in the marine environment. In this review, we have listed only the information that is currently available.

The half-life of Tralopyril as a measure of its persistency at different environmental conditions is listed in Table 3. The half-life of Tralopyril at 18 °C in seawater, river water, and E3 medium (control medium for toxicity test) has been calculated to be 6.1 hours, 8.1 hours, and 7.4 hours, respectively. This chemical compound can decompose quickly in seawater; however, it can also quickly accumulate in marine organisms due to its high bioconcentration potential, with the value of $LogK_{ow}$ equal to 4.69 [26].

A parallel trend was observed in a separate investigation conducted by the Danish Environmental Protection Agency. This study revealed that Tralopyril can undergo complete hydrolysis within 48 hours when exposed to deionized water, with the reaction exhibiting even greater rate in seawater. Consequently, the transformation products resulting from this process were identified as 3-bromo-5-(4-chlorophenyl)-4-cyano-1H-pyrrole-2-carboxylic acid (BCCPCA). However, the researchers also found that BCCPCA demonstrated remarkable stability to biodegradation due to its notably high $K_{ow}$ value and extended half-life. This stability raises potential concerns over its persistence in the environment, warranting further investigations into the long-term ecological impact of this compound. Additional studies may be required to evaluate the potential risks associated with the presence of BCCPCA and its effects on ecosystems and human health [59].

Table 3. Half-life of Tralopyril at different environmental conditions.

| Factor | t1/2 | Environmental condition | Reference |
|---|---|---|---|
| pH 7.8 | 6.1 hours | seawater, 18 °C | [60] |
| pH 8.1 | 8.1 hours | river water, 18 °C | [60] |
| 17 °C | 7.4 hours | artificial seawater | [61] |
| 20 °C | 4 hours | artificial seawater | [61] |

## Toxicity

A recent study aimed to evaluate the toxicological effects of short-term exposure to Tralopyril on turbot (*Scophthalmus maximus*) [62]. Turbot were exposed to Tralopyril at concentrations of 5 µg/L, 15 µg/L, and 30 µg/L for 7 days. The results showed that Tralopyril induced oxidative stress and affected energy metabolism in turbot, as evidenced by a decrease in superoxide dismutase activity and an increase in Ca2+ -Mg2+ -ATPase activity in the gills.

Tralopyril also disrupted the thyroid endocrine system, inducing hyperthyroidism and upregulating the expression of hypothalamus–pituitary–thyroid axis-related genes. The

integrated biomarker response index showed that 15 μg/L Tralopyril had the greatest effect on turbot.

Additionally, the main reason for induced oxidative stress was that Tralopyril significantly inhibited the activity of superoxide dismutase (SOD), leading to an imbalance in intracellular reactive oxygen species (ROS) levels and affecting the activity of catalase (CAT). However, glutathione (GSH) and malondialdehyde (MDA) content were not significantly affected by Tralopyril exposure.

Another study evaluated the effect of Tralopyril on the locomotor activity of zebrafish larvae and its related mechanisms [63]. The study found that Tralopyril significantly reduced locomotor activity of zebrafish larvae after 168-hour exposure. It also caused adverse modifications in tail muscle tissue, the nervous system, and energy metabolism in larvae. Furthermore, the change in metabolites involved in carbohydrate and lipid metabolism indicates that Tralopyril may disrupt energy metabolism. The study also found that Tralopyril may disrupt the nervous system in zebrafish larvae by causing changes in dopamine (DA), acetylcholine (ACh), and acetylcholinesterase (aChE) activity, and the expression of genes involved in neurodevelopment. As shown in Figure 4, exposure to Tralopyril at 2.0 μg/L led to significantly decreased DA, Ach and aChE activities in zebrafish larvae.

The effects of Tralopyril exposure on adult pacific oysters were investigated [64]. It was found that mantle mucus secretion coverage ratio was increased in a dose-dependent pattern by Tralopyril, and the antioxidant defense systems, digestive enzyme, and biomineralization capacity in oysters were also affected. Moreover, the mRNA expression levels of biomineralization related genes were disrupted by Tralopyril exposure. It was suggested by the study that pacific oysters could be severely damaged by Tralopyril exposure, and these findings provided new insights for understanding the toxicity of Tralopyril in marine mollusks. The toxicological effect of Tralopyril on various marine species are listed in Table 4.

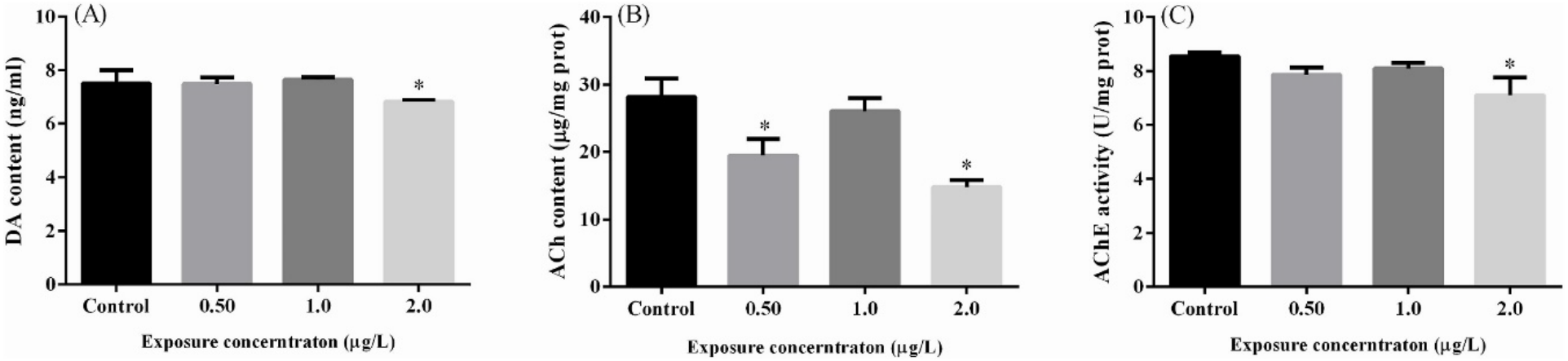



Figure 4. The effects of exposure to Tralopyril on the levels of (A) dopamine (DA), (B) acetylcholine (ACh), as well as (C) acetylcholinesterase (AchE) activity in 168-hour post-fertilization (hpf) larvae [63]. Correction: X-axis label should read “Exposure concentration.” Figure permissions by Elsevier.

Table 4. The toxicological effect of Tralopyril on commercial sea species.

| Species | Exposure time | Concentration | Toxicological effect | Reference |
|---|---|---|---|---|
| Turbot | 7 days | 15 µg/L | oxidative stress, affected energy metabolism, and disrupted the thyroid endocrine system | [62] |
| Zebrafish | 168 h | 0.5 to 2 µg/L | inhibition of locomotor activity, impairment of tail muscle tissue, disruption of the nervous system, and interference with carbohydrate and lipid metabolism. | [63] |
| Pacific oyster | 6 days | Up to 160 µg/L | increased mantle mucus secretion and affected oysters' antioxidant defense systems, digestive enzyme, and biomineralization capacity | [64] |
| Pacific oyster | 48 h | 2409 µg/L | $LC_{50}$ | [64] |
| Pacific oyster | 72 h | 1702 µg/L | $LC_{50}$ | [64] |
| Pacific oyster | 96 h | 911 µg/L | $LC_{50}$ | [64] |

# Zinc Pyrithione

## Persistence, bioaccumulation and biomagnification

The persistency of zinc pyrithione (ZnPT) is influenced by environmental conditions such as temperature, pH, and sunlight, as well as its solubility in water. Higher temperatures, extreme pH levels, and sunlight can impact its stability and degradation. ZnPT's limited solubility allows it to settle on the seafloor or adhere to suspended particles, reducing its availability.

A study was conducted to examine the degradation of ZnPT and CuPT in seawater [65]. The researchers monitored the reduction in toxicity resulting from degradation over a two-day period using a bioassay. The bioassay utilized natural assemblages of coastal marine bacteria collected from Roskilde Fjord in Denmark. To investigate photodegradation of the compounds, bacteria were exposed to sterile ZnPT- and CuPT-dilution that had either been exposed to sunlight or darkness. Biodegradation was examined by diluting ZnPT and CuPT in sterile

seawater or natural seawater. The photodegradation half-life for ZnPT was estimated to be 8.3 ± 0.9 min, and for CuPT, it was estimated to be 7.1 ± 0.2 min. It was observed that total and microbial degradation, in combination with photodegradation, did not further shorten the degradation time, suggesting no biodegradation. It was also found that biodegradation without the influence of sunlight was negligible over the time-period investigated.

As shown in Table 5, it is widely agreed upon by all reported findings that the fate and distribution of ZnPT in the environment is directly affected by sunlight penetration in seawater [27, 66]. When direct sunlight can penetrate a deeper depth in clear water with low turbidity and calm conditions, ZnPT can easily undergo photodegradation. However, in moderately turbid coastal water, where a depth of 1-2m is sufficient to remove or weaken UV light, ZnPT tends to accumulate in seawater or sediment.

Table 5. Half-life of zinc pyrithione (ZnPT) at different environmental conditions.

| Factor | $t_{1/2}$ | Environmental condition | Reference |
|---|---|---|---|
| Dark | > 48 h | sterile seawater | [27] |
| Sunlight | 7–8 min | sterile seawater | [27] |
| Dark | 8.3 ± 0.9 min | artificial seawater | [65] |
| Sunlight | 210 min | artificial seawater | [65] |
| Dark | 90 d | abiotic artificial seawater | [66] |
| Dark | 4 d | biotic natural seawater | [66] |
| Dark | 7-8 h | biotic river/pond water | [66] |
| Sunlight | < 2 min | abiotic artificial seawater | [66] |

## Toxicity

The toxicity effect of zinc pyrithione (ZnPT) on the suspension-cultured fish cell line CHSE-sp derived from chinook salmon and juvenile rainbow trout was found to be significant, as determined by the in vitro and in vivo toxicity tests [22]. The in vitro acute toxicity test showed that zinc pyrithione had a high toxicity level to the fish cells (24-h $EC_{50}$: 180 μg/L). After exposure for 28 days to the highest concentration used (3000 μg/L), it was observed that the control compound, surfactant sodium n-dodecyl sulphate, did not reach 50% lethality (28-day $LC_{50}$) for juvenile rainbow trout, while for ZnPT, the $LC_{50}$ value was found to be 4.6 μg/L. These results suggest that zinc pyrithione can pose a potential risk to the survival and health of chinook salmon and juvenile rainbow trout.

The effects of different concentrations (0, 1, 10, and 50 µg/L) of ZnPT on the blood of the olive flounder over a 30-day period was investigated in another study [67]. The results revealed that exposure to higher concentrations of ZnPT led to reduced immune function, increased stress, and changes in liver enzymes. The fish exposed to 10 and/or 50 µg/L of ZnPT for 20 days exhibited a reduction in alternative complement activity and lysozyme activity, as well as a decrease in total Ig levels. Furthermore, it caused a decrease in red and white blood cells, as well as in total protein and albumin concentrations, as shown in Figure 5. On the other hand, higher concentrations of ZnPT induced lipid peroxidation resulted in an increased antioxidant response, with intracellular glutathione levels significantly increasing in response to ZnPT exposure, as shown in Figure 6. The findings suggest immunotoxicity of ZnPT and changes in hematological homeostasis in olive flounder blood.

In another study, the toxic effects of CuPT and ZnPT on embryonic olive flounder (*Paralichthys olivaceus*) were compared based on developmental morphogenesis and transcriptional variation [68]. As shown in Table 6, the survival rates of embryonic flounder were found to be ≥ 95% in the negative control and DMSO control (0.1% DMSO). A statistically significant pyrithione-concentration-dependent pattern was observed in the mortality of embryos, with mortality reaching 100% in those exposed to 1000 µg/L CuPT. Although the mortality of embryos exposed to 1000 µg/L ZnPT was 75 ± 35%, no concentration-dependent pattern of embryo mortality was observed among the three exposure groups. The toxic potency of CuPT was greater with respect to developmental malformation and mortality than that of ZnPT. Higher expression levels of genes related to tail fin malformation were observed in embryonic flounder exposed to CuPT compared to those exposed to ZnPT.

Moreover, the genes related to muscle and nervous system development also exhibited significant changes in differential gene expression profiles using RNA sequencing (cutoff value $P < 0.05$). Additionally, cellular respiration and kidney development were affected in embryos exposed to CuPT, as revealed by gene ontology analysis, while the genes associated with cell development, nervous system development, and heart development showed significant variation in embryonic flounder exposed to ZnPT. The results suggested the common and unique developmental toxic effects of CuPT and ZnPT on embryonic flounder through transcriptomic analyses.

The median lethal concentrations ($LC_{50}$) of CuPT and ZnPT antifoulants were evaluated on red sea bream and toy shrimp [69]. The $LC_{50}$ values, based on actual concentrations, were 9.3 and

98.2 µg/L for CuPT and ZnPT, respectively, in red sea bream and 2.5 and 120 µg/L, respectively, in toy shrimp. The gill filaments of the experimental fish were severely damaged after exposure, indicating fatal hypoxemia as a cause of death. The joint toxicity of the pyrithiones with copper (Cu) was also estimated by combining the $LC_{50}$ values of the pyrithiones and copper. The $LC_{50}$ values for copper were 84.4 and 113 µg/L for red sea bream and toy shrimp, respectively.

The toxicity of ZnPT–Cu mixtures was higher than the additive toxicity of the two compounds separately. Even at ZnPT concentrations lower than its $LC_{50}$ value, the addition of copper caused significant mortality (approximately 50%) in both red sea bream and toy shrimp. This suggests that copper enhances the toxicity of ZnPT. It is likely that copper facilitates the conversion of ZnPT to the more toxic CuPT, which significantly contributes to the increased toxicity observed in the presence of copper for both organisms. These findings underscore the importance of considering the synergistic toxicological effects when assessing the environmental impact of chemical mixtures. The toxic effect of ZnPT was also observed for other non-target species such as brine shrimp, prussian carp, mediterranean mussels, bivalvia, eastern mosquitofish, and Asian clam, as listed in Table 7 [70–75].

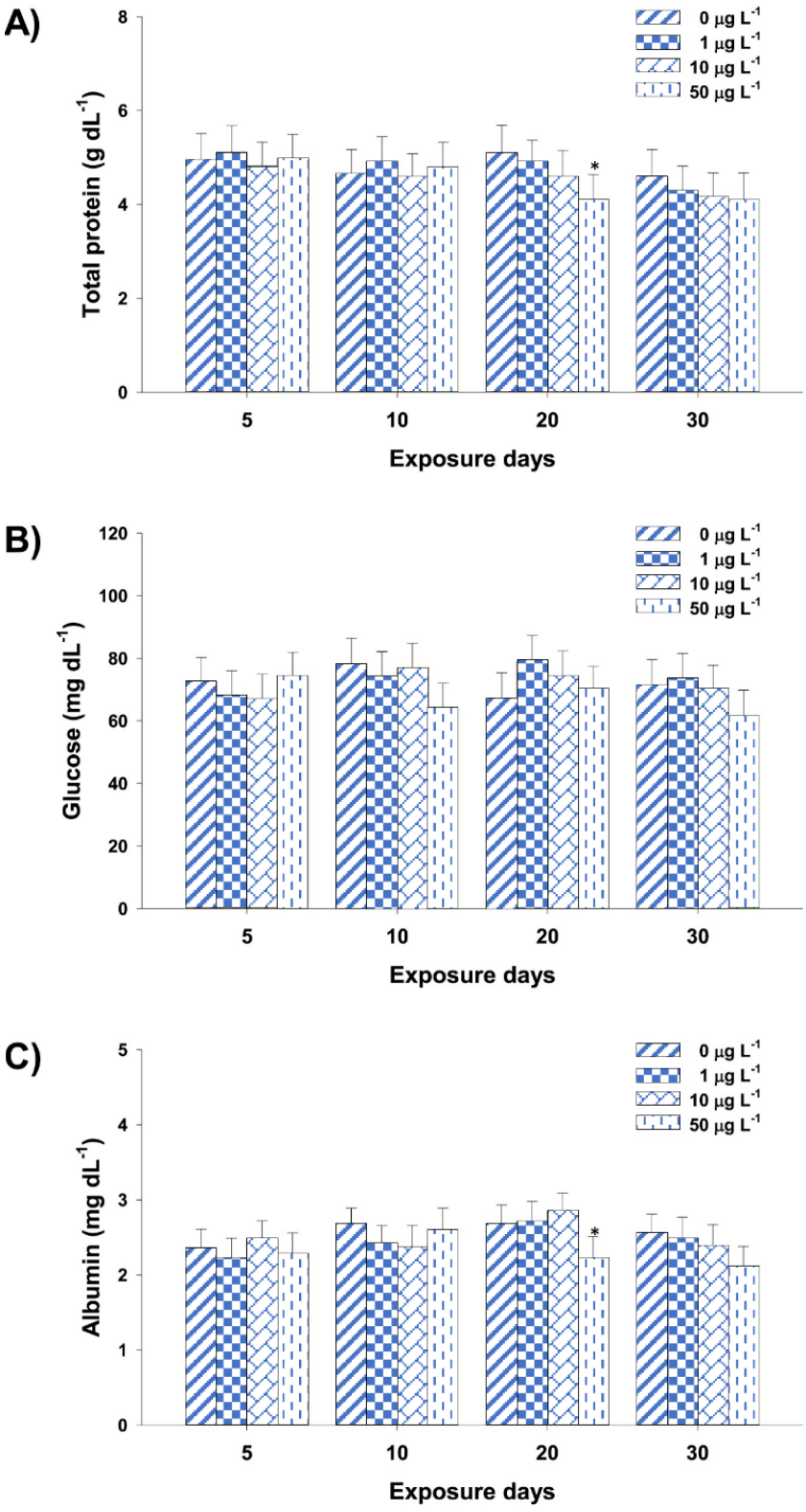



Figure 5. The influence of waterborne zinc pyrithione (ZnPT) exposure at various concentrations (0, 1, 10, and 50 µg/L) on biochemical parameters, A) total protein, B) glucose, and C) albumin

in the serum of olive flounder *P. olivaceus* [67]. Figure permissions by Elsevier; modified for accessibility.

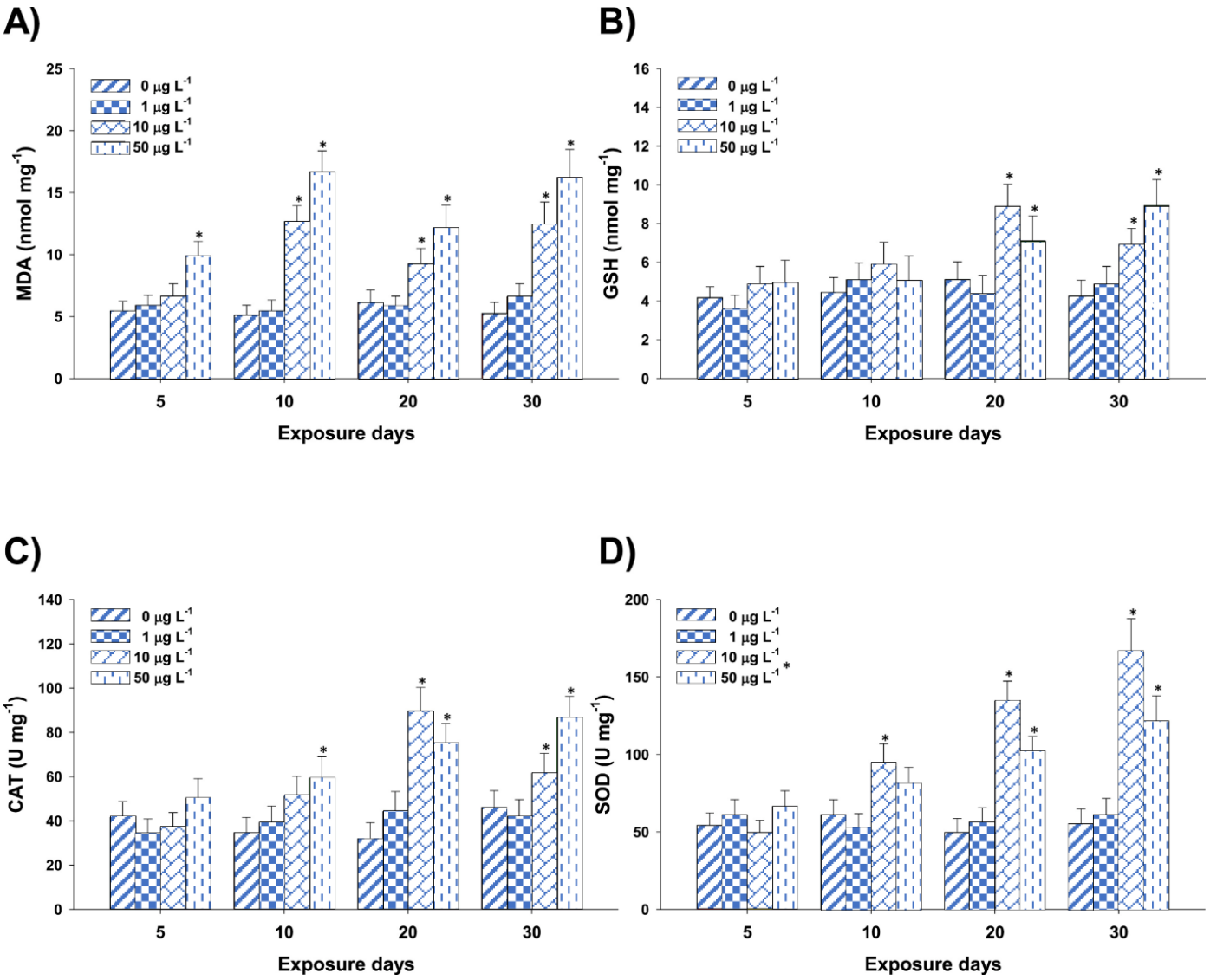



Figure 6. Impact of zinc pyrithione (ZnPT) exposure at different concentrations (0, 1, 10, and 50 µg/L) on the antioxidant defense system of olive flounder (*P. olivaceus*) over 30 days. The antioxidant defense system was evaluated by measuring four parameters: A) malondialdehyde (MDA); B) glutathione (GSH); C) catalase (CAT); and D) superoxide dismutase (SOD) in the fish's serum [67]. Figure permissions by Elsevier; modified for accessibility.

Table 6. The effect of zinc pyrithione (ZnPT) and copper pyrithione (CuPT) on survival rates of embryonic flounder [68].

| Exposure condition | Mortality (mean ± SD) | Pericardial edema (mean ± SD) | Dorsal curvature (mean ± SD) | Caudal finfold defect (mean ± SD) |
|---|---|---|---|---|
| Negative Control | 5 ± 1 | 5 ± 6 | 5 ± 5 | 26 ± 7 |
| Control (DMSO) | 6 ± 0 | 15 ± 4 | 12 ± 2 | 45 ± 16 |
| CuPT at 10 µg/L | 4 ± 1 | 14 ± 9 | 21 ± 13 | 56 ± 8 |
| CuPT at 100 µg/L | 68 ± 22** | 90 ± 14** | 88 ± 17** | 90 ± 14** |
| CuPT at 1000 µg/L | 100** | All died | All died | All died |
| ZnPT at 10 µg/L | 4 ± 3 | 2 ± 4 | 4 ± 4 | 39 ± 12 |
| ZnPT at 100 µg/L | 2 ± 2 | 26 ± 24 | 16 ± 13 | 17 ± 19 |
| ZnPT at 1000 µg/L | 75 ± 35** | 100** | 100** | 100** |

*Cutoff value $P < 0.05$
** Cutoff value $P < 0.01$
SD is standard deviation.

Table 7. The toxicological effect of zinc pyrithione (ZnPT) on commercial sea species.

| Species | Exposure time | Concentration | Toxicological effect | Reference |
|---|---|---|---|---|
| Chinook salmon cell | 24 h | 180 µg/L | $EC_{50}$ | [22] |
| Rainbow trout | 7 d | 8.4 µg/L | $LC_{50}$ | [22] |
| Rainbow trout | 28 d | 4.6 µg/L | $LC_{50}$ | [22] |
| Flounder | 30 days | 0 to 50 µg/L | reduced immune function, increased stress, and changes in liver enzymes, decrease in red and white blood cells, alterations in the antioxidant defense system | [67] |
| Flounder | 48 h | 512.86 µg/L | $LC_{50}$ | [68] |
| Red sea bream | 96 h | 98.2 µg/L | $LC_{50}$ | [69] |
| Toy shrimp | 96 h | 120 µg/L | $LC_{50}$ | [69] |
| Brine shrimp | 48 h | 1370 µg/L | $LC_{50}$ | [70] |
| Prussian carp | 96 h | 163 µg/L | $LC_{50}$ in freshwater | [71] |
| Prussian carp | 96 h | 126 µg/L | $LC_{50}$ in water with 1.5 % salinity | [71] |
| Prussian carp | 96 h | 113 µg/L | $LC_{50}$ in water with 3 % salinity | [71] |
| Zebrafish | 96 h | 0.073 µM | $LC_{50}$ | [72] |
| Mediterranean mussels | 96 h | 20 and 40 µg/L | Increasing in SOD*, decreasing in GSH† | [73] |
| Bivalvia | 7 days | 10 µg/L | decreasing in GSH and MDA‡, hemocytic infiltration | [74] |
| Asian clam | 96 h | 2170 µg/L | $LC_{50}$ | [75] |

* SOD stands for superoxide dismutase.

† GSH stands for glutathione.

‡ MDA stands for malondialdehyde.

# Copper Pyrithione

## Persistence, bioaccumulation and biomagnification

The environmental fate of CuPT in seawater is an important concern, as it can potentially harm marine organisms and ecosystems. The half-life of CuPT in seawater has been investigated

under different conditions, including sunlight exposure and absence of sunlight [65, 66]. Under sunlight exposure, the half-life of CuPT was found to be 7.1 minutes in both sterile and natural seawater [65]. This indicates that the presence of sunlight greatly speeds up the degradation of CuPT in seawater. In the absence of sunlight, CuPT has longer half-lives: 37 hours in natural seawater, 12.9 days in abiotic artificial seawater, and 4 days in biotic natural seawater (Table 8) [65, 66]. Other factors such as the presence of microorganisms or the composition of the seawater can also affect the rate of degradation.

In general, the half-life values of CuPT in seawater suggest that it is a relatively unstable compound that can rapidly degrade under certain conditions, particularly in the presence of sunlight. However, the longer half-life values observed in the absence of sunlight indicate that CuPT may persist in seawater for a significant amount of time under certain conditions, potentially leading to environmental accumulation and adverse effects on marine organisms. Further research is needed to fully understand the fate and behavior of CuPT in seawater and to develop effective strategies to mitigate its environmental impact.

The accumulation of copper in gill tissues following exposure to CuPT at various concentrations was investigated in a recent study using ICP-AAS analysis [76, 77]. The researchers found that significant increases in copper concentration were observed in the gill tissues after a 2-hour treatment with doses of 16-64 μg/L of CuPT when compared to control groups (Figure 7). Results from light microscopy showed the formation of club-shaped lamella, edema, fusion of secondary lamella, loss of micro-ridge structures, and epithelial exfoliation (Figure 8). Transmission electron microscopy (TEM) analysis also revealed changes in the morphology of chloride cells, such as the swollen appearance of mitochondria, internal cristae disruption, and lipid membrane disruption (Figure 9). These findings suggest that exposure to CuPT at these concentrations can lead to the accumulation of copper in gill tissues, potentially posing a risk to aquatic organisms.

A recent study conducted by Hobbs et al. [78] addresses the accumulation of copper inside marinas and its contribution to elevated concentrations of copper in marine waters. Marinas have been found to be a significant source of copper, mainly originating from antifouling paints used to prevent biofouling of boat hulls. With legislation being developed to regulate copper diffusion from antifouling paints in Washington State, USA, this study provides baseline data for copper concentrations in five marinas of varying sizes and configurations within Puget Sound, a large fjord estuary.

Over a year, multiple environmental media samples were collected, revealing that copper accumulates at higher concentrations inside marinas than outside, with more enclosed marinas accumulating more copper than more open marinas. The authors also employed a power analysis to evaluate the suitability of the baseline dataset for measuring future progress towards reducing copper levels in Puget Sound from marinas. Overall, this study serves as an important baseline for future research in assessing the effectiveness of copper reduction legislation in the marine environment.

Table 8. Half-life of copper pyrithione (CuPT) at different environmental conditions.

| Factor | t1/2 | Environmental condition | Reference |
|---|---|---|---|
| Sunlight | 7.1 min | sterile seawater | [65] |
| Sunlight | 7.1 min | natural seawater | [65] |
| Dark | 37 h | natural seawater | [65] |
| Dark | 12.9 d | abiotic artificial seawater | [66] |
| Dark | 4 d | biotic natural seawater | [66] |

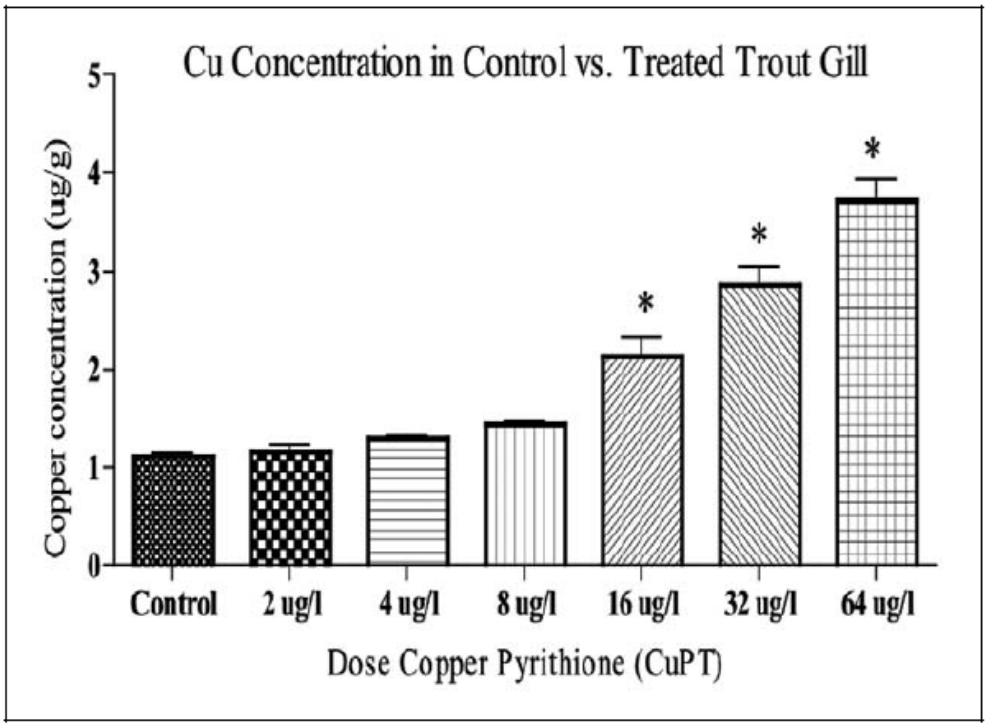



Figure 7. Copper (Cu) concentration in the gill tissues of trout that were treated with copper pyrithione (CuPT) and control groups. The figure shows that there was a significant increase in copper concentration in the gill tissues at CuPT treatment doses of 16 µg/L, 32 µg/L, and 64 µg/L as compared to controls [76]. Figure courtesy of L.D. Trombetta.

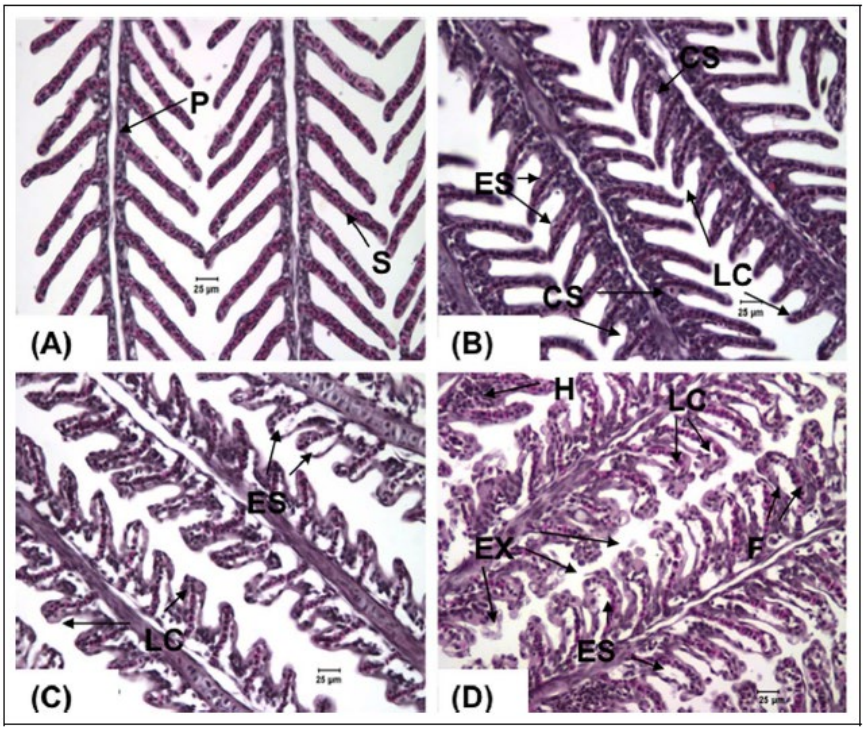



Figure 8. Light micrographs of the gill tissues of trout (A) untreated (control) or (B-D) treated with copper pyrithione (CuPT) at different concentrations of 16, 32, and 64 µg/L. In the control group, primary and secondary lamellae were clearly visible. However, the micrographs for the treated tissues demonstrated various abnormal features, including swelling of chloride cells (CS), exfoliation of epithelial cells (EX), epithelial swelling (ES), lamellar clubbing (LC), and fusion of secondary lamellae (F). These changes were observed at CuPT treatment doses of 16, 32, and 64 µg/L. The magnification used for the images was 20 times [76]. Figure courtesy of L.D. Trombetta.

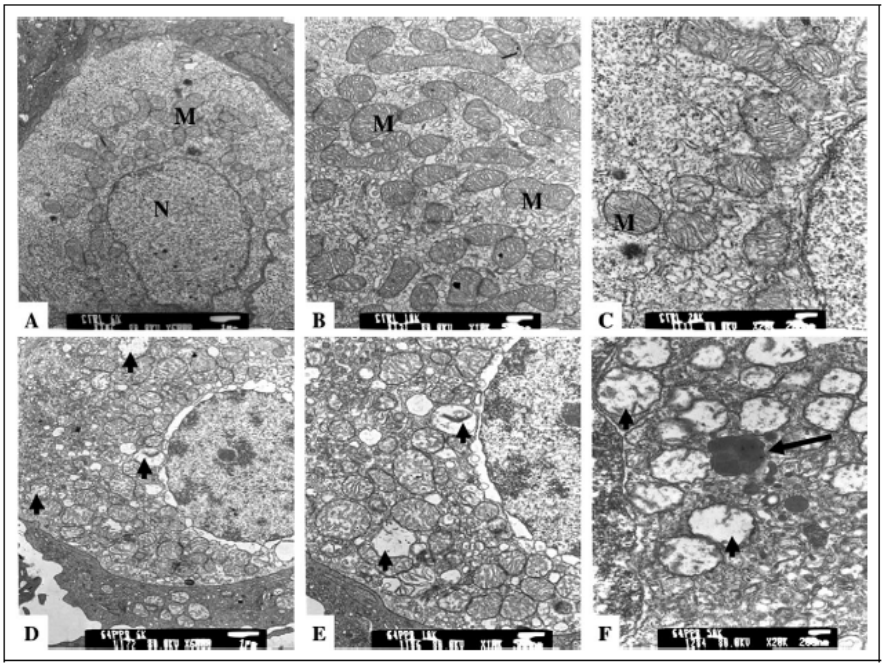



Figure 9. Transmission electron micrographs of trout gill chloride cells under control conditions and following treatment with 64 μg/L copper pyrithione (CuPT). In the control group (A-C), the chloride cells have a normal ultrastructure, with well-defined cristae and membranes in numerous mitochondria within the cytoplasm. However, in the 64 μg/L CuPT-treated group (D-F), the images reveal swollen mitochondria with loss of internal membrane structure (arrowheads), and widespread vacuolization. Moreover, there are signs of lipid accumulation (arrow). These findings provide insights into the potential mechanisms underlying the toxic effects of CuPT on gill tissues, particularly on chloride cells. Further research is needed to better understand these mechanisms and to develop effective strategies for mitigating the potential environmental impact of CuPT. The magnification range for the images is 6000-20000 [76]. Figure courtesy of L.D. Trombetta.

## Toxicity

According to in vitro and in vivo toxicity tests, the toxicity effect of CuPT on the suspension-cultured fish cell line CHSE-sp, derived from chinook salmon and juvenile rainbow trout, was significant [22]. The acute toxicity test conducted in vitro indicated that CuPT exhibited a high level of toxicity to the fish cells, with a 24-hour $EC_{50}$ of 100 μg/L. Furthermore, after a 28-day exposure to the highest concentration used (3000 μg/L), it was observed that surfactant sodium n-dodecyl sulphate (SDS), which was used as the control compound, did not cause more than 50% lethality (28-day $LC_{50}$) for juvenile rainbow trout, whereas the $LC_{50}$ value for CuPT was determined to be 1.3 μg/L. Based on these results, it can be concluded that CuPT has the potential to pose a risk to the health and survival of chinook salmon and juvenile rainbow trout.

Borg et al. conducted a study investigating the toxicological effects of CuPT on juvenile brook trout (*Salvelinus fontinalis*) [76]. The fish were exposed to varying doses of CuPT (2-64 µg/L) for two hours, while a control group was also included. The study revealed a significant increase in copper levels in the gill tissue of the CuPT-exposed fish compared to the control group. This was determined through analysis using inductively coupled plasma atomic absorption spectrophotometry (ICP-AAS).

The researchers also observed morphological changes in the gill tissue of the exposed fish. Scanning electron microscopy (SEM) and histological analysis revealed the formation of club-shaped lamellae, edema, fusion of secondary lamellae, loss of microridge structures, and epithelial exfoliation. Transmission electron microscopy (TEM) revealed altered morphology of chloride cells, including the swollen appearance of mitochondria with disruption of internal cristae and lipid membrane disruption.

The study also found evidence of oxidative stress in the gill tissue of the exposed fish. Thiobarbituric acid reactive substance (TBARS) assays demonstrated increased levels of lipid peroxidation products in the gill tissue, while assays for the total antioxidant capacity of gill tissue revealed significantly lowered antioxidant levels. These results suggest that CuPT exposure at environmentally relevant doses is potentially harmful to juvenile brook trout and other aquatic organisms.

A study conducted by Tai et al. [79] examined the area-specific toxicity of copper on two important fisheries species, pacific oyster (*Crassostrea gigas*) and sea squirt (*Halocynthia roretzi*), using natural seawaters collected from three sites in the coastal area of Miyagi Prefecture, Japan. The study found that the effects of copper toxicity were species- and area-specific, with the 10% effect concentration ($EC_{10}$) for copper ranging from 12.8-17.0 µg/L for *C. gigas* and 15.0-22.0 µg/L for *H. roretzi*, while the 50% effect concentration ($EC_{50}$) ranged from 20.3-22.6 µg/L for *C. gigas* and 45.6-47.2 µg/L for *H. roretzi*.

In another study, the effects of CuPT on *Litopenaeus vannamei*, a widely farmed white shrimp species, were investigated in relation to apoptotic cell ratio, production of reactive oxygen species (ROS), and gene expression in hemocytes after exposure to different concentrations of CuPT (0, 64, and 128 µg/L) over a period of 48 hours [80]. The study found the $LC_{50}$ value equal to 827 µg/L after 48 h exposure as shown in Figure 10. Additionally, the findings demonstrated that exposure to CuPT induced ROS production in a concentration-dependent manner, with a significant increase observed only in the 128 µg/L groups from 3 to 48 hours (Figure 11). The

apoptotic cell ratio was significantly increased at 12 and 24 hours in the 64 µg/L groups and from 3 to 48 hours in the 128 µg/L groups. The expression of several genes in hemocytes was also affected by CuPT exposure, including up-regulation of Mn-superoxide dismutase (MnSOD) at 12 hours in the 64 µg/L groups, glutathione peroxidase at 24 and 48 hours in the 64 µg/L groups, caspase-3 at 24 hours in the 64 µg/L and 128 µg/L groups, metallothionein and HSP70 at 3 hours in the 64 µg/L groups, and MnSOD at 3 hours in the 128 µg/L groups.

These results indicated that CuPT exposure induces oxidative stress, activates caspase-3, and leads to hemocyte apoptosis in *L. vannamei*. However, the expression of certain genes, such as MnSOD, glutathione peroxidase, and metallothionein were up-regulated to provide protective mechanisms against CuPT-induced stress. These findings highlight the potential ecological risks associated with the use of CuPT in aquaculture, and underscore the importance of continued research into alternative, more environmentally friendly antifouling agents. The toxic effect of CuPT was also observed for other non-target species such as brine shrimp and guppy fish, as listed in Table 9 [70, 81].

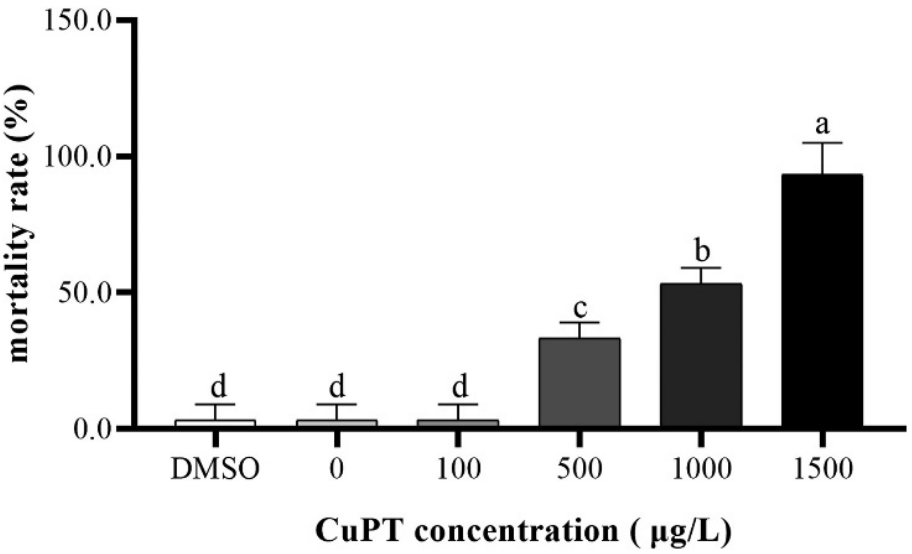



Figure 10. The mean mortality rate of *L. vannamei* after a 48-hour exposure to various concentrations of copper pyrithione (CuPT) [80]. Figure permissions by Elsevier.

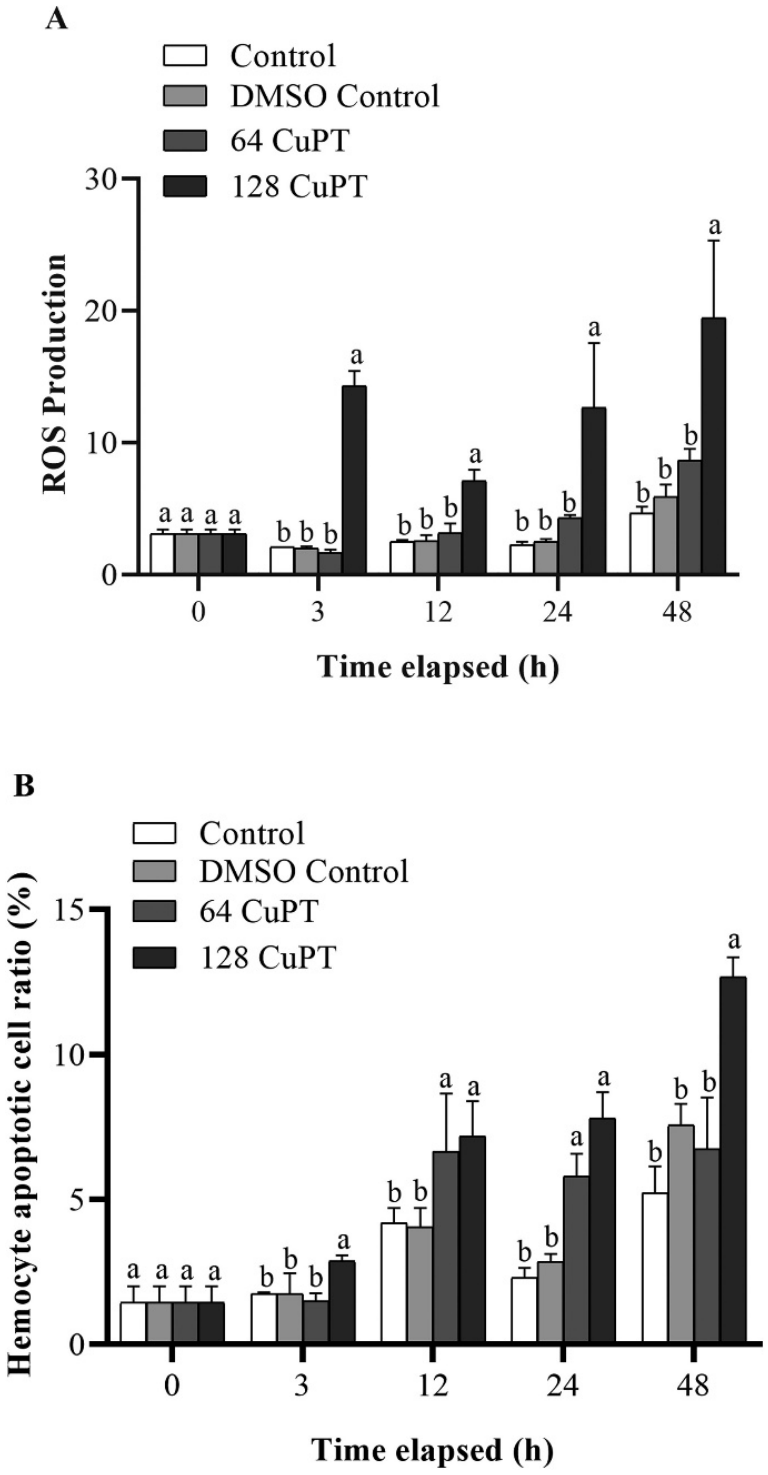



Figure 11. The results of reactive oxygen species (ROS) production (A) and hemocyte apoptotic ratio (B) in *L. vannamei* in response to varying copper pyrithione (CuPT) exposures [80]. DMSO stands for dimethyl sulfoxide. Figure permissions by Elsevier.

Table 9. The toxicological effect of copper (Cu) and copper pyrithione (CuPT) on commercial sea species.

| Species | Exposure time | Concentration | Toxicological effect | Reference |
|---|---|---|---|---|
| Chinook Salmon cell | 24 h | 100 µg/L CuPT | $EC_{50}$ | [22] |
| Rainbow trout | 7 d | 7.6 µg/L CuPT | $LC_{50}$ | [22] |
| Rainbow trout | 28 d | 1.3 µg/L CuPT | $LC_{50}$ | [22] |
| Brook trout | 2 h | 2 to 64 µg/L CuPT | increased oxidative stress, increased levels of lipid peroxidation products, lowered antioxidant levels | [76] |
| Flounder | 48 h | 42.65 µg/L CuPT | $LC_{50}$ | [68] |
| Pacific Oyster | 24 h | 20.3–22.6 µg/L Cu | $EC_{50}$ | [79] |
| White shrimp | 48 h | 827 µg/L CuPT | $LC_{50}$ | [80] |
| Brine shrimp | 48 h | 4580 µg/L CuPT | $LC_{50}$ | [70] |
| Guppy | 96 h | 2245 µg/L 2,2′-Dithiobis-pyridine* | $LC_{50}$ | [81] |

*2,2′-Dithiobis-pyridine is one of the main degradation products of CuPT.

## Non-Biocidal Antifouling Coatings

Fouling-release coatings are designed to be non-toxic and do not incorporate harmful biocides. Instead, these coatings function by creating a smooth surface that inhibits the attachment of fouling organisms like barnacles, mussels, and algae. By preventing their adhesion, fouling-release coatings help maintain the optimal performance of marine vessels and structures, reducing the potential degradation caused by these organisms.

While fouling-release coatings are generally considered to be more environmentally friendly than traditional antifouling coatings, there is still some concern about their potential toxicity. Certain fouling-release coatings may contain silicone oils or other additives that have the potential to leach into the surrounding water, posing a potential risk to marine life [82]. These coatings consist of a silicone resin matrix and may also include unbound silicone oils, such as

polydimethylsiloxanes (PDMS), which have the ability to leach out and potentially affect marine environments.

The physicochemical properties of PDMS, such as its low water solubility and high stability, make it an attractive ingredient for antifouling/foul-release products. However, its persistence and ability to adsorb suspended particulate matter mean that it can settle into sediment and potentially inhibit pore water exchange. Furthermore, undissolved silicone oil films or droplets can cause physical-mechanical effects that can trap and suffocate organisms at higher exposures. Existing toxicological studies have shown that PDMS does not bioaccumulate in marine organisms, and the soluble fractions have low toxicity to aquatic and benthic organisms. However, these studies have not yet assessed the potential impacts of undissolved silicone oil films or droplets, which can have different toxicological effects than the soluble fraction [83, 84].

A study conducted by Feng et al. [85] investigated the effect of fouling-release coatings on embryonic development of a sea urchin (*Arbacia punctulata*) and a fish (*Oryzias latipes*). The study revealed that the commercial fouling-release coatings, which contained undisclosed components and were claimed to be non-toxic, hindered the growth of sea urchin embryos. It also had significant adverse effects on fish embryos after a one-month immersion of coatings in seawater, including decreased hatching success, decreased hatchling survival, and inability to inflate the swim bladder. These findings suggest that compounds leaching from silicone coatings can impact the development of marine organisms. While fouling-release coatings are generally considered to be more environmentally friendly than traditional antifouling coatings, this study highlights the importance of understanding the potential impacts of these coatings on marine life.

# Comparative Studies

Comparing the toxicity of different biocides used in antifouling paints is crucial for several reasons. Firstly, it helps to determine the potential environmental impact of biocides on non-target marine organisms. This is particularly important, as marine ecosystems are sensitive and complex, and even low levels of toxicity can have significant consequences on the food chain and biodiversity. Secondly, comparing the toxicity of different biocides can aid in the development of safer and more environmentally friendly antifouling paints. By identifying the biocides with the lowest toxicity, it is possible to formulate antifouling paints that are less harmful to the marine environment. Thirdly, a comparison of biocide toxicity can help in the selection of appropriate biocides for specific applications. For instance, a biocide with high toxicity may not be suitable for use in a sensitive marine ecosystem, while a less toxic alternative may be more appropriate.

A study conducted by Bao et al. [86] aimed to comprehensively investigate the acute toxicities of copper, tributyltin (TBT), and five commonly used biocides, including Irgarol, diuron, zinc pyrithione (ZnPT), copper pyrithione (CuPT), and chlorothalonil, on the growth or survival of 12 marine species. The study found that TBT was highly toxic to both autotrophic and animal species, although it was not the most toxic biocide for all test species.

As shown in Table 10, Irgarol, ZnPT, and CuPT were found to be more toxic than TBT on the growth of *Chroococcus, Synechococcus sp., Skeletonema costatum, Thalassiosira pseudonana, Pyrocystis lunula* (except Irgol), *Aiptasia sp., Hydroides elegans* (larvae*), Balanus amphitrite* (larvae)*, Elasmopus rapax* (juvenile), *Tigriopus japonicus* (adult)*, Oryzias melastigma* (larvae)*,* male *Gametophytes,* and female *Gametophytes*, while CuPT was the most toxic to fish larvae. Chlorothalonil was less toxic than TBT, ZnPT, and CuPT on the survival of test animal species. ZnPT and CuPT showed similar toxicities to autotrophic species, and ZnPT was consistently less toxic than CuPT to the test animal species except *Aiptasia sp*. Irgarol and diuron are highly toxic to autotrophic aquatic species but less toxic to crustaceans and fish. Both biocides could cause severe impacts on the growth of microalgae and corals in the marine ecosystem at their highest detectable concentrations. Continuous monitoring and risk assessment of these biocides in the marine environment are necessary.

Okamura et al. [22] conducted a comparative study to assess the toxicity of potential marine antifouling compounds as alternatives to organotin compounds. They used fish cell lines from chinook salmon embryos and juvenile rainbow trout for their evaluations. The compounds

examined were CuPT, Diuron, Irgarol 1051, DCOIT/Sea-Nine 211, and ZnPT, tested in vitro using Alamar Blue™ dye and correlated with in vivo $LC_{50}$ values in rainbow trout. The study confirmed that fish cell lines are effective for rapid toxicity screening. Toxicity rankings, from highest to lowest, were CuPT > ZnPT > DCOIT/Sea-Nine 211 > Diuron > Irgarol 1051. Surprisingly, Diuron and Irgarol 1051 were the least toxic, suggesting their potential as safer alternatives, while pyrithiones showed the highest toxicity. This study serves as a valuable reference, potentially being the sole comprehensive investigation covering a wide spectrum of biocides in marine antifouling research. Further investigation is crucial before considering pyrithiones as replacements for organotin compounds in marine antifouling paints.

Researchers compared the toxicity of CuPT and ZnPT on embryonic olive flounder (*Paralichthys olivaceus*). CuPT was found to be more toxic than ZnPT. This was evident in both developmental effects (malformation and mortality) and gene expression profiles. The study also highlighted differences in the specific pathways affected by each compound, with CuPT impacting cellular respiration and kidney development. ZnPT affected cell development, nervous system development, and heart development [68].

Table 10. The toxicological effect of different antifouling biocides on commercial sea species.

| Species | Endpoint | DCOIT (µg/L) | Tralopyril/ Econea (µg/L) | ZnPT (µg/L) | CuPT (µg/L) | TBT (µg/L) | Irgarol (µg/L) | Reference |
|---|---|---|---|---|---|---|---|---|
| Chinook Salmon cell | 24 h $EC_{50}$ | 2600 | NA | 180 | 100 | NA | NA | [22] |
| Rainbow trout | 7 d $LC_{50}$ | 14 | NA | 8.4 | 7.6 | NA | NA | [22] |
| Rainbow trout | 28 d $LC_{50}$ | 14 | NA | 4.6 | 1.3 | NA | NA | [22] |
| Chroococcus minor | 7 d $EC_{50}$ | NA | NA | 51 | 50 | 100 | 5.7 | [86] |
| Synechococcus sp. | 96 h $EC_{50}$ | NA | NA | 22 | 22 | 50 | 23 | [86] |
| *Skeletonema costatum* | 96 h $EC_{50}$ | NA | NA | 1.7 | 1.8 | 290 | 1.1 | [86] |
| *Thalassiosira pseudonana* | 96 h $EC_{50}$ | NA | NA | 0.51 | 0.70 | 770 | 1.4 | [86] |
| *Pyrocystis lunula* | 24 h $EC_{50}$ | NA | NA | 44 | 23 | 140 | 220 | [86] |
| *Aiptasia sp.* | 96 h $EC_{50}$ | NA | NA | 410 | 2000 | NA | 9.4 | [86] |
| *Hydroides elegans* (larvae) | 48 h $LC_{50}$ | NA | NA | 7.6 | 5.7 | 100 | NA | [86] |
| *Balanus amphitrite* (larvae) | 24 h $EC_{50}$ | NA | NA | 210 | 63 | NA | 34 | [86] |
| *Elasmopus rapax* (juvenile) | 96 h $LC_{50}$ | NA | NA | 29 | 11 | 77 | 9.4 | [86] |
| *Tigriopus japonicus* (adult) | 96 h $LC_{50}$ | NA | NA | 170 | 30 | 580 | 18 | [86] |
| *Oryzias melastigma* (larvae) | 96 h $LC_{50}$ | NA | NA | 43 | 8.2 | 7300 | 25 | [86] |
| Male *Gametophytes* | 48 h $EC_{50}$ | 1015 | NA | NA | >400 | NA | 96 | [87] |
| Female *Gametophytes* | 48 h $EC_{50}$ | 890 | NA | NA | >400 | NA | 172 | [87] |

# Conclusion

Antifouling coatings are essential for preventing the attachment of fouling organisms to marine structures and vessels, minimizing degradation and maintaining optimal performance. However, it is crucial to understand the potential toxicity and ecological risks associated with these compounds.

DCOIT/Sea-Nine 211 presents significant environmental and toxicological concerns. It can bioaccumulate in aquatic organisms and has been found to induce adverse effects on marine life, including oxidative stress, reproductive impairments, and interference with the endocrine system. The vulnerability of commercial species highlights potential risks to human health and the economy. Further research and regulation are necessary to mitigate the environmental impact of DCOIT and other antifouling compounds. Urgent action is needed to adopt sustainable and eco-friendly alternatives in antifouling practices to protect marine ecosystems and human well-being.

Tralopyril/Econea exhibits a relatively short half-life in seawater and river water, suggesting rapid decomposition in these environments. However, the transformation products resulting from the hydrolysis procedure, specifically identified as 3-bromo-5-(4-chlorophenyl)-4-cyano-1H-pyrrole-2-carboxylic acid (BCCPCA), exhibited exceptional resistance to biodegradation, due to its notably high $K_{ow}$ value and extended half-life. This inherent stability raises significant concerns over the potential persistence of BCCPCA in the environment. Studies have demonstrated various toxicological effects of Tralopyril on marine species, including oxidative stress, disruption of energy metabolism, thyroid endocrine system interference, locomotor activity reduction, tissue structure modifications, and gene expression alterations.

These findings highlight the potential ecological risks associated with Tralopyril in marine environments. Further research is needed to understand its persistence, bioaccumulation, and long-term effects, and comprehensive monitoring and regulatory measures should be implemented to mitigate Tralopyril's potential adverse impacts and protect marine ecosystems.

Zinc pyrithione (ZnPT) is commonly used as a biocide in antifouling paints. Its persistence in the environment is influenced by factors such as temperature, pH, sunlight, and water solubility. ZnPT tends to settle on the seafloor or adhere to suspended particles, limiting its availability in the water column. Studies have revealed significant toxicity of ZnPT to fish cells, potentially impacting the survival and health of fish species. Comparative assessments have also

highlighted the toxic effects of ZnPT on various non-target species, emphasizing the need for understanding its long-term effects and ecological consequences in marine environments.

Copper pyrithione (CuPT) has been found to exhibit high toxicity, including developmental malformation and mortality, in various marine species. Comparative studies have indicated that CuPT may be more toxic than other compounds, including ZnPT, in terms of specific effects on fish larvae and gene expression related to different developmental processes.

Fouling-release coatings, which create a smooth surface to inhibit fouling organism attachment, are generally considered more environmentally friendly than traditional antifouling coatings. Nonetheless, concerns remain regarding the potential toxicity of certain fouling-release coatings containing additives like silicone oils. The leaching of silicone oils, including undissolved films or droplets, can have physical-mechanical effects on organisms, leading to entrapment and suffocation. Further research is necessary to assess the specific toxicological effects of these undissolved silicone oil components.

In conclusion, assessing the environmental impact and toxicity of antifouling coatings and their constituent compounds, including DCOIT, Tralopyril, ZnPT, and CuPT, is crucial. The development of safer and more sustainable alternatives with lower toxicity is essential to minimize adverse effects on marine ecosystems. Continued research, comprehensive toxicological assessments, and continuous monitoring are necessary to mitigate the potential ecological risks associated with the use of these compounds in antifouling coatings.

# References


[1] J.C. Almeida, Í.B. Castro, B.Z. Nunes, E. Zanardi-Lamardo, Antifouling booster biocides in Latin America and the Caribbean: A 20-year review, Marine Pollution Bulletin. 189 (2023) 114718. https://doi.org/10.1016/j.marpolbul.2023.114718.

[2] M.R. Detty, R. Ciriminna, F.V. Bright, M. Pagliaro, Xerogel coatings produced by the Sol–Gel process as anti-Fouling, fouling-release surfaces: From lab bench to commercial reality, ChemNanoMat. 1 (2015) 148–154. https://doi.org/10.1002/cnma.201500056.

[3] B.G. de Campos, J. Figueiredo, F. Perina, D.M. de S. Abessa, S. Loureiro, R. Martins, Occurrence, effects and environmental risk of antifouling biocides (EU PT21): Are marine ecosystems threatened?, Critical Reviews in Environmental Science and Technology. 52 (2022) 3179–3210. https://doi.org/10.1080/10643389.2021.1910003.

[4] I. Amara, W. Miled, R.B. Slama, N. Ladhari, 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.

[5] C. Bressy, J.-F. Briand, S. Lafond, R. Davy, F. Mazeas, B. Tanguy, C. Martin, L. Horatius, C. Anton, F. Quiniou, C. Compère, What governs marine fouling assemblages on chemically-active antifouling coatings?, Progress in Organic Coatings. 164 (2022) 106701. https://doi.org/10.1016/j.porgcoat.2021.106701.

[6] L. Tian, Y. Yin, H. Jin, W. Bing, E. Jin, J. Zhao, L. Ren, Novel marine antifouling coatings inspired by corals, Materials Today Chemistry. 17 (2020) 100294. https://doi.org/10.1016/j.mtchem.2020.100294.

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

[8] I.K. Konstantinou, T.A. Albanis, Worldwide occurrence and effects of antifouling paint booster biocides in the aquatic environment: A review, Environment International. 30 (2004) 235–248. https://doi.org/10.1016/S0160-4120(03)00176-4.

[9] N. Malhotra, T.-R. Ger, B. Uapipatanakul, J.-C. Huang, K.H.-C. Chen, C.-D. Hsiao, Review of copper and copper nanoparticle toxicity in fish, Nanomaterials. 10 (2020) 1126. https://doi.org/10.3390/nano10061126.

[10] L. Burridge, J.S. Weis, F. Cabello, J. Pizarro, K. Bostick, Chemical use in salmon aquaculture: A review of current practices and possible environmental effects, Aquaculture. 306 (2010) 7–23. https://doi.org/10.1016/j.aquaculture.2010.05.020.

[11] D.H. Baldwin, C.P. Tatara, N.L. Scholz, Copper-induced olfactory toxicity in salmon and steelhead: Extrapolation across species and rearing environments, Aquatic Toxicology. 101 (2011) 295–297. https://doi.org/10.1016/j.aquatox.2010.08.011.

[12] D. Hernández-Moreno, M. Blázquez, O. Andreu-Sánchez, A. Bermejo-Nogales, M.L. Fernández-Cruz, Acute hazard of biocides for the aquatic environmental compartment from a life-cycle perspective, Science of The Total Environment. 658 (2019) 416–423. https://doi.org/10.1016/j.scitotenv.2018.12.186.

[13] J. Figueiredo, T. Oliveira, V. Ferreira, A. Sushkova, S. Silva, D. Carneiro, D. N. Cardoso, S. F. Gonçalves, F. Maia, C. Rocha, J. Tedim, S. Loureiro, R. Martins, Toxicity of innovative anti-fouling nano-based solutions to marine species, Environmental Science: Nano. 6 (2019) 1418–1429. https://doi.org/10.1039/C9EN00011A.

[14] B.G. de Campos, M.B.M. do Prado e Silva, F. Avelelas, F. Maia, S. Loureiro, F. Perina, D.M. de S. Abessa, R. Martins, Toxicity of innovative antifouling additives on an early life stage of the oyster Crassostrea gigas: short- and long-term exposure effects, Environ Sci Pollut Res. 29 (2022) 27534–27547. https://doi.org/10.1007/s11356-021-17842-3.

[15] J. Figueiredo, S. Loureiro, R. Martins, Hazard of novel anti-fouling nanomaterials and biocides DCOIT and silver to marine organisms, Environmental Science: Nano. 7 (2020) 1670–1680. https://doi.org/10.1039/D0EN00023J.

[16] F. Cima, R. Varello, Potential disruptive effects of copper-based antifouling paints on the biodiversity of coastal macrofouling communities, Environ Sci Pollut Res. 30 (2023) 8633–8646. https://doi.org/10.1007/s11356-021-17940-2.

[17] I. Banerjee, R.C. Pangule, R.S. Kane, Antifouling coatings: Recent developments in the design of surfaces that prevent fouling by proteins, bacteria, and marine organisms, Advanced Materials. 23 (2011) 690–718. https://doi.org/10.1002/adma.201001215.

[18] M. Liu, S. Li, H. Wang, R. Jiang, X. Zhou, Research progress of environmentally friendly marine antifouling coatings, Polymer Chemistry. 12 (2021) 3702–3720. https://doi.org/10.1039/D1PY00512J.

[19] M. Lejars, A. Margaillan, C. Bressy, Fouling release coatings: A nontoxic alternative to biocidal antifouling coatings, Chem. Rev. 112 (2012) 4347–4390. https://doi.org/10.1021/cr200350v.

[20] T.A. Kochina, Yu.A. Kondratenko, O.A. Shilova, D.Yu. Vlasov, Biocorrosion, biofouling, and advanced methods of controlling them, Prot Met Phys Chem Surf. 58 (2022) 129–150. https://doi.org/10.1134/S2070205122010129.

[21] S.E. Martins, G. Fillmann, A. Lillicrap, K.V. Thomas, Review: Ecotoxicity of organic and organo-metallic antifouling co-biocides and implications for environmental hazard and

risk assessments in aquatic ecosystems, Biofouling. 34 (2018) 34–52. https://doi.org/10.1080/08927014.2017.1404036.

[22] H. Okamura, T. Watanabe, I. Aoyama, M. Hasobe, Toxicity evaluation of new antifouling compounds using suspension-cultured fish cells, Chemosphere. 46 (2002) 945–951. https://doi.org/10.1016/S0045-6535(01)00204-1.

[23] V. Silva, C. Silva, P. Soares, E.M. Garrido, F. Borges, J. Garrido, Isothiazolinone biocides: Chemistry, biological, and toxicity profiles, Molecules. 25 (2020) 991. https://doi.org/10.3390/molecules25040991.

[24] F.A. Guardiola, A. Cuesta, J. Meseguer, M.A. Esteban, Risks of using antifouling biocides in aquaculture, International Journal of Molecular Sciences. 13 (2012) 1541–1560. https://doi.org/10.3390/ijms13021541.

[25] R.A. Downs, J.R. Dean, A. Downer, J.J. Perry, Determination of the biocide Econea® in artificial seawater by solid phase extraction and high performance liquid chromatography mass spectrometry, Separations. 4 (2017) 34. https://doi.org/10.3390/separations4040034.

[26] A.R. Neves, J.R. Almeida, F. Carvalhal, A. Câmara, S. Pereira, J. Antunes, V. Vasconcelos, M. Pinto, E.R. Silva, E. Sousa, M. Correia-da-Silva, Overcoming environmental problems of biocides: Synthetic bile acid derivatives as a sustainable alternative, Ecotoxicology and Environmental Safety. 187 (2020) 109812. https://doi.org/10.1016/j.ecoenv.2019.109812.

[27] Z.Y. Soon, J.-H. Jung, M. Jang, J.-H. Kang, M.-C. Jang, J.-S. Lee, M. Kim, 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 Pollut. 230 (2019) 310. https://doi.org/10.1007/s11270-019-4361-0.

[28] C. Faggio, V. Tsarpali, S. Dailianis, Mussel digestive gland as a model tissue for assessing xenobiotics: An overview, Science of the Total Environment. 636 (2018) 220–229. https://doi.org/10.1016/j.scitotenv.2018.04.264.

[29] X. Chen, Q. Yang, L. Xiao, D. Tang, Q.P. Dou, J. Liu, Metal-based proteasomal deubiquitinase inhibitors as potential anticancer agents, Cancer Metastasis Rev. 36 (2017) 655–668. https://doi.org/10.1007/s10555-017-9701-1.

[30] H. Yamada, Toxicity and preliminary risk assessment of alternative antifouling biocides to aquatic organisms, in: I.K. Konstantinou (Ed.), Antifouling Paint Biocides, Springer, Berlin, Heidelberg, 2006: pp. 213–226. https://doi.org/10.1007/698_5_056.

[31] J.W. Readman, Development, occurrence and regulation of antifouling paint biocides: Historical review and future trends, in: I.K. Konstantinou (Ed.), Antifouling Paint

Biocides, Springer, Berlin, Heidelberg, 2006: pp. 1–15. https://doi.org/10.1007/698_5_047.

[32] A.M.C. Maan, A.H. Hofman, W.M. de Vos, M. Kamperman, Recent developments and practical feasibility of polymer-based antifouling coatings, Advanced Functional Materials. 30 (2020) 2000936. https://doi.org/10.1002/adfm.202000936.

[33] M. Matthies, S. Beulke, Considerations of temperature in the context of the persistence classification in the EU, Environ Sci Eur. 29 (2017) 15. https://doi.org/10.1186/s12302-017-0113-1.

[34] Regulation (EC) No 1907/2006 - Registration, evaluation, authorisation and restriction of chemicals (REACH) | Safety and health at work EU-OSHA, (n.d.). https://osha.europa.eu/en/legislation/directives/regulation-ec-no-1907-2006-of-the-european-parliament-and-of-the-council (accessed May 12, 2023).

[35] L. Chen, Y. Xu, W. Wang, P.-Y. Qian, Degradation kinetics of a potent antifouling agent, butenolide, under various environmental conditions, Chemosphere. 119 (2015) 1075–1083. https://doi.org/10.1016/j.chemosphere.2014.09.056.

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

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

[38] J.M. Armitage, L. Toose, L. Camenzuli, A.D. Redman, T.F. Parkerton, D. Saunders, J. Wheeler, A. Martin, E. Vaiopoulou, J.A. Arnot, A critical review and weight of evidence approach for assessing the bioaccumulation of phenanthrene in aquatic environments, Integrated Environmental Assessment and Management. 17 (2021) 911–925. https://doi.org/10.1002/ieam.4401.

[39] Í.B. Castro, Improper environmental sampling design bias assessments of coastal contamination, Trends in Environmental Analytical Chemistry. 24 (2019) e00068. https://doi.org/10.1016/j.teac.2019.e00068.

[40] J.R. Almeida, M. Correia-da-Silva, E. Sousa, J. Antunes, M. Pinto, V. Vasconcelos, I. Cunha, Antifouling potential of nature-inspired sulfated compounds, Sci Rep. 7 (2017) 42424. https://doi.org/10.1038/srep42424.

[41] W.D. Shade, S.S. Hurt, A.H. Jacobson, K.H. Reinert, Ecological risk assessment of a novel marine antifoulant, Environmental Toxicology and Risk Assessment: Second Volume, ASTM International (n.d) 381–408. https://doi.org/10.1520/STP13169S.

[42] H. Harino, M. Kitano, Y. Mori, K. Mochida, A. Kakuno, S. Arima, Degradation of antifouling booster biocides in water, Journal of the Marine Biological Association of the United Kingdom. 85 (2005) 33–38. https://doi.org/10.1017/S0025315405010799h.

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

[44] K.V. Thomas, M. McHugh, M. Waldock, Antifouling paint booster biocides in UK coastal waters: inputs, occurrence and environmental fate, Science of the Total Environment. 293 (2002) 117–127. https://doi.org/10.1016/S0048-9697(01)01153-6.

[45] V.A. Sakkas, I.K. Konstantinou, T.A. Albanis, 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 (2002) 215–227. https://doi.org/10.1016/S0021-9673(02)00430-2.

[46] L. Chen, R. Ye, Y. Xu, Z. Gao, D.W.T. Au, P.-Y. Qian, 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 (2014) 116–125. https://doi.org/10.1016/j.aquatox.2014.01.023.

[47] L. Chen, J. Sun, H. Zhang, D.W.T. Au, P.K.S. Lam, W. Zhang, V.B. Bajic, J.-W. Qiu, P.-Y. Qian, Hepatic proteomic responses in marine medaka (*Oryzias melastigma*) chronically exposed to antifouling compound butenolide [5-octylfuran-2(5H)-one] or 4,5-Dichloro-2-N-Octyl-4-Isothiazolin-3-One (DCOIT), Environ. Sci. Technol. 49 (2015) 1851–1859. https://doi.org/10.1021/es5046748.

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

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

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

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

[52] Y.-S. Moon, M. Kim, C.P. Hong, J.-H. Kang, J.-H. Jung, Overlapping and unique toxic effects of three alternative antifouling biocides (Diuron, Irgarol 1051®, Sea-Nine 211®) on non-target marine fish, Ecotoxicology and Environmental Safety. 180 (2019) 23–32. https://doi.org/10.1016/j.ecoenv.2019.04.070.

[53] M. Ito, K. Mochida, K. Ito, T. Onduka, K. Fujii, 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 (2013) 1053–1060. https://doi.org/10.1016/j.chemosphere.2012.08.052.

[54] H. Okamura, T. Watanabe, I. Aoyama, M. Hasobe, Toxicity evaluation of new antifouling compounds using suspension-cultured fish cells, Chemosphere. 46 (2002) 945–951. https://doi.org/10.1016/S0045-6535(01)00204-1.

[55] L. Chen, R. Ye, Y. Xu, Z. Gao, D.W.T. Au, P.-Y. Qian, 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 (2014) 116–125. https://doi.org/10.1016/j.aquatox.2014.01.023.

[56] A.R. da Silva, A. da S. Guerreiro, S.E. Martins, J.Z. Sandrini, DCOIT unbalances the antioxidant defense system in juvenile and adults of the marine bivalve Amarilladesma mactroides (Mollusca: Bivalvia), Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 250 (2021) 109169. https://doi.org/10.1016/j.cbpc.2021.109169.

[57] B.G. de Campos, M.K. Fontes, P.K. Gusso-Choueri, G.P. Marinsek, C.R. Nobre, B.B. Moreno, F.E.L. Abreu, G. Fillmann, R. de Britto Mari, D.M. de S. Abessa, A preliminary study on multi-level biomarkers response of the tropical oyster Crassostrea brasiliana to exposure to the antifouling biocide DCOIT, Marine Pollution Bulletin. 174 (2022) 113241. https://doi.org/10.1016/j.marpolbul.2021.113241.

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

[59] Antifouling Biocides Leaching, Degradation and Fate, (2023). https://mst.dk/service/publikationer/publikationsarkiv/2023/jun/antifouling/.

[60] I.B. Oliveira, R. Schönenberger, C.M. Barroso, M.J.-F. Suter, LC-MS/MS determination of Tralopyril in water samples, Chemosphere. 145 (2016) 445–449. https://doi.org/10.1016/j.chemosphere.2015.11.098.

[61] V. Lavtizar, H. Okamura, Early developmental responses of three sea urchin species to Tralopyril and its two degradation products, Chemosphere. 229 (2019) 256–261. https://doi.org/10.1016/j.chemosphere.2019.04.202.

[62] B. Liu, P. Li, S. He, S. Xing, Z. Cao, X. Cao, X. Wang, Z.-H. Li, Effects of short-term exposure to Tralopyril on physiological indexes and endocrine function in turbot (Scophthalmus maximus), Aquatic Toxicology. 245 (2022) 106118. https://doi.org/10.1016/j.aquatox.2022.106118.

[63] X. Chen, J. Zheng, M. Teng, J. Zhang, L. Qian, M. Duan, Y. Cheng, W. Zhao, Z. Wang, C. Wang, Tralopyril affects locomotor activity of zebrafish (Danio rerio) by impairing tail muscle tissue, the nervous system, and energy metabolism, Chemosphere. 286 (2022) 131866. https://doi.org/10.1016/j.chemosphere.2021.131866.

[64] X. Wang, P. Li, S. He, S. Xing, Z. Cao, X. Cao, B. Liu, Z.-H. Li, Effects of Tralopyril on histological, biochemical and molecular impacts in Pacific oyster, Crassostrea gigas, Chemosphere. 289 (2022) 133157. https://doi.org/10.1016/j.chemosphere.2021.133157.

[65] K. Maraldo, I. Dahllöf, Indirect estimation of degradation time for zinc pyrithione and copper pyrithione in seawater, Marine Pollution Bulletin. 48 (2004) 894–901. https://doi.org/10.1016/j.marpolbul.2003.11.013.

[66] P.A. Turley, R.J. Fenn, J.C. Ritter, Pyrithiones as antifoulants: Environmental chemistry and preliminary risk assessment, Biofouling. 15 (2000) 175–182. https://doi.org/10.1080/08927010009386308.

[67] B.-H. Min, M. Saravanan, S.-E. Nam, H.-J. Eom, J.-S. Rhee, Waterborne zinc pyrithione modulates immunity, biochemical, and antioxidant parameters in the blood of olive flounder, Fish & Shellfish Immunology. 92 (2019) 469–479. https://doi.org/10.1016/j.fsi.2019.06.048.

[68] D. Shin, Y. Choi, Z.Y. Soon, M. Kim, D.-J. Kim, J.-H. Jung, Comparative toxicity study of waterborne two booster biocides (CuPT and ZnPT) on embryonic flounder (Paralichthys olivaceus), Ecotoxicology and Environmental Safety. 233 (2022) 113337. https://doi.org/10.1016/j.ecoenv.2022.113337.

[69] K. Mochida, K. Ito, H. Harino, A. Kakuno, K. Fujii, Acute toxicity of pyrithione antifouling biocides and joint toxicity with copper to red sea bream (Pagrus major) and toy shrimp (Heptacarpus futilirostris), Environmental Toxicology and Chemistry. 25 (2006) 3058–3064. https://doi.org/10.1897/05-688R.1.

[70] E. Gutner-Hoch, R. Martins, F. Maia, T. Oliveira, M. Shpigel, M. Weis, J. Tedim, Y. Benayahu, 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 (2019) 530–537. https://doi.org/10.1016/j.envpol.2019.05.031.

[71] T. Ren, G.-H. Fu, T.-F. Liu, K. Hu, H.-R. Li, W.-H. Fang, X.-L. Yang, Toxicity and accumulation of zinc pyrithione in the liver and kidneys of Carassius auratus gibelio: association with P-glycoprotein expression, Fish Physiol Biochem. 43 (2017) 1–9. https://doi.org/10.1007/s10695-016-0262-y.

[72] Y. Zhao, Y. Liu, J. Sun, H. Sha, Y. Yang, Q. Ye, Q. Yang, B. Huang, Y. Yu, H. Huang, Acute toxic responses of embryo-larval zebrafish to zinc pyrithione (ZPT) reveal embryological and developmental toxicity, Chemosphere. 205 (2018) 62–70. https://doi.org/10.1016/j.chemosphere.2018.04.010.

[73] S. Katalay, A. Guner, M. Dagdeviren, G. Yigitturk, A. Yavasoglu, A.C. Gunal, N.U. Karabay Yavasoglu, F. Oltulu, Oxidative stress-induced apoptotic changes after acute exposure to antifouling agent zinc pyrithione (ZnPT) in Mytilus galloprovincialis Lamark (Mediterranean mussels) tissues, Chemistry and Ecology. 38 (2022) 356–373. https://doi.org/10.1080/02757540.2022.2047951.

[74] N. Třešňáková, A. Çağlan Günal, G. Başaran Kankılıç, E. Paçal, Ü. Nihan Tavşanoğlu, R. Uyar, F. Erkoç, Sub-lethal toxicities of zinc pyrithione, copper pyrithione alone and in combination to the indicator mussel species Unio crassus Philipsson, 1788 (Bivalvia, Unionidae), Chemistry and Ecology. 36 (2020) 292–308. https://doi.org/10.1080/02757540.2020.1735377.

[75] A.F. Nogueira, J.L. Pereira, S.C. Antunes, F.J.M. Gonçalves, B. Nunes, Effects of zinc pyrithione on biochemical parameters of the freshwater Asian clam Corbicula fluminea, Aquatic Toxicology. 204 (2018) 100–106. https://doi.org/10.1016/j.aquatox.2018.08.021.

[76] D.A. Borg, L.D. Trombetta, Toxicity and bioaccumulation of the booster biocide copper pyrithione, copper 2-pyridinethiol-1-oxide, in gill tissues of Salvelinus fontinalis (brook trout), Toxicol Ind Health. 26 (2010) 139–150. https://doi.org/10.1177/0748233710362381.

[77] K.M. Almond, L.D. Trombetta, The effects of copper pyrithione, an antifouling agent, on developing zebrafish embryos, Ecotoxicology. 25 (2016) 389–398. https://doi.org/10.1007/s10646-015-1597-3.

[78] W.O. Hobbs, M. McCall, J. Lanksbury, K. Seiders, P. Sandvik, M. Jones, H. Chuhran, D. Momohara, D. Norton, A baseline of copper associated with antifouling paint in marinas within a large fjord estuary, Marine Pollution Bulletin. 178 (2022) 113547. https://doi.org/10.1016/j.marpolbul.2022.113547.

[79] R. Tai, K. Chiba, Y. Nishimura, S. Han, S. Masunaga, W. Naito, The toxicity of copper to pacific oyster and sea squirt from Japan using regional seawater as a test medium, Journal of Water and Environment Technology. 20 (2022) 118–127. https://doi.org/10.2965/jwet.22-046.

[80] T. Chen, S. Li, Z. Liang, L. Li, H. Guo, Effects of copper pyrithione (CuPT) on apoptosis, ROS production, and gene expression in hemocytes of white shrimp Litopenaeus vannamei, Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 256 (2022) 109323. https://doi.org/10.1016/j.cbpc.2022.109323.

[81] X. Li, J. Wang, M. Yu, X. Zhang, W. Wang, H. Tian, S. Ru, 2,2′-Dithiobis-pyridine induced reproductive toxicity in male guppy (Poecilia reticulata), Ecotoxicology and Environmental Safety. 169 (2019) 778–785. https://doi.org/10.1016/j.ecoenv.2018.11.076.

[82] K. Truby, C. Wood, J. Stein, J. Cella, J. Carpenter, C. Kavanagh, G. Swain, D. Wiebe, D. Lapota, A. Meyer, E. Holm, D. Wendt, C. Smith, J. Montemarano, Evaluation of the performance enhancement of silicone biofouling-release coatings by oil incorporation, Biofouling. 15 (2000) 141–150. https://doi.org/10.1080/08927010009386305.

[83] M. Nendza, Hazard assessment of silicone oils (polydimethylsiloxanes, PDMS) used in antifouling-/foul-release-products in the marine environment, Marine Pollution Bulletin. 54 (2007) 1190–1196. https://doi.org/10.1016/j.marpolbul.2007.04.009.

[84] C. Muller-Karanassos, W. Arundel, P.K. Lindeque, T. Vance, A. Turner, M. Cole, Environmental concentrations of antifouling paint particles are toxic to sediment-dwelling invertebrates, Environmental Pollution. 268 (2021) 115754. https://doi.org/10.1016/j.envpol.2020.115754.

[85] D. Feng, D. Rittschof, B. Orihuela, K.W.H. Kwok, S. Stafslien, B. Chisholm, The effects of model polysiloxane and fouling-release coatings on embryonic development of a sea urchin (Arbacia punctulata) and a fish (Oryzias latipes), Aquatic Toxicology. 110–111 (2012) 162–169. https://doi.org/10.1016/j.aquatox.2012.01.005.

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

[87] H. Lee, S. Depuydt, S. Choi, T. Han, J. Park, Rapid toxicity assessment of six antifouling booster biocides using a microplate-based chlorophyll fluorescence in Undaria pinnatifida gametophytes, Ecotoxicology. 29 (2020) 559–570. https://doi.org/10.1007/s10646-020-02207-2.

# Appendix A. Acronyms and Abbreviations

Table 11. Acronyms, abbreviations, and their meanings.

| Term | Meaning |
|---|---|
| ACh | acetylcholine |
| AChE | acetylcholinesterase |
| ATP | adenosine triphosphate |
| BCCPCA | 3-bromo-5-(4-chlorophenyl)-4-cyano-1H-pyrrole-2-carboxylic acid |
| BCF | bioconcentration factor |
| BMF | biomagnification factor |
| Cu | copper |
| CuPT | copper pyrithione |
| CAT | catalase |
| DA | dopamine |
| DCOIT | 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one, known commercially as Sea-Nine 211 |
| DMSO | dimethyl sulfoxide |
| DO | dissolved oxygen |
| Econea | 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile |
| e.g. | for example |
| et al. | and others |
| GSH | glutathione |
| GST | glutathione-S-transferase |
| HE | hematoxylin-eosin |
| hpf | hour post-fertilization |
| ICP-AAS | inductively coupled plasma atomic absorption spectrophotometry |
| KH101 | pyridine triphenyl borane |
| LC | Median lethal concentration |
| MDA | malondialdehyde |
| MnSOD | Mn-superoxide dismutase |
| PEG | polyethylene glycol |
| PDMS | polydimethylsiloxane |
| ROS | reactive oxygen species |
| Sea-Nine 211 | 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT) |
| SD | Standard deviation |
| SDS | surfactant sodium n-dodecyl sulphate |
| SEM | scanning electron microscopy |

| Term | Meaning |
|---|---|
| SOD | superoxide dismutase |
| TBARS | thiobarbituric acid reactive substance |
| TBT | tributyltin |
| TEM | transmission electron microscopy |
| Tralopyril | 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile, known commercially as Econea |
| ZnPT | zinc pyrithione |