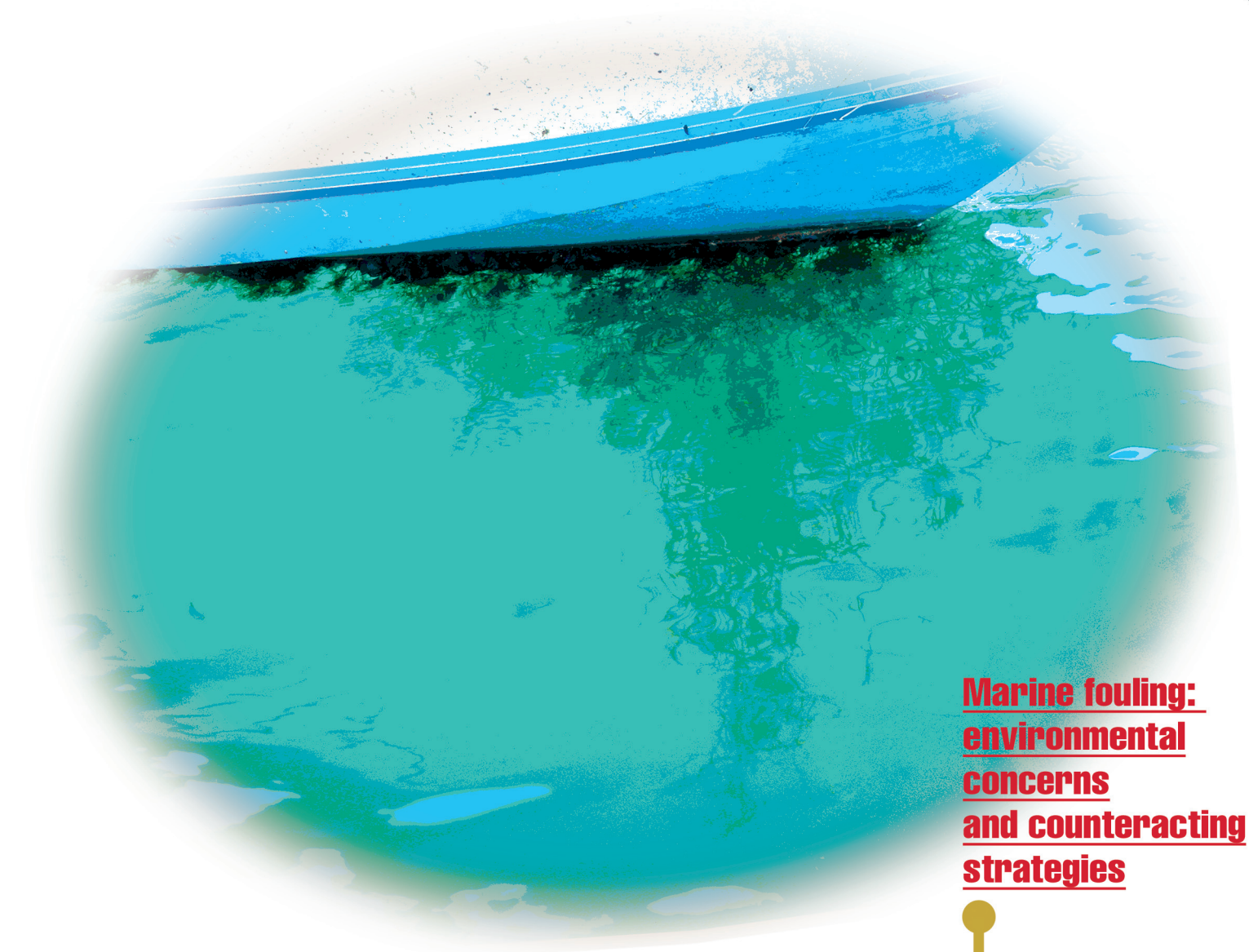
bimestrale dell'ENEA
anno 60
gennaio - febbraio 2014

# Energia, Ambiente e Innovazione

1/2014



## Marine fouling: environmental concerns and counteracting strategies

EA
Energia, Ambiente e Innovazione

RIVISTA BIMESTRALE DELL'ENEA
ANNO 60 - N. 1 GENNAIO-FEBBRAIO 2014

**Direttore Responsabile**
Diana Savelli

**Comitato di Direzione**
Pietro Agostini, Vincenzo Artale, Giacobbe Braccio, Marco Casagni, Gian Piero Celata, Vincenzo Cincotti, Carlo Cremisini, Pierino De Felice, Roberta Delfanti, Francesco Di Mario, Roberta Fantoni, Elena Fantuzzi, Massimo Forni, Massimo Iannetta, Carmela Marino, Paride Meloni, Silvio Migliori, Roberto Morabito, Aldo Pizzuto, Vincenzo Porpiglia, Rino Romani, Sergio Sangiorgi, Massimo Sepielli, Leander Tapfer, Ezio Terzini, Carlo Tricoli, Francesco Troiani, Marco Vittori Antisari, Gabriele Zanini

**Comitato tecnico-scientifico**
Osvaldo Aronica, Ilaria Bertini, Paolo Clemente, Paolo Di Lazzaro, Andrea Fidanza, Stefano Giammartini, Giorgio Graditi, Massimo Maffucci, Laura Maria Padovani, Paolo Ruti, Emilio Santoro

**Coordinamento editoriale**
Giuliano Ghisu

**Collaboratori**
Daniela Bertuzzi, Paola Carrabba, Orietta Casali, Antonino Dattola, Barbara Di Giovanni

**Edizione web**
Antonella Andreini, Serena Lucibello, Concetta Manto

**Promozione**
Paola Crocianelli

**Traduzioni**
Carla Costigliola

**Progetto grafico**
Paola Carabotta, Bruno Giovannetti

**Per informazioni e contatti:** infoeai@enea.it

**Immagine di copertina:** Carmine Minopoli

**Foto negli interni:** Carmine Minopoli (pag. 3, pag. 4); F. Lamiot, Wikimedia Commons http:// commons.wikimedia.org (pag. 44); Remi Jouan, Wikimedia Commons http://commons.wikimedia.org (pag. 89)

*Gli articoli riflettono le opinioni degli autori e non necessariamente quelle dell'ENEA*

**Pre-stampa**
FGE Srl - Fabiano Gruppo Editoriale
Regione Rivelle, 7/F - 14050 Moasca (AT)
e-mail: *info@fgeditore.it*

**Stampa**
Varigrafica Alto Lazio
Via Cassia, km 36,300 (Zona industriale) - 01036 Nepi (VT)

**Registrazione**
Tribunale Civile di Roma
Numero 148 del 19 aprile 2010 del Registro Stampa

Finito di stampare nel aprile 2014

Prodotto realizzato impiegando carta Symbol Freelife certificata FSC

N. 1/2014

# table of contents

## Foreword



## Rd Research & development

### (bio)Fouling and antifouling measures



### Environmental occurrence and concerns of antifouling biocides



### National/international legislation on antifouling

*by Sonia Manzo and Paolo Massanisso*

# Marine fouling: environmental concerns and counteracting strategies

Biofouling is ubiquitous in the marine environment and is a major problem for the shipping industry. The widespread use of toxicants in antifouling paints has resulted in high levels of contamination in the environment and has raised concerns about their effects on marine communities

The accumulation of unwanted matter on surfaces is a problem plaguing a variety of industries and human activities and is recognized by the term "fouling", which is related to both biofouling and inorganic fouling.

In particular, inorganic fouling is referred to deposits from corrosion, crystallization, suspended particles, oil and ice, whereas biofouling describes the growth of micro- and macro-organisms on surfaces.

Biofouling is ubiquitous in the marine environment and is a major problem for the shipping industry. Indeed, the growth of organisms on a vessel hull increases the frictional drag which reduces the ship speed and consequently requires increased power and fuel consumption to maintain the same cruising speed.

The need for effective antifoulants preventing against the settlement and growth of marine organisms on all submerged structures – i.e., not only ship hulls but also oil rig supports, buoys and fish cages – is recognized worldwide as being of significant economic importance. This requirement has been a driving force for the development of antifouling (AF) paints technologies, a global industry that is now worth approximately US$ 5 billion annually.

Consolidated antifouling measures include the use of coatings based on toxicants, traditionally incorporated into a paint matrix, that gradually leach from the surface layer. The widespread use of toxicants in AF paints, tributyltin in particular, has resulted in high levels of contamination in the environment and has raised concerns about their effects on



marine communities (shell malformation in oysters, mortality of mussel larvae and imposex in gasteropods), leading to policy actions to regulate their utilization. Therefore, to avoid these environmental alarms, the need has arisen for the continuous development of new non-toxic AF formulations, from non-toxic silicone-based coatings, known as foul release coatings, to innovative and new promising lines of research inspired by biomimetic solutions.

This special issue of EAI was born from the fundamentals of the Carisma project (Characterization and ecological risk analysis of antifouling biocides in the Southern Adriatic Sea), funded the by the Italian Ministry of Foreign Affairs, that aims to assess the quality of the portion of the Adriatic Sea between Italy (Apulia region) and Albania and, in particular, the environmental impact due to the use of antifouling paints. Actually, a large expertise in this field both in the analytical and ecotoxicological areas is far-back present at ENEA, as highlighted in the following section that provides a brief description of the "ENEA primary activities on antifouling biocides" and "ENEA main articles concerning antifouling biocides". This publication represents a wide-ranging reporting in the fouling/antifouling field and addresses a broad spectrum of the environmental issues. It wants to tackle and analyze the various aspects of fouling, with particular emphasis to biofouling, starting from the description of the biological phenomenon and of the main AF strategies, to the environmental impact, in terms of the amount of AF biocides released and the unwanted effects observed, reaching the definition of the ecological risk for the marine community. In addition, the legislative aspect is also addressed from different points of view: transposition and application, environmental protection and the REACH Regulation. In order to help readers, this issue is divided into three sections:

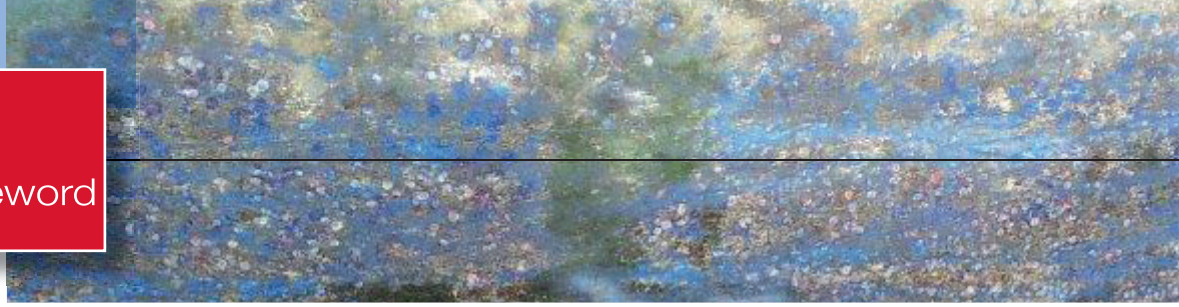
1) general characteristics of (bio)fouling and AF measures;
2) analytical aspects and environmental concerns of AF biocides;
3) national/international legislation.

We would like to thank all the contributors to this special issue and all experts in the field, coming from different public (such as ISPRA, CNR, the General Command of the Harbour, ASA and ENEA) and private Institutions (Boero Group and Shoreline), for sharing their expertise and experience with our readers. Moreover, we would like to extend our thanks to all the staff of EAI, Dr. Diana Savelli, Dr. Giuliano Ghisu and Dr. Carla Costigliola for their advice and assistance, and to Dr. Carlo Cremisini, Head of the Technical Unit for Environmental Characterization, Prevention, and Remediation (ENEA-UTPRA). Finally, it is our hope that readers will enjoy reading this special issue, the content of which will constitute a significant resource for all the Scientific actors and stakeholders, interested in this interdisciplinary field.

This publication represents a wide-ranging reporting in the fouling/antifouling field and addresses a broad spectrum of the environmental issues

*Sonia Manzo*
*ENEA, Technical Unit for Technologies Development - Portici Research Centre*

*Paolo Massanisso*
*ENEA, Technical Unit for Environmental Characterization, Prevention, and Remediation*

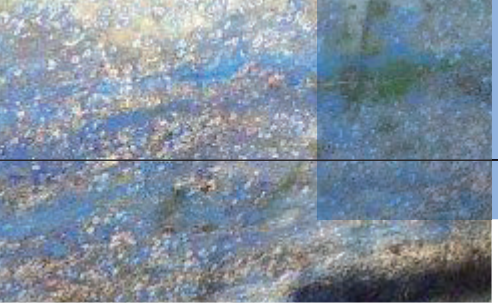


## ENEA primary activities on antifouling biocides

| International funded Projects | Years | Description and rule |
|---|---|---|
| HIC-TBT (EU-LIFE, 98ENV/NL//000199) | 1999-2001 | Assessment of the environmental distribution of TBT in Spain, Italy, Portugal in the North Sea in relation to its impact on marine life; development of communication strategies in order to sensitize the non-scientific community to the problem of TBT. Partner |
| OT-SAFE (EU – 5th FP, QLK1-CT-2001-01437) | 2001-2004 | EU-wide monitoring of contamination of fish products by organotin compounds and the related effect on human health, through the evaluation of the effect that cooking has on organotin compounds present in mussels. Partner |
| TBTIMPACTS (EU – 6th FP, INCO 510658) | 2005-2009 | Implications of TBT pollution and its ban, costs and benefits of TBT based antifoulants and other alternatives; environmental impact of organotin compounds in Europe and India coastlines and awareness towards this contaminant. Coordinator |
| CARISMA (Italian Ministry of Foreign Affairs, Projects of major importance in the Scientific and Technological Collaboration Executive Programmes with Albania, PGR00123) | 2012-2014 | Environmental impact of antifouling paints in the portion of the Adriatic Sea between Italy (Apulia region) and Albania and. Coordinator |

| Certification and stability studies | Years | Description |
|---|---|---|
| BCR 424, BCR 462, BCR 477, BCR 646, BCR 710 | 1991-2002 | Preparation and certification of reference materials for organotin compounds in several environmental matrices (sediment, biota tissues). ENEA has acted as coordinator in BCR 447 and BCR 710 projects and as partners in the others certification campaigns. |
| BCR 462, BCR 477, BCR 710 | 2000 - present day | Stability studies on behalf of IRMM |

## ENEA main articles concerning antifouling biocides published in peer-reviewed journals in the analytical and ecotoxicological areas





references


1. T. Ferri, R. Morabito and A. Perini: “Organotin determination by GFAAS”, in Heavy Metals in the Hydrological Cycle, Ed. M. Astruc and J.N. Lester, p. 413 - 418, London, (1988).
2. S. Chiavarini, C. Cremisini, T. Ferri, R. Morabito and A. Perini: “Monitoring of organotins in the La Spezia gulf - I - Analytical methods and preliminary results (summer 1989)”, Sci. Tot. Environ., 101, 217-227 (1991).
3. M. Caricchia, S. Chiavarini, C. Cremisini, R. Morabito and R. Scerbo: “Organotin compounds in marine mussel collected from Italian coasts” Analytical Sciences, 7, 1193-1196 (1991).
4. M. Caricchia, S. Chiavarini, C. Cremisini, M. Fantini and R. Morabito: “Monitoring of organotins in the La Spezia gulf - II. Results of the 1990 sampling campaigns and concluding remarks”, Sci. Tot. Environ., 121, 133-144 (1992).
5. S. Chiavarini, C. Cremisini, T. Ferri, R. Morabito and C. Ubaldi: “Liquid-solid extraction of butyltin compounds from marine samples” Appl. Organomet. Chem., 6, 147-153 (1992).
6. M. Caricchia, S. Chiavarini, C. Cremisini, R. Morabito and C. Ubaldi: “Analytical methods for the determination of organotins in the marine environment” Int. J. Env. An. Chem., 53, 37-52 (1993).
7. M. Caricchia, S. Chiavarini, C. Cremisini, R. Morabito and R. Scerbo: “Influence of the storage conditions on the organotin determination in mussel samples”, Anal. Chim. Acta, 286, 329-334 (1994).
8. Ph. Quevauviller, R. Ritsema, R. Morabito, W.M.R. Dirkx, S. Chiavarini, J.M. Bayona and O.F.X. Donard: “Critical Consideration with respect to the identification of tin species in the environment” Appl. Organomet. Chem., 8, 541-549 (1994).
9. T. Ferri, F. Roberti, S. Chiavarini, C. Cremisini and R. Morabito: “Influence of tropolone on voltammetric speciation analysis of butyltin compounds”, Appl. Organomet. Chem., 8, 587-593 (1994).
10. R. Morabito, S. Chiavarini and C. Cremisini: “GC-MS for the speciation of organotin compounds in environmental samples” In: “Quality assurance of Environmental Analysis within the BCR-programme”, Ph. Quevauviller, E. Maier and B. Griepink Eds., Elsevier Publ., Chapter 17, 435-464 (1995).
11. R. Morabito: “Speciation of organotin compounds in environmental matrices”, Microchem. J., 51, 198-206 (1995).
12. Ph. Quevauviller, S. Chiavarini, C. Cremisini, R. Morabito, M. Bianchi and H. Muntau: “Preparation and homogeneity study of a mussel candidate reference material for the certification of organotin compounds”, Mikrochim. Acta, 120, 281-287 (1995).
13. Ph. Quevauviller, M. Astruc, L. Ebdon, H. Muntau, W. Cofino, R. Morabito and B. Griepink: “A programme to improve the quality of butyltin determinations in environmental matrices”, Mikrochim. Acta, 123, 163-173 (1996).
14. F. Pannier, A. Astruc, M. Astruc and R. Morabito: “Determination of butyltin compounds in mussel samples: a comparative study of analytical procedures”, Appl. Organomet. Chem., 10, 471-476 (1996).
15. M.B. de la Calle-Guntinàs, R. Scerbo, S. Chiavarini, Ph. Quevauviller and R. Morabito: “Comparison of different derivatization methods for the determination of butyl- and phenyltin compounds in mussel by gas chromatographic methods”, Appl. Organomet. Chem., 11, 693-702 (1997).
16. R. Morabito, P. Soldati, M.B. de la Calle and Ph. Quevauviller: “An attempt to certify phenyltin compounds in a mussel reference material”, Appl. Organomet. Chem., 12, 621-634, (1998).
17. R. Morabito, P. Massanisso and Ph. Quevauviller: “Derivatization methods for the determination of organotin compounds in environmental samples”, TrAC, 19, 97-106, (2000).
18. Pellegrino, P. Massanisso and R. Morabito: “Comparison of 12 selected extraction methods for the determination of butyl- and phenyl-tin compounds in mussel samples” TrAC, 19, 97-106 (2000).
19. S. Chiavarini, P. Massanisso, P. Nicolai, C. Nobili and R. Morabito: “Butyltins concentration levels and imposex occurence in snails from the sicilian coasts (Italy)”, Chemosphere , 50, 311-319 (2003).
20. M.M. Santos, L. Filipe C. Castro, M.N. Vieira, J. Micael, R. Morabito, P. Massanisso, M.A. Reis-Henriques, “New insights into the mechanism of imposex induction in the dogwhelk Nucella lapillus”, Comparative Biochemistry and Physiology, Part C 141 (1), 101 – 109 (2005).
21. C. Brunori, I. Ipolyi, P.Massanisso, R. Morabito, “New trends in sample preparation methods for the determination of organotin compounds in marine matrices”, Handbook of Environmental Chemistry, Vol. 5: Water Pollution, 51-70, (2006).
22. Manzo S., Buono S., Cremisini C., “Toxic effects of Irgarol and Diuron on sea urchin Paracentrotus lividus early development, fertilization, and offspring quality”, Archives of Environmental Contamination and Toxicology, 51, 61–68 (2006).
23. G.Di Landa, G. Ansanelli, R. Ciccoli, C.Cremisini., “Occurrence of antifouling paint booster biocides in selected harbors and marinas inside the Gulf of Napoli”, Marine Pollution Bulletin, 52, 1541–1546 (2006)
24. N.S. Thomaidis, S. Stasinakis, G. Gatidou, R. Morabito, P. Massanisso, T. D. Lekkas, “Occurrence of Organotin Compounds in the Aquatic Environment of Greece”, Water, Air, & Soil Pollution 181 (1-4), 201-210 (2007).
25. Manzo S., Buono S. and Cremisini C. Predictability of Copper, Irgarol and Diuron combined effects on sea urchin Paracentrotus lividus. Archives of Environmental Contamination and Toxicology, 54 (1): 57-68 (2008).
26. S. Jadhav, N.B Bhosle, P. Massanisso, R. Morabito, “Organotins in the sediments of the Zuari estuary, west coast of India”, Journal of Environmental Management, 90 (suppl. 1), 4-7 (2009).
27. G. Di Landa, L.Parrella, S. Avagliano, G. Ansanelli, E. Maiello, C. Cremisini, “Assessment of the Potential Ecological Risks Posed by Antifouling Booster Biocides to the Marine Ecosystem of the Gulf of Napoli (Italy)”. Water, Air, & Soil Pollution, 200, 305–321, (2009)
28. S. Manzo, G. Ansanelli, L. Parrella, G. Di Landa, P. Massanisso, S. Schiavo, C. Minopoli, B. Lanza, R.Boggia, P. Aleksi, A. Tabaku, “A first evaluation of the threat posed by antifouling biocides in the Southern Adriatic sea”, accepted for publication in Environmental Science: Processes & Impacts (2014).

## (BIO)FOULING AND ANTIFOULING MEASURES

# Fouling: an overall issue

The term fouling refers to the accumulation of unwanted material on a surface, with the result of reducing the efficiency and functionality of the surface and/or of the device it belongs to. Fouling affects many more fields than one would expect – medical, marine and industrial – always creating severe losses of money. Here some examples are presented, as well as some methods adopted as fouling countermeasures, also mimicking ingenious strategies derived from nature

*DOI: 10.12910/EAI2014-38*

*Giovanna Armiento*

## Introduction

In its broadest sense, fouling is any accumulation of unwanted material on a surface, which causes a side effect or impairs the functionality and efficiency of the surface and/or of the device it belongs to.

Several types of fouling and their combinations may occur: 1) crystalline or precipitation fouling, 2) corrosion fouling, 3) particulate fouling, 4) chemical reaction fouling, and 5) biological fouling or biofouling. Biological fouling results from a) development of a biofilm consisting of microorganisms and their products (microbial fouling), b) deposition and growth of macroorganisms (macrobial fouling), and c) assorted detritus. Microbial fouling usually precedes colonization of the surface by macroorganisms.

The importance given to fouling phenomena is ultimately due to the fact that they result in severe energy losses, either if the deposits increase the fluid frictional resistance at a surface or impede the flow of heat across surfaces, or of a fluid across membranes, or increase the rate of corrosion at a surface [1].

A remarkable number of papers in scientific literature deal with the problem of fouling, reflecting the fact that many are the fields in which this phenomenon creates concern. Just to mention the most remarkable, fouling affects the long-term functionality of implantable bioelectronics and malfunction of biosensors in the medical field, while in industrial applications it can give unwanted effects in power plants (e.g., geothermal), water-treatment systems (e.g., for desalination or wastewater reclamation), heating exchangers, sensors (and other devices) used for river and marine monitoring and even in the food processing industry.

This paper summarizes the principal aspects related to fouling in various fields, with the aim to give an overview of the problem and of the methods adopted as countermeasures.

## Devices for environmental applications

### *Sensors for environmental monitoring*

Typically, water quality is assessed by monitoring parameters such as pH, conductivity, dissolved oxygen, temperature, turbidity, nitrate and phosphate concentrations.

A variety of sensors is available for these purposes and a wide range of antifouling measures must be developed to ensure that sensor performance is not impeded by biofouling (e.g., biofilm formation on the glass membrane - a specially formulated, ultrathin glass - of the proton-selective electrode used for *in situ* pH measurement).

**Giovanna Armiento**
*ENEA, Technical Unit for Environmental Characterization, Prevention, and Remediation*

Generally, for marine and riverine sensors, biofouling decreases the operating lifetime and increases the cost of maintenance of the sensor, since the latter must be removed from the sampling location to be cleaned. Biofouling will also introduce a degree of error into the collected data, e.g., if a fluorimeter is used to quantitate the chlorophyll concentration in water, accumulation of other absorbing species on the sensor will reduce the amount of light which can be absorbed by the analyte. Biofouling also poses problems for the platforms on which the sensors are deployed.

Sensors employed for marine and riverine monitoring primarily undergo aquatic biofouling, which comprises four stages: i) adsorption of a conditioning layer, ii) adhesion of bacteria, iii) growth of a biofilm and iv) macrofouling.

Among the methods used in the past to combat fouling there is the mechanical cleaning by high pressure water jets, but it is not suitable for delicate sensor components; chlorination has also been used, but it has been shown that byproducts of chlorine in water include carcinogenic compounds such as trihalomethanes.

As a consequence of Tributyltin banning, due to its extreme toxicity, research on antifouling coatings has focussed on two different types of materials. The first type, non-stick coatings, resists adhesion by fouling organisms, thus preventing the growth of biofilms at a surface; they are materials with low surface energy, usually silicones and fluorinated polymers.

The second type of materials is prepared by incorporating a compound, which is biologically active against those organisms settling on the surface (antimicrobial activity).

Mechanical antifouling methods are a more benign approach to antifouling than leaching of biocides from surface coatings into the water. The U.S. Navy patented an oceanographic sensor, which vibrates upon excitation by an electric potential, thus removing fouling material from the surface [2]. However, the power required is quite high and this makes it unsuitable for use in battery-powered remote sensors; moreover, the sensitivity of the sensor can be decreased as a result of the coating.

Alternatively, the sensor can be exposed for the minimum time required to sample, and then the sensor is removed from the fouling environment [3].

Electrochemistry can also be used to kill fouling organisms, e.g., the generation of chlorine and hypochlorous acid by electrolysis of seawater has been proposed as a method for preventing marine sensors from fouling [4]. Otherwise, electrochemistry can be used to kill microorganisms by direct transfer of electrons from the electrode to the fouling organisms [5].

Another method used as antifouling is the irradiation of surfaces, e.g., ultraviolet light has been used on marine sensors, but also on filtration membranes, valves, intake gratings and also for wastewater disinfection [6]. However, this method is not practical to use with remote sensors due to demanding power requirements. Instead, no energy supply is needed when coating surface with a photocatalytic material, for the photocatalytic inhibition, e.g., of algal growth [7]. Photolysis of water in the presence of the zinc oxide photoactive material leads to the formation of hydrogen peroxide, a known toxicant [8].

Laser irradiation was also investigated as a means of preventing biofouling by barnacles and diatoms [9] and ultrasonic irradiation for control of biofilm formation on glass tubing [10], and low frequency sound, too, has been tested to prevent zebra mussel fouling.

As far as sensors are concerned, a strategy is to render the membrane more hydrophilic, e.g., by polymerization of the surfaces. The interaction of the cationic polymer chains with negatively charged areas on the bacterial cell membrane is claimed to explain the efficacy of the treatment.

Also hybrid organic/inorganic reverse osmosis membranes, containing aromatic polyamide thin films underneath titanium dioxide nanoparticles, have been tested to inhibit membrane fouling [11]. $TiO_2$ photocatalysis is known to generate various active oxygen species, such as hydroxyl radicals and hydrogen peroxide that kill bacteria by destruction of the bacterial cell membrane.

The ideal antifouling strategy for sensors would provide a low cost, easily implemented, environmentally benign solution to fouling, which would allow sensors to operate unattended for a sufficient time span, but at present the methods described above cannot satisfy all of these criteria. Further research is needed in deter-

mining the long-term environmental effects of substances tested to this aim, and to completely understand the mechanism of action of many naturally antifouling compounds [12].

*Permeable reactive barriers*

The permeable reactive barrier (PRB) is a passive treatment technology used to treat contaminated groundwater. PRBs are generally used for long-term treatment (decades) and during their lifetime fouling caused by mineral precipitation is a major concern. Fouling causes loss of pore space and reactive surface area of the reactive medium, consequently flow paths and residence time can be altered, thus influencing the effectiveness of the barrier. Changes in residence time are particularly important, as contaminants must remain within the reactive medium long enough to ensure that the treatment will effectively react with contaminants [13].

Most PRBs use granular zero-valent iron (ZVI) to create redox conditions, resulting in degradation or immobilization of chlorinated solvents and herbicides, heavy metals, and radionuclides. The involved reactions also cause the precipitation of secondary minerals, such as iron oxides, (oxy)hydroxides and carbonates [14]. Accumulation of minerals in ZVI reduces the porosity and hydraulic conductivity, affects the surface area for reactivity, and alters flowpaths, resulting in preferential flow and/or blockage of flow [15]. The rate of porosity reduction is a function of the ground water chemistry and flow rate, with greater amounts of minerals accumulating when the inflowing ground water has higher concentrations of dissolved mineral-forming ions [16].

Simulations of ground water flow and reactive transport have been used to evaluate how mineral fouling may affect the hydraulic behaviour of PRBs over decades of continuous flow in carbonate-rich alluvial aquifers. Results of the simulations show that a little change in hydraulic behaviour occurs within 10 years from the time of installation, which is consistent with field experience to date. Significant changes in hydraulic behaviour should be expected after ~30 years due to larger reductions in porosity and hydraulic conductivity. After 50 years, large regions of PRBs may become clogged and the PRB is likely to become less permeable than the aquifer, resulting in appreciable bypassing of the barrier by groundwater.

Li and Benson [17] proposed some strategies to limit the impact of fouling in PRBs. Residence times are less affected by mineral precipitation when a pre-treatment zone is employed. pH adjustment limits the total amount of hydroxide ions in groundwater to reduce porosity reduction and to retain larger residence times. Larger ZVI particles reduce porosity reduction as a result of the smaller iron surface area for iron corrosion, and retain longer residence time. Mechanical treatment redistributes the porosity uniformly throughout the PRB over time, which is effective in maintaining the residence time. These findings are predicted with numerical models, additional research and monitoring are necessary to confirm that the performances anticipated can be used in practical in situ application.

*Membranes fouling*

**Reverse osmosis for desalination**

Problems with water are expected to grow worse in the coming decades, therefore, many researchers have focused on methods suitable to obtain freshwater by saltwater desalination and water reuse to sustain future generations. The reverse osmosis (RO) technology is considered as a promising solution and is gaining worldwide acceptance at present [18]. RO is a pressure-driven process whereby a semi-permeable membrane (i.e., RO membrane) rejects dissolved constituents in the feeding water while allowing water to pass through. The progress in RO technology is greatly dependent on the development of RO membranes, which has become both possible and practical after the invention of the thin-film composite (TFC) aromatic polyamide membrane.

Despite its many advantages, one of the obstacles to the widespread use of TFC polyamide RO membrane is the proneness to fouling [19]. Fouling is a process where solutes or particles in feeding water deposit onto RO membrane surface in a way that causes flux decline and affects the quality of the water produced. This will inevitably make the operation difficult and decrease the membrane lifetime, which will be translated into higher costs.

To prevent RO membrane fouling, a number of methods

for antifouling RO membranes have been developed, including the selection of new starting monomers, the improvement of interfacial polymerization process, surface modification of conventional RO membrane and the incorporation of inorganic particles [20].
There are mainly four types of foulants in RO membrane fouling: inorganic (salt precipitations such as metal hydroxides and carbonates), organic (natural organic matters such as humic acid), colloidal (suspended particles such as silica) and biological (such as bacteria and fungi). Physicochemical properties of RO membrane surface, such as hydrophilicity, roughness and electrostatic charge, are major factors influencing the membrane fouling [21].
The development of fouling-resistant RO membranes takes these major factors into account.
Increase in hydrophilicity offers better fouling resistance since many foulants, such as protein, are hydrophobic in nature [22].
A smoother surface is commonly expected to experience less fouling, presumably because foulant particles are more likely to be entrained by rougher topologies than by smoother membrane surfaces [23].
Finally, surface-bound long-chain hydrophilic molecules (e.g., polyethyleneglycol) are very effective in preventing the adsorption of macromolecules, such as protein onto membrane surface, due to the steric repulsion mechanism [24].
Most research is aimed to face the factors listed above, e.g., by the introduction of hydrophilic layer, the reduction of surface roughness, the improvement of charge property and the utilization of the steric repulsion effect. Nonetheless, fouling cannot be thoroughly prevented, since there are no membranes that are free from fouling under any circumstances [22].

**Reverse osmosis and nanofiltration for effluent reclamation**

RO is also increasingly used, together with nanofiltration (NF), in the advanced treatment of municipal secondary effluents for the production of high-quality reuse water [25]. However, membrane fouling is a major obstacle in the development of membrane technology in this field.
These systems undergo fouling occurrences similar than the RO membranes described above, but their nature is linked to the particular media treated. Thus the main fouling agents are: effluent organic matter (EfOM), microbial and inorganic membrane fouling.
EfOM represents a large group of structurally complex, heterogeneous, and poorly defined organic compounds [26].
Biofouling originates from the following processes: microorganisms irreversibly attach on the membrane surface and then grow, reproduce, and secrete substances by utilizing the nutrients in wastewater before a biofilm is finally formed [27]; this biofilm decreases the membrane flux, increases the transmembrane pressure, and causes the membrane biodegradation and salt flux increase [28].
Colloidal natural organic matter, colloidal calcium phosphate, and sometimes colloidal silicates are the main components of the inorganic foulant, all of which have great affinity towards aggregation with one another.
These fouling processes and their interrelations are still poorly understood, so further studies are necessary to examine their mechanism, identify their properties, and take the relevant control measures.

## Energy production and delivery

*Geothermal plants*

Geothermal energy is one of most promising energy supply source and many geothermal power stations have been set up and operated in several countries, furnishing houses and industries with energy.
The present challenge is to continue to lower production costs without compromising safety, in order to remain competitive with other power sources. Among the factors involved in lowering the cost of geothermal utilization, significant fouling and corrosion are two control issues that have not been satisfactorily settled [29].
Scaling (term used to indicate mineral fouling) and corrosion of highly saline and corrosive geothermal water are often observed within plants or in reservoirs in which the cooled fluid is reinjected into formations, thereby decreasing the fluid flow by clogging the pipes of the plant and the pores of the rock. Fouling simultaneously results in an increase in fluid resistance, as well as extra energy consumption and wastewater dischar-

ge; furthermore, an incomplete fouling layer can lead to local corrosion [30].
The most corrosion - and scaling-relevant compounds in geothermal fluids are scales of carbonates, silica, sulfides, oxides and also soluble salt minerals (halite) originating from, e.g., evaporite formations [31].
Among them, the main contributors to geothermal fouling are the scalings of silica and calcium carbonate, since they are primary components of the earth's crust.
Calcium scaling in geothermal plants is largely driven by pressure reduction through fluid transmission devices, thus the geothermal hot water scale deposits onto heat transfer surfaces of heat exchangers and onto the surfaces of the flowing conduits. When the pressure of the brine solution decreases rapidly, $CO_2$ gas is evolved from the brine due to its decrease in solubility. This increases the pH of the brine and causes the deposition and crystal growth of calcium carbonate. The kinetics of this reaction is very fast, causing scale formation immediately downstream of such pressure drops and the plugging of, e.g., valves, pressure taps and flow instruments. Calcium carbonate is also found on heated surfaces (see next paragraph), since its solubility decreases as temperature increases (retrograde solubility).
For silica scale, the deposition mechanism is more complicated than that of carbonate. Silica solubility increases as brine temperature increases (prograde solubility), and is saturated in geothermal brines in the downhole environment. Consequently it can become supersaturated as the brine is cooled through the heat exchange, or when part of the brine is flashed into steam. Supersaturation causes the precipitation of silica in an amorphous form on heat exchanger surfaces, separators, well lines and discharged lines. The scale formed by silica is hard and not easily removed by mechanical or chemical methods.
The scale so deposited deteriorates the heat transfer capability dramatically and, at the same time, it remarkably increases the pumping power needed to flow geothermal hot water, to the detriment of the stable and long operation of the system.
Several technologies for inhibiting fouling have been developed over the past decades based on the fouling and corrosion categories and severity, including crystallizer-clarifiers scale inhibitors [29], plant and fitting material selections, electrical submersible pumps [29], steam cleaning and various coatings, such polyphenylenesulfide-based, or epoxy resin [32], or $SiO_2$ on copper substrate [30].
Even if many improvements have been achieved so far, further work is still needed to protect the plant components against corrosion, oxidation, and scaling in the harsh, hostile geothermal environment, and to develop a system for the effective use of this natural "high density" energy.

*Mineral fouling in heat exchangers*

As mentioned for the geothermal plants, mineral fouling (scaling) is also experienced in heat exchangers, especially with the use of cooling water systems. It is the deposition of precipitated mineral salt crystals on a heat transfer surface. The formed fouling layer decreases the thermal efficiency of the heat exchanger, increasing the operating cost. Fouling demands billions of dollars annually for cleaning and maintaining the equipment: studies show that 1 mm limestone deposit could double the energy consumption in a heat power plant [33 and references therein].
When a heat pump is used as an air-conditioning system, the outside heat exchanger is used as a condenser, where heat has to be rejected to the surroundings. The mineral ions contained in circulating water are accumulated, and their concentration increases with time, creating fouling problems. The precipitated solids form both soft and hard scale deposits on the heat transfer surfaces, increasing the resistance to heat transfer and subsequently decreasing the thermal efficiency of the equipment.
The concentration of fouling materials (foulants), temperature, pH, pressure, time, flow velocity, mechanical motions, radiation, and impurities are factors affecting nucleation and subsequent crystal formation.
Fouling can be "soft" and "hard": the former is due to particulate accumulation, prevalently particulate matter, bacteria, corrosion products and so on [34], the latter is due to mineral crystallization, mostly calcium carbonate.
To date, chemical treatments have been the most effective approach for scaling prevention, however water pollution may derive from the chemicals employed.

Alternative methods have been tested and proposed, e.g., the use of oscillating electric field and of devices such as permanent magnets, solenoid coil device, high-voltage electrode [35], electro-flocculation mechanisms [36] and [33].

## Medical devices affected by fouling

Medical biofouling occurs in areas such as prosthetic implants, biosensors, catheters, dental implants and medical equipment, and can cause problems such as implant rejection, malfunction of biosensors and spread of infectious diseases. As far as medical implants are concerned, more than 45% of hospital-contracted infections are linked to biofilm-infected medical devices. For instance, catheters are the most commonly used medical device and the second highest cause of infection [37].

Biofouling in these cases is due to the adhesion of proteins or microorganisms (biofilm) to the device and begins soon after implantation. Treating biofilms on infected medical devices often requires surgical replacement, which increases the risk of mortality and antibody resistance.

The affected medical devices can be permanent (implanted and intended for long-term use) or temporary (intended for short-term use). Permanent implant devices include biosensors, heart valves, bone plates, fasteners, orthopaedic implants, dental implants, pacemakers, drug-delivery devices and ventilation tubes [38]. Immediately after surgery, the permanent implant is flooded with blood followed by the adsorption of proteins onto the surface [39]. Such adsorption on a biosensor may lead to sensor 'blindness', reduced lifespan and increased power consumption. Mechanical heart valve biofilms can lead to tissue inflammation from microorganisms, which can also enter the bloodstream by the surrounding skin or other devices. A severe trauma often requires bone plates and fastener implants, that are susceptible to biofilm formation because of the high concentration of microorganisms in the contaminated wound area, and once infected they generally require removal [40].

Temporary implant devices include biosensors, catheters, drug-delivery devices, bone plates, fasteners, needleless connectors and ventilator tubes [38]. The most common biosensor is the single-use blood glucose monitoring device for diabetic patients, this device operates through a membrane, where biofouling starts upon bodily contact when micro-organisms, proteins and other components adhere to the surface, impeding the sensor's diffusion ability. Failures of this biosensor can be also caused by fibrous encapsulation, electrode passivation and biodegradation [41].

Furthermore, urinary catheter calcification from bacterial colonization may cause bladder stone formation and urinary tract infections [42]. Pulmonary, transdermal, intravenous and subcutaneous drug delivery implanted devices are limited, owing to biofouling of electrode surfaces or membranes.

Needless to mention how important are the effects of fouling in the medical field, since, in addition to huge losses of money, in this case risks are posed for human health.

## Conclusions

Although the most widely known form of fouling is found in the marine environment - where biofouling colonizes ships, buoys, offshore structures, oil installations, cables, etc. – a large number of other fields are affected by this phenomenon. Fouling is recognised as a most critical factor affecting natural aquatic systems, water distribution systems, wastewater treatment systems, heat exchangers, fuel consumption by ships, and even human health.

The development of antifouling methods is an important research path and has attracted wide attention in recent years. To achieve effective solutions, fouling has to be tackled in terms of the fundamental physical, chemical, and biological processes involved, as well as by analysing its influence on energy losses and stimulating fundamental investigations on the relevant topics. Continued work in this research field is expected to deliver cheaper, more reliable solutions to this age-old problem, also drawing inspiration from nature, where flora and fauna demonstrate a multitude of antifouling lessons that can be mimicked for engineering purposes.

Rd

references


1. W.G. Characklis (1981), “Fouling Biofilm Development: A Process Analysis”, Biotechnol. Bioeng., 23, 1923-1960.
2. U.S. Pat. 4092858, 1978.
3. U.S. Pat. 6185988, 2001.
4. U.S. Pat. 5633460, 1997.
5. A. Abou-Ghazala and K. H. Schoenbach (2000), “Biofouling prevention with pulsed electric fields”, *IEEE Trans. Plasma Sci., 28, 115-121.*
6. B. Mounaouer and H. Abdennaceur (2012), “Ultraviolet radiation for microorganism inactivation in wastewater”, *J. Environ. Prot., 3(2), 194-202.*
7. C. A. Linkous, G. J. Carter, D. B. Locuson, A. J. Ouellette, D. K. Slattery and L. A. Smitha (2000), “Photocatalytic inhibition of algae growth using TiO2, WO3, and cocatalyst modifications”, *Environ. Sci. Technol., 34, 4754-4758.*
8. U.S. Pat. 6063849, 2000.
9. K. Nandakumar, H. Obika, T. Shinozaki, T. Ooie, A. Utsumi and T. Yano (2003) “Lethal and sub-lethal impacts of pulsed laser irradiations on the larvae of the fouling barnacle Balanus Amphitrite”. *Biofouling,19, 169-176.*
10. I. E. C. Mott, D. J. Stickler, W. T. Coakley and T. R. Bott. (1998), “The removal of bacterial biofilm from water-filled tubes using axially propagated ultrasound”, *J. Appl. Microbiol., 84, 509-514.*
11. S. Y. Kwak, S. H. Kim and S. S. Kim (2001), “Hybrid organic/inorganic reverse osmosis (RO) membrane for bactericidal anti-fouling”, *Environ. Sci. Technol., 35, 2388-2394.*
12. A. Whelan and F. Regan (2006), “Antifouling strategies for marine and riverine sensors”, *J. Environ. Monit., 8, 880–886.*
13. L. Li, C.H. Benson, E.M. Lawson (2005), “Impact of Mineral Fouling on Hydraulic Behavior of Permeable Reactive Barriers”, *Groundwater, 43(4), 582-596.*
14. P.D. Mackenzie, D.P. Horney, T.M. Sivavec (1999), “Mineral precipitation and porosity losses in granular iron columns”, *J. Hazard. Mater., 68(1/2), 1–17.*
15. D. Sarr (2001), “Zero-valent iron permeable reactive barriers. How long will they last?”, *Remediation, 11(2), 1–18.*
16. *D.H. Phillips, D.B. Watson, Y. Roh, and B. Gu (2003), “Mineralogical characteristics and transformations during long-te*rm operation of a zerovalent iron reactive barrier”, *J. Environ. Qual., 32(6) 2033–2045.*
17. L. Li, C.H. Benson (2010), “Evaluation of five strategies to limit the impact of fouling in permeable reactive barriers”, *J. Hazard. Mater., 181, 170–180.*
18. L.F. Greenlee, D.F. Lawler, B.D. Freeman, B. Marrot, P. Moulinc (2009), “Reverse osmosis desalination: water sources, technology, and today’s challenges”, *Water Res., 43, 2317-2348.*
19. A. Subramani, E.M.V. Hoek (2010), “Biofilm formation, cleaning, re-formation on polyamide composite membranes”, Desalination, 257, 73-79.
20. G. Kang and Y. Cao (2012), “Development of antifouling reverse osmosis membranes for water treatment: A review”, Water Res., 46, 584 -600.
21. J.S. Louie, I. Pinnau, I. Ciobanu, K.P. Ishida, A. Ng, M. Reinhard (2006), “Effects of polyether-polyamide block copolymer coating on performance and fouling of reverse osmosis membranes”, *J. Membrane Sci., 280, 762-770.*
22. D. Rana, Y. Kim, T. Matsuura, H.A. Arafat (2011), “Development of antifouling thin-film-composite membranes for seawater desalination”, *J. Membrane Sci., 367, 110-118.*
23. A.C. Sagle, E.M. Van Wagner, H. Ju, B.D. McCloskey, B.D. Freeman, M.M. Sharma (2009), “PEG-coated reverse osmosis membranes: desalination properties and fouling resistance”, *J. Membrane Sci., 340, 92-108.*
24. F.Q. Nie, Z.K. Xu, Y. Peng, J. Wu, P. Seta (2004), “Acrylonitrile-based copolymer membranes containing reactive groups: effects of surface-immobilized poly(ethylene glycol)s on antifouling properties and blood compatibility. *Polymer 45, 399-407.*
25. X. K. Ke, R. Hongqiang, D. Lili, G. Jinju, Z. Tingting (2013), “A review of membrane fouling in municipal secondary effluent reclamation”, *Environ. Sci. Pollut. Res., 20, 771-777.*
26. D.J. Barker, D.C. Stuckey (1999), “A review of soluble microbial products (SMP) in wastewater treatment systems”, *Water Res., 33, 3063–3082.*
27. M.F.A. Goosen, S.S. Sablani, H. Ai-Hinai et al. (2004), “Fouling of reverse osmosis and ultrafiltration membranes: a critical review”, *Sep. Sci. Technol., 39, 2261–2297.*
28. M.M.T. Khan, P.S. Stewart, D.J. Moll et al. (2010), “Assessing biofouling on polyamide reverse osmosis (RO) membrane surfaces in a laboratory system”, *J. Membr. Sci., 349, 429–437.*
29. D. L. Gallup (2009), “Production engineering in geothermal technology: A review”, *Geothermics, 38, 326-334.*
30. C. Ning, L. Mingyan, Z. Weidong (2012), “Fouling and Corrosion Properties of SiO2 Coatings on Copper in Geothermal Water”, *Ind. Eng. Chem. Res., 51, 6001–6017.*
31. A. Hesshaus, G. Houben, R. Kringel (2013), “Halite clogging in a deep geothermal well. Geochemical and isotopic characterisation of salt origin”, *Phys. Chem. Earth, 64, 127-139.*
32. T. Sugama (2006), “Polyphenylenesulfied/montomorillonite clay nanocomposite coatings: Their efficacy in protecting steel against corrosion”, *Mater. Lett., 60, 2700-2706.*
33. L. D. Tijing, H. Y. Kim, D. H. Lee, C. S. Kim & Y. I. Cho (2009), “Use of an Oscillating Electric Field to Mitigate Mineral Fouling in a Heat Exchanger”, *Exp. Heat Transfer, 22(4), 257-270.*
34. T.R. Bott (1995), “The Fouling of Heat Exchangers”, Elsevier Science, New York.
35. Y.I. Cho, S. Lee, W. Kim, and S. Suh (2003), “Physical water treatment for the mitigation of mineral fouling in cooling-tower water applications”, in *Refereed Proceedings: Heat Exchanger Fouling and Cleaning: Fundamentals and Applications, 1-12.*
36. Y. I. Cho, W. T. Kim & D. J. Cho (2007), “Electro-Flocculation Mechanism of Physical Water Treatment for the Mitigation of Mineral Fouling in Heat Exchangers”, *Exp. Heat Transfer, 20(4), 323-335.*
37. J. Chan, S. Wong (eds) (2010), “Biofouling types, impact and anti-fouling”, Nova Science Publishers, New York.
38. M. Millar (2005), “Microbial biofilms and clinical implants. In Surfaces and interfaces for biomaterials”, (ed. P. Vadgama), 619–631, CRC Press, Boca Raton.
39. R. L. Reis, & S. Weiner (eds) (2004), “Learning from nature how to design new implantable biomaterials”, Kluwer Academic Publishers, Norwell.
40. M. S. Khan, S. Rehman, M.A. Ali, B. Sultan & S. Sultan (2008), “Infection in orthopedic implant surgery, its risk factors and outcomes”, *J. Ayub. Med. Coll. Abbottabad.*
41. J.D. Newman & A.P.F. Turner (2005), “Home blood glucose biosensors: a commercial perspective”, *Biosens. Bioelectron., 20, 2435–2453.*
42. G.A. O’May, S.M. Jacobsen, D.J. Stickler, H.T.L Mobley & M.E. Shirtliff (eds M. Shirtliff & J. G. Leid) (2009), “Complicated urinary tract infections due to catheters”. In *The role of biofilms in device-related infections,* 123–164. Springer-Verlag, Berlin, Germany.

(BIO)FOULING AND ANTIFOULING MEASURES

# Antifouling agents for marine applications: a NeverEnding Story

The use of something toxic to combat specific biological species causing adverse effects on the human activities is quite unavoidable, but sooner or later problems caused to the environment and to not target organisms must be faced. In the case of the antifouling coatings, there is a cyclical development/production of new "products", initially considered as the final solution of the problem, and then discovered responsible of new unpredicted adverse effects. A NeverEnding Story

*DOI: 10.12910/EAI2014-39*

*Carlo Cremisini*

Marine biological fouling, usually called marine bio-fouling, can be defined as the accumulation of microorganisms, plants, and animals on artificial surfaces (ships, submerged pipelines, …) immersed in sea water. In the case of ships, the adverse effects caused by this biological settlement are well known:

- Frictional resistance, which leads to subsequent potential speed reduction. As a result, higher fuel consumption is needed, with less energy-efficient systems, and the consequent increase in emissions and transport overall costs.
- Increase in the frequency of dry-docking operations. A large amount of toxic wastes is easily generated during this process.
- Introduction of species into environments where they are not naturally present (invasive or non-native species).

The antifouling (AF) technology has developed in close association with increased maritime transportation of people and goods but, as for many other technologies, its development can be considered a NeverEnding Story. This is typical of the approach based on the use of something toxic for specific biological species causing adverse effects on human activities (agriculture, industry, transport, …). Sooner or later, problems caused to the environment and to not target organisms (sometimes modifying biological equilibria and diversity) must be faced.

This historical development of AF strategies has been very well resumed by Diego Meseguer Yebra, Søren Kiil and Kim Dam-Johansen in *Antifouling technology – past, present and future steps towards efficient and environmentally friendly antifouling coatings* [1]. In the following, a rapid summary of the main stages.

Problems caused by bio-fouling for the maritime transportation system were rapidly understood by ancient people, and so were the strategies to combat these adverse effects for more than 2000 years. In a broad sense, as already suggested in literature [2], we can find something that could be considered as the earliest citation of coating used for extending the life of vessels and preventing against bio-fouling in the first book of the Bible (Genesi 6:14)! God said to Noah "…..make yourself an ark with ribs of cypress; cover it with reeds and coat it inside and outside with pitch" (Figure 1).

Many authors and historians (e.g., P. Cintas in several studies on ancient civilizations in the Mediterranean Sea, and F. Braudel in *Les Mémoires de la Mediterranée* [3]) attribute the incredible fame of Phoenicians as

**Carlo Cremisini**
*ENEA, Head of Technical Unit for Environmental Characterization, Prevention, and Remediation*

the best sailors in the world to the use of pitch from the Black Sea for protecting the hulls of their boats. In fact, Phoenicians and Carthaginians widely used pitch. There is some evidence that metal sheets on wooden vessels were probably used also in the 1500–300 BC period [4], but this is more difficult to prove. In a translation from the Aramaic of a papyrus dated about 412 BC, concerning boat repairs, the following note was found: "And the arsenic and sulphur have been well mixed with Chian oil thou broughtest back on thy last voyage and the mixture evenly applied to the vessel's sides that she may speed through the blue waters freely and without impediment" [5].

Greeks and Romans used similar approaches sometimes, including arsenic and sulphur mixed with oils to prevent against the attack of shipworms [6].

The Chinese Admiral Cheng Ho had the hulls of his junks coated with lime mixed with poisonous oil to protect wood from worms [5]. From the 13th to 15th century pitch, blended with several other components such as oils, resin, tallow, were widely used.

It is interesting to remind that Leonardo da Vinci invented a rolling mill for making sheet lead. One of the first attested reference about underwater use of copper was in 1618, during the reign of the Danish King Christian IV, mentioning the use of copper for sheltering keel and rudder. In the same period we can find one of the first record of the use of copper (copper sulphide or a copper/arsenic compound) as an antifoulant in a British patent (William Beale, 1625).

In the second part of 1700's copper was widely used, especially in British Navy, even if only later its antifouling mechanism of action (based on the dissolution of copper in the seawater) was studied and demonstrated (sir H. Davy). The good results of copper sheltering were evident in the famous Trafalgar battle. Among the factors contributing to the victory of the British Navy, the use of copper was considered one of the most important. 3923 copper shelters were fixed to the hull with more than 550.000 rivets on the vessel Victory, commanded by Adm. Nelson. Actually, copper is an effective and (still) widely used biocide, however its effectiveness is relatively short (maximum 2 years, but often a few months ), so dry dockings of vessels for cleaning and paint reapplication are frequently required.


**FIGURE 1** God said to Noah "….. .make yourself an ark with ribs of cypress; cover it with reeds and coat it inside and outside with pitch" (Genesis 6:14)

After the introduction of iron ships at the end of the 18th century, the use of copper sheathing was drastically reduced [4, 7, 8], due to its corrosive effects on iron, and several alternatives were tried, including sheathings of zinc, lead, nickel, arsenic, galvanised iron and alloys of antimony, zinc and tin, followed by wooden sheathing, which was then coppered [1, 6].

Consequently, in this period a variety of paints based on the mixing of one or more toxicants in a "polymeric" matrix started to be developed. So, by the late 18th and into the 19th centuries, coatings containing copper, arsenic and mercury were increasingly applied to vessel hulls [5]. It is easily understandable that until recent times, the environmental concern on the use of these toxicants was absolutely disregarded.

Mallet in 1841, William John Hay in 1847, James McInness in 1860 patented antifouling paints based on the use of different "poisonous materials", mixed with or applied over a coat of varnish, and James Tarr and Augustus Wonson in 1863 patented an A/F paint using copper oxide in tar with naphtha [5].

The "Italian Moravian" and McInness' "hot-plastic paints", shellac type paints (active in the prevention

of rust), and various copper paints have been widely used for a long time.
For about 50 years a considerable number of products based on these principles have been developed, thereafter substituted by the so-called "cold-plastic paints", easier to apply and effectively decreasing fouling and extending up to 18 months the period between dry-dock times for re-painting.
After World War II, important changes took place in the AF paints industry. During this period, studies on organotins and their AF properties improved the performance of AF paints and offered a great contribution to the solution of the problem. Van de Kerk and co-workers [9, 10] already described the efficacy of the TBT-containing products in the 1950s. Organotins have been widely used in copper-based paints, at first in the so-called "free association form" [11]. The paints used at that time can be classified into insoluble matrix type and soluble matrix type, according to their water solubility.
In the following, a rapid description of different types of TBT paints, based on different approaches, just to give an idea of the level of complexity of the technologies investigated.
Tributyltin Free Association Paints: in these paints the antifouling agents are dispersed in a resinous matrix from which they can, more or less, slowly leach. The control of the rate of release of biocides from a free association paint system and the constant leaching level is quite complex to achieve and it is difficult to make theoretical previsions in terms of environmental risks. However, results of monitoring programs suggest that paints containing freely associated biocides (the most widely used copper compounds and TBT), can be considered as the main cause of relatively high initial concentrations of biocides in the marine environment.
TBT Self Polishing Copolymer Paints (SPC): in these paints copolymer systems are based on a combination of biologically active resins and antifouling agents, such as TBT copolymer resins and copper compounds. TBT react by hydrolysis with the seawater, resulting in the slow release which combats fouling. The remaining surface of the paint is continuously eroded by the seawater action, resulting in the exposure of a fresh surface of TBT polymer. This hydrolysis/erosion process continues until no paint is left on the surface and this process confers two key properties on the TBT copolymer paint system: increased ability to control/regulate the biocide leaching rate and smoother surfaces as a result of the erosion process [12-13].
As already mentioned, the development of TBT (tributyltin) as an antifouling agent in conventional coatings started in1960s. TBT-based coatings allowed to control the biocide release rates, but quite early adverse effects on the marine ecosystems appeared: already in 1974, oyster farmers reported abnormal shell growth while in the 1980s TBT was clearly demonstrated to be linked to shell abnormalities in oysters *(Crassostrea gigas)* and imposex in dogwhelks *(Nucella lapillus)*. So in 1987–90 TBT coatings were prohibited on vessels <25 m in France, UK, USA, Canada, Australia, EU, NZ and Japan, followed by other Countries worldwide.
Several studies demonstrated the problems caused to the marine environment and monitoring campaigns also started in Italy [14-16].
In the meanwhile, from the 1990s to present time, copper release rate restrictions were introduced in Denmark and considered elsewhere (e.g., California, USA).
The International Maritime Organisation (IMO) adopted (2001) the "AFS Convention" to eliminate TBT from AF coatings from vessels imposing the following steps: 2003 – prohibition of further application of TBT; 2008 – prohibition of active TBT presence; finally the IMO "AFS Convention" entered-into-force (2008).
Coming back to the NeverEnding Story, starting from the 2000's, the research into "environmentally friendly" AF alternatives increased, but as frequently happens in these situations, the alternatives themselves started to pose new "alternative" problems [16-17]. Again, in the last few years eco-toxicological assessments have been made in Italy's marine coastal environment [18-24].
One of the approaches widely used, considering that some algal groups are tolerant to copper [25], was based on the fortifying paints with additional 'booster' biocides, aimed at targeting hull colonisations by micro- and macro-algae. Several algal toxic compounds have been tested worldwide including chlorothalonil, dichlofluanid, Irgarol 1051, TCMS pyridine, thiocyanatomethylthiobenzothiazole (TCMTB), diuron, dichloro-octylisothiazolin (DCOIT, Sea Nine 211), zinc and copper pyrithione (Zinc and Copper Omadine) and zineb [26-29].

These are often herbicides (e.g., Irgarol 1051 and diuron, but also fungicides) that have negative effects on the growth rate of photosynthetic organisms. Legislation now exists in some countries to regulate the use of some 'booster' biocides in AF paints such as, for example, diuron and Irgarol 1051. In the UK, a review of booster biocides in 2000 resulted in only four biocides gaining approval (dichlofluanid, DCOIT (Trade name: Sea Nine 211), zinc pyrithione and zineb). Approvals of chlorothalonil, diuron and Irgarol 1051 were revoked due to their high toxicity at low concentrations and their persistence in the environment [30]; Irgarol 1051 and diuron are also banned in Denmark (DEPA, 2008), and diuron is banned in the Netherlands. The use of Irgarol 1051 in AF paints is not permitted in Australia as it was not granted approval for use as an AF biocide by the Australian Pesticides and Veterinary Medicines Authority (APVMA), when its presence was detected and the risks it posed assessed in the 1990s. Applications for approval have been submitted to the European Union for eleven AF biocides, including copper (II) oxide, copper thiocyanate and Irgarol 1051, but not diuron [31].

The increased consciousness of the impacts on the marine environment resulting from the use of toxic AF paints has induced investments on research and development of non-toxic alternatives, such as foul-release coatings that incorporate silicone elastomers, waxes or silicone oils, and "natural" coatings in which AF compounds are sourced from algae and other marine organisms [32].

Foul-release coatings currently on the market include silicone (e.g., Intersleek 700, Sealion and Bioclean), fluoropolymer (e.g., Intersleek 900), hybrid (e.g., Phasecoat UFR) and hydrogel silicone (e.g., Hempasil X3) coatings (Townsin and Anderson, in [32]).

"Natural" coatings however are not currently in commercial use due to the difficulties in sourcing a supply of natural AF compounds at a reasonable cost in addition to meeting the requirements of environmental regulation agencies [1].

At the moment no alternatives seem to be promising to replace biocide-based A/F coatings [33]. Hence, a considerable part of the efforts are still concentrated on the study of new binder systems better regulating the release of booster biocides. Future regulatory decisions in favour of non-toxic alternatives in antifouling paints could shift the balance and force these products into commercial use.

One possibility is the attempt to prevent the adhesion of fouling organisms by developing ultra-smooth surfaces, making the settling of organisms difficult. Brady made a summary of the most significant properties of coatings necessary to obtain satisfactory results [34], but again the main requirement is to be physically and chemically stable for prolonged periods in the marine environment. These properties are owned by fluoropolymers and silicones, but many other materials are being continuously developed. Nevertheless, modest results evidenced the still limited efficacy of fouling release properties of these coatings; moreover, the advantages of these technology seems limited to fast-moving vessels, at the moment.

The other interesting approach is the study of the AF natural protection of marine living organisms such as wales. The attempt to reproduce the microtexture of the surface of their body is fascinating, but again results are modest so far.

The use of microstatically charged microfibres to obtain the "furry" surface effect was supposed to prevent hard biofouling from settling. Again doubtful results were obtained.

In theory the application of UV, ultrasonic, laser beams could be used by automated systems (robot technology): underwater cleaning is potentially cost-effective with respect to the cleaning procedures in a dry-dock. This approach needs further developments.

The last research frontier could be the development of a coating capable of selectively releasing bioactive substances after artificial (electricity, ultrasound..) or natural (temperature or fouling adhesives themselves) stimulation [1].

## Conclusions

Two main topics, of scientific/technological and philosophical/ethical nature and both related to the environmental concerns, will probably drive research on A/F coatings. The optimization of a reliable A/F paint performance model could be a powerful tool for a rational screening of new ideas eliminating the weak ones at the early stages of the development process. At the same time, studies on the adhesion mechanisms and biological characte-

ristics of the fouling processes need to be continued. It is however fundamental to find a compromise between industrial and academic needs: environmental eco-toxicological assessment as well as scientific investigations are necessary even if costly and time-consuming. This can only be achieved defining clearly the acception of the term "sustainabilty" on a global scale, also in the case of A/F coatings development, production and use addressing research towards acceptable alternative solutions, balancing economic and environmental sustainability.


references

1. Diego Meseguer Yebra, Søren Kiil, Kim Dam-Johansen, (2004), Antifouling technology—past, present and future steps towards efficient and environmentally friendly antifouling coatings. *Progress in Organic Coatings* 50, 75–104.
2. Peter Walters, (2011), A selection of historical paint references Painted Memories articles from– March – Antifouling. Coatings Societies International Surface coatings association New Zealand. http://www.scanz.org.nz _ Genesi 6: 14.
3. Fernand Braudel, (1998) Les Mémoires de la Mediterranée, édition de Fallois ISBN 978-88-58-72560-3 (2010 in Tascabili Bombiani – Le memorie del Mediterraneo).
4. I. Lunn, (1974), Antifouling: a brief introduction to the origins and developments of the marine antifouling industry. BCA Publications, Thame, UK.
5. From http://www.international-marine.com/antifoulings/history-of-fouling control.aspx
6. M.E. Callow (1990) Ship fouling: Problems and solutions. *Chem Ind,* 5, 123-127.
7. WHOI, (1952), Marine Fouling and Its Prevention. Woods Hole Oceanographic Institute, Annapolis, Iselin, COD, USA.
8. J.A.Lewis, 1998, Marine biofouling and its prevention on underwater surfaces. *Mater. Forum* 22, 41–61.
9. M.H. Gitlitz, (1981), *J. Coat. Technol.,* 53(678) 46-52.
10. Van der Kerk, G. J. M., and Luijten, J. G. A. (1954). Investigations on organotin compounds. III. The biocidal properties of organotin compounds. *J. Appl. Chem.* 4, 314-319
11. I. Omae, (2003) "Organotin antifouling paints and their alternatives" in *Appl. Organomet. Chem.,* 17(2) 81-105.
12. Organotin Environmental Programme (ORTEP Association) http://www.ortepa.org/pages/b1pt5.htm
13. R.F. Bennett (1996). Industrial manufacture and applications of tributyltin compounds. In: Tributyltin: Case Study of an Environmental Contaminant, editor S.J. de Mora. Cambridge University Press, Cambridge, 21-61 pp.
14. Champ M., (2000), A review of organotin regulatory strategies, pending actions, related costs and benefits. *Sci Total Environ* 258 21–71.
15. James W. Readman, (2006), Development, Occurrence and Regulation of Antifouling Paint Biocides: Historical Reviewand Future Trends. *Hdb Env Chem* Vol. 5(p.O), 1–15.
16. Katherine A. Dafforn, John A. Lewis, Emma L. Johnston, (2011), Antifouling strategies: History and regulation, ecological impacts and mitigation. *Marine Pollution Bulletin* 62, 453–465.
17. S. Chiavarini, C. Cremisini and R. Morabito, (1996), Organotin compounds in marine organisms. In: "Element speciation in bioinorganic chemistry", S. Caroli Ed., J. Wiley & Sons Publ., Chap. 9, pp. 287-329.
18. S. Chiavarini, C. Cremisini, R. Morabito, A.M. Caricchia, R. Scerbo, (1991), Organotin compounds in marine mussels from italian coastal environment. *Analytical Sciences,* 7 1193-1196.
19. S. Manzo, S. Buono, C. Cremisini, (2008), Predictability of copper, Irgarol, and Diuron combined effects on sea Urchin Paracentrotus lividus. *Arch. Environ. Contam. Toxicol.* 54, 57–68.
20. S. Manzo, S. Buono, C. Cremisini, (2006), Toxic effects of Irgarol and Diuron on sea urchin Paracentrotus lividus early development, fertilisation and offspring quality. *Archives Environ. Contam. Toxicol.,* 51(1), 61-68.
21. G. Di Landa, G. Ansanelli, R. Ciccoli, C. Cremisini, (2006), Occurence of antifouling paint booster biocides in selescted harbours and marinas inside the Gulf of Napoli: a preliminary survey. *Marine Pollution Bulletin* 52(119) 1541-1546.
22. S. Manzo, S. Buono, C. Cremisini, (2008), Predictability of copper, Irgarol and Diuron combined effects on sea urchin. *Archives Environ. Contam. Toxicol.,* 54(1) 57-68.
23. G. Di Landa, L. Parrella, S. Avagliano, G. Ansanelli, E. Maiello, C. Cremisini, (2009), Assessment of the potential ecological risk posed by antifouling booster biocides to the marine ecosystem of the Gulf of Napoli (Italy). *Water Air and Soil Pollution.* 200 (1-4) 305-321.
24. Manzo S., Buono S., Cremisini C. Cadmium, (2010), Lead and their mixtures with Copper: Paracentrotus lividus embryotoxicity assessment, prediction and offspring quality evaluation. *Ecotoxicology* 19(7) 1209-1223.
25. A.B.A. Boxall, S.D. Comber, A.U. Conrad, J. Howcroft, N. Zaman, (2000), Imputs, monitoring and fate modelling of antifouling biocides in UK estuaries, *Marine Pollution Bulletin* 40 898-905.
26. R.H. Reed, L. Moffat (1983). Copper toxicity and copper tolerance in Enteromorpha compressa (L.) *Grev. J. Exp. Mar. Biol. Ecol.* 69 85–103.
27. N. Voulvoulis (2006) Antifouling paint booster biocides: occurrence and partitioning in water and sediments. In: Konstantinou I (ed) The handbook of environmental chemistry - 5.0 Antifouling Paint Biocides. Springer, New York, pp 155–170.
28. Voulvoulis N, Scrimshaw MD, Lester JN (1999) Alternative antifouling biocides. *Appl Organomet Chem* 13 135–143
29. K.V. Thomas, M. McHugh, M. Waldock (2002) Antifouling paint booster biocides in UK coastal waters: inputs, occurrence and environmental fate. *Sci Total Environ* 293 117–127.
30. T. Cresswell, J.P. Richards, G.A. Glegg, J.W. Readman (2006) The impact of legislation on the usage and environmental concentrations of Irgarol 1051 in UK coastal waters, *Marine Pollution Bulletin* 52 1169–1175.
31. M. Pereira, C. Ankjaergaard (2009) Advances in Marine Antifouling Coatings and Technologies. Woodshead Publishing, Cambridge, UK p. 240.
32. C. Hellio, J.P. Marechal, B.A.P.Da Gama, R.C. Pereira and A.S. Clare (2009). Marine natural products with antifouling activities. In C. Hellio and Yebra, DM. (Eds.). *Advances in Antifouling Coatings and Technologies,* p. 572-622.
33. Chambers L D, Stokes K R, Walsh F C, et al., (2006), Modern approaches to marine antifouling coatings. *Surf Coat Technol,* 201 3642–3652.
34. R. F. Brady (1999), *Prog. Org. Coat,* 35 31-35.

(BIO)FOULING AND ANTIFOULING MEASURES

# Initial phase of biofouling: the microbial biofilm formation

The biofouling formation is a sequential process that starts with the adsorption of organic macromolecules (proteins, glycoproteins and polysaccharides). The second step, is characterized by the adhesion of prokaryotes and the subsequent development of a bacterial biofilm starting to produce a matrix of Extracellular Polymeric Substances (EPS). Here we will discuss how the bacterial community composition can be assessed during the initial phases of the biofilm development by the CAtalyzed Reporter Deposition Fluorescence In Situ Hybridization (CARD–FISH), in combination with Confocal Laser Scanning Microscopy (CLSM).
Understanding the first steps of the biofilm development process is of crucial importance for micro and macro fouling control and prevention

*DOI: 10.12910/EAI2014-40*

*Stefano Fazi, Ilaria Pizzetti, Francesca Di Pippo*

## Introduction

In the marine environment, all immersed surfaces (natural or artificial) are rapidly colonized by a succession of organisms, the outcome being known as 'biofouling' (Figure 1). Within the first hours, surfaces are covered by microbial biofilms (microfouling) [1], that influence the successive settlement, colonization and growth of macroorganisms (macrofouling) [2]. Biofilm formation is then followed within a week by diatoms (microalgae), spores of macroalgae (seaweeds), protists, fungi and protozoa, followed in turn by larvae of invertebrates such as barnacles (Linear successional surface colonization model) [3, 4, 5, 6, 7].
The implications of microbial biofilms in marine biology, and especially in relation to biofouling, have also been extensively studied, and a wide type of specific interactions between microbial biofilms (Figures 1 and 2) and fouling organisms [8, 9, 10, 11, 12] have been revealed. Microbial biofilms were shown to influence the settlement of marine organisms decades ago [13]. More recently, the formation, composition and physiology of bacterial biofilms have been studied, including their role in the environment [14, 15, 16, 17]. It is now well established that density-dependent, cell-to-cell communication processes between bacteria, generally referred to as 'quorum sensing', control several important features of biofilms (e.g., development, virulence and dispersal stages) [18, 19, 20, 21].

## Initial stage of biofilm development: bacterial colonization, matrix formation and maturation

Bacteria are considered to be the primary colonizers of substrata, constituting the initial stage of biofilm development. By encountering surfaces, free-swimming microbial cells can switch from a planktonic to a benthonic lifestyle exuding a slimy matrix and forming complex and dynamic communities with high phenotypic diversification and high degree of cellular coordination [22].

**Stefano Fazi, Ilaria Pizzetti**
*Water Research Institute - National Research Council (IRSA-CNR), Monterotondo – Rome*

**Francesca Di Pippo**
*Water Research Institute - National Research Council (IRSA-CNR) Institute for Coastal Marine Environment - National Research Council (IAMC-CNR), Sa Mardini, Torregrande, Oristano*

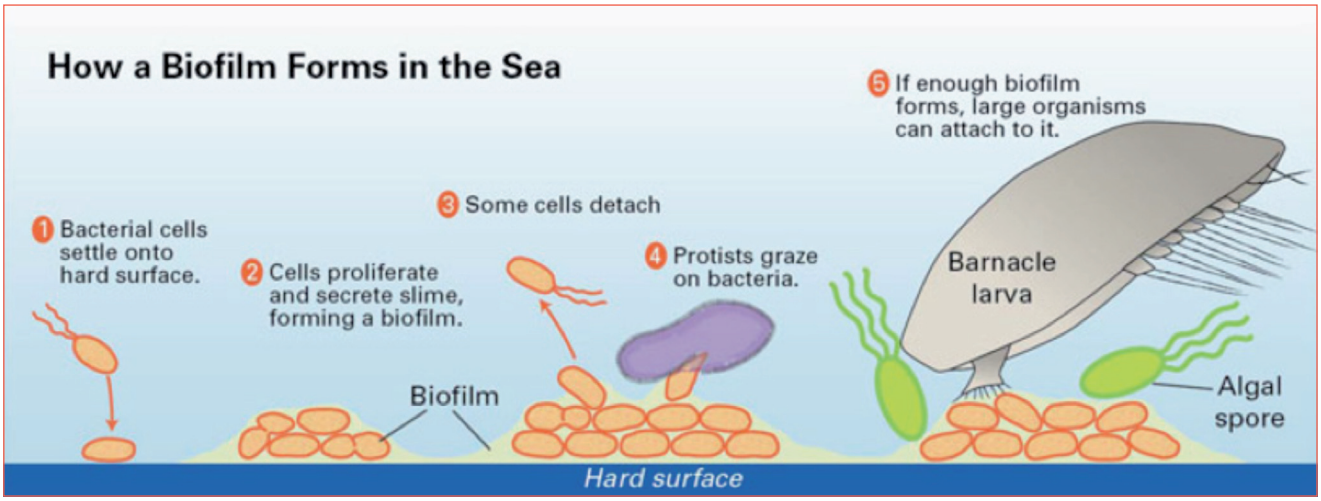



**FIGURE 1** Microbial biofilm formation. Modified from http://www.whoi.edu/oceanus/illustrations

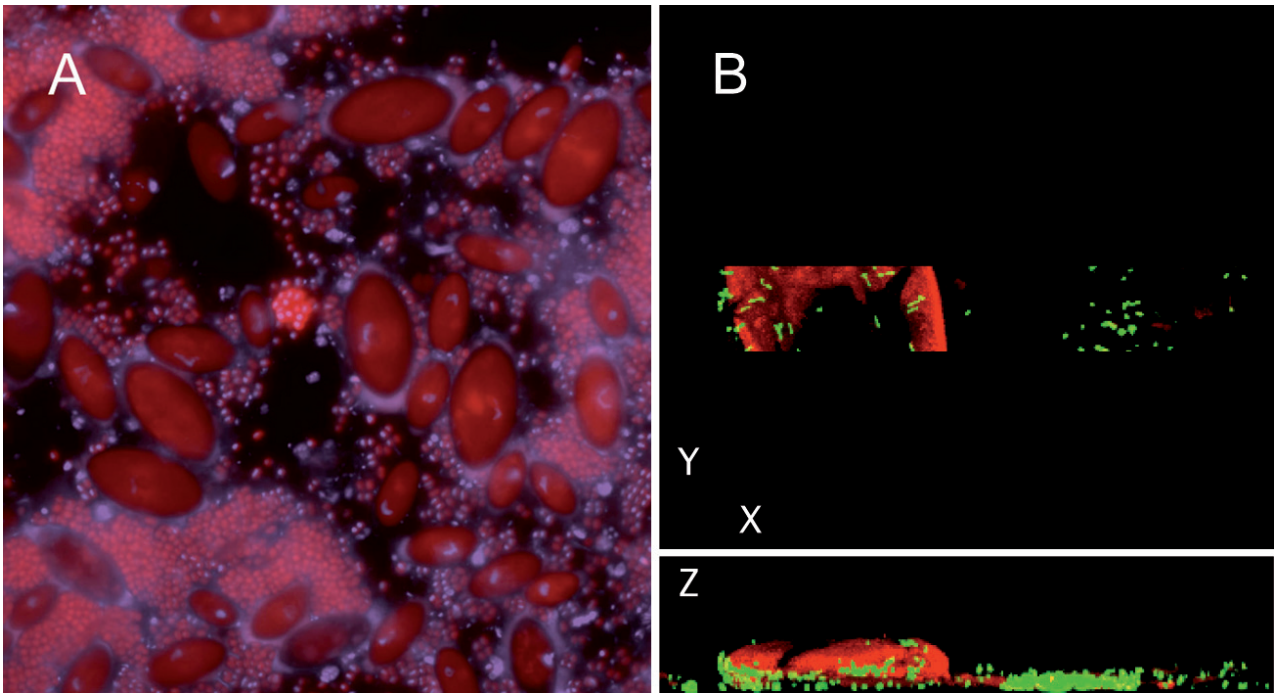



**FIGURE 2** A) Epifluorescence micrograph of biofilm. Blue, DAPI signals of bacteria; the red signal was due to the Chlorophyll a autofluorescence in cyanobacterial and microalgal cells. B) CLSM images showing the spatial distribution of bacteria (X-Y plane) and the biofilm thickness (X-Z plane), as determined by CARD-FISH. The autofluorescence of the photosynthetic pigments (Chl a) was detected with the 633-nm line of an Ar/HeNe laser (excitation) and observed in the red and far-red channels at 590 to 800 nm (emission). The hybridized cells were excited with the 488-nm line of an Ar laser and observed in the green channel from 490 to 530 nm (adapted from Lupini et al. [39])

Rd

Biofilms are now recognized as matrix-enclosed, attached microbial communities that can develop highly differentiated architectures, including mushroom-like structures, ripples and ridges, or filamentous streamers floating in the bulk liquid. The extracellular matrix is a key factor for the overall biofilm functionality. It is a highly hydrated system composed of extracellular polymeric substances (EPS) comprising exopolysaccharides, along with a wide variety of proteins, nucleic acids, glycoproteins, phospholipids, glycolipids and humic substances [14, 15, 23, 24]. Although the precise and molecular interactions of the various secreted biofilm matrix polymers have not been defined, and the contributions of these components to matrix integrity are poorly understood at the molecular level [25], several functions of EPS have been determined. Independent of the EPS composition, the matrix typically features a hydrogel-like structure, which embeds the biofilm cells and determines the physico-chemical and biological properties of the whole biofilm [26, 27].

The matrix network encloses and holds together the microorganisms in the biofilm, providing mechanical stability to the community [26, 27], which is the major advantage of the biofilm mode of life for microorganisms [28]. In addition, EPS are thought to play an important role in the adhesion of cells to substrata. This allows the formation of stable and functional microconsortia with a low expense of energy, allowing cells to metabolise, reproduce and communicate between each other more efficiently [29]. In addition to the advantages of mechanical stability, the matrix also provides protection against heavy metals, other toxic substances and grazing by predators. The intense research on single- or multi-species biofilms grown in flow cells have also unravelled many microbial interactions (competition, cooperation), largely deterministic in nature, due to the coexistence of niche differentiation [22].

In the wild, biofilms are open and dynamic communities and are part of a larger network; some authors suggested a new ecological concept of biofilms, and by viewing biofilms as microbial landscapes, studied their community assembly according to the metacommunity ecology theory [19, 22, 30]. The formation of phototrophic biofilms is a complex process, regulated by diverse hydrodynamic and chemical characteristics of the surrounding water, preconditioning of the substratum, cell surface characteristics, EPS secretion [30, 31, 32]. As biofilms develop, competition for resources such as nutrients, light, and space, is believed to select those species that are more competitive for a limiting resource. Oxygenic phototrophic microorganisms such as benthic diatoms, unicellular and filamentous cyanobacteria, and benthic green algae generate energy and reduce carbon dioxide, providing organic substrates and oxygen. This photosynthetic activity fuels metabolic processes and conversions in the entire biofilm community, including the heterotrophic fraction [33].

The utilization of $CO_2$ during photosynthesis results in steep vertical redox and chemical gradients that enforce the stratification in these communities along the microenvironments, restricting phototrophic microorganisms to the upper layer of the biofilm, most anoxygenic phototrophs and anaerobic chemotrophs to the lower part. With the increasing complexity of maturing biofilms, competition for resources is likely to support high species diversity and spatial heterogeneity, as a result of concurrent functional niche diversification within the biofilm [19].

## Single-cell approach and CLSM to study the biofilm 3D architecture

Currently, increasing attention is being paid to biofilms that develop on artificial substrata immersed in seawater [34, 35, 36, 37, 38]. However, microbial biofilms in aquatic environments are very heterogeneous and dynamic systems, which makes them difficult to model and investigate. In marine biofilms developed on unpainted artificial surfaces, microbial communities mainly consist of bacteria and diatoms [39]. Proteobacteria, especially a-proteobacteria, appear dominant among these bacterial communities [40, 41, 42, 43], but the population dynamics depends on several environmental factors. Marine biofilm communities have also been reported as a potential source of pathogenic bacteria [44, 45]. However, bacterial communities grown on dissimilar surfaces appeared to evolve and become more similar over time, as determined by Denaturing Gradient Gel Electrophoresis (DGGE) and Fluorescence In Situ Hybridization (FISH) [41,42]. By

using Fluorescence In Situ Hybridization techniques (e.g. CARD-FISH), the bacterial community composition can be documented, but loosing information on the spatial distribution of specific bacterial clusters, which is due to the destruction of the biofilm structure by scraping and filtering [46, 47, 48]. When it comes to the possibility of visualizing specific cells while maintaining the 3D structure of the biofilm unaltered, there have been substantial improvements made by utilizing FISH in combination with Confocal Laser Scanning Microscopy (see CLSM-FISH in [49]). A limited number of studies have demonstrated the direct use of CLSM-FISH on a biofilm attached to an artificial or natural substratum (e.g. polycarbonate slides – [50]; clay beads – [51]; polystyrene beads – [52]; marine algae – [53]). Several attempts have recently been made by embedding biofilms on gel pads [54] or by using crio-sectioning [55, 56, 57, 58]. However, such additional manipulation can potentially lead to a loss of mass and/or distortion of the in situ perspective [52].

We optimized a straightforward CARD-FISH protocol in combination with CSLM for the hybridization and the inspection of biofilms attached to the original substrate [59]. Thus, the protocol allows the simultaneous identification and the spatial localization of cells, while maintaining the natural architecture of the biofilm unal-

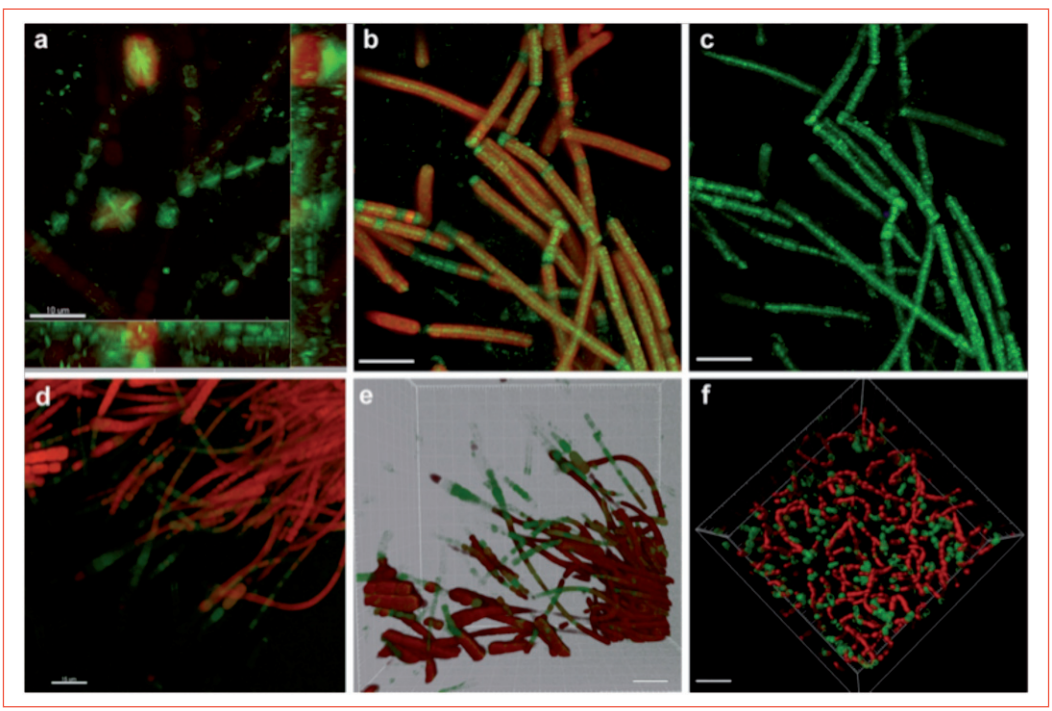



**FIGURE 3** CLSM images after staining with fluorochromes. (a) Reaction of *Anabaena augstumalis* biofilm to concanavalin A-Alexa Fluor 488-conjugate, showing neutral polysaccaridic material (green signal, Capsular Polysaccharides matrix-forming) deposited around the vegetative cells and akinetes, where filaments attached to the substratum; (b, c) fine neutral polysaccharidic envelope surrounding the filament of *P. autumnale* biofilm; (d, e) *Calothrix* sp. biofilm after staining with concanavalin A-Alexa Fluor 488-conjugate, showing the positive reaction of the basal part of the filament and the 3D reconstruction image of the filaments of *Calothrix* sp. biofilm and the envelopes around the basal part; (f) 3D reconstruction of the reaction of *Nostoc* sp. biofilm with concanavalin A-Alexa Fluor 488-conjugate, showing the presence of an envelope around the heterocysts (green). The red signal was due to the Chl *a* autofluorescence in vegetative cells and akinetes. Scale bars: 10 μm in a-c and 15 μm in d-f (adapted from Di Pippo et al. [19])

tered. Due to the impracticality of applying the CARD-FISH protocol to the biofilm grown on flat microscope glass slides, traditionally used for the analysis of the epiphytic community in flowing water systems [60], we utilized chambered slides (10-well diagnostic microscope epoxy coated slides; well diameter: 6.7 mm - Thermo Scientific, Germany). The slides were randomly collected in triplicate and then fixed in formaline (2% final concentration). The slide surfaces around the wells were gently cleaned with a small tampon to remove the biofilm grown in-between the wells, thus avoiding buffer scramble during the procedure. CARD-FISH was performed, optimising the protocol for the analysis of bacterial cells on polycarbonate membrane after sample filtration described by Fazi et al. [61, 62] (for details, see [59]).

CLSM can also be utilized to study the following stages of microfouling, when microalgal and cyanobacterial microconsortia colonize the bacterial layers [63], allowing the formation of phototrophic biofilms. Confocal microscopy provides information on the morphology of the biofilm-forming microorganisms, their spatial distribution, relationships with substrata and the interactions among microbial members. The use of CLSM in a multichannel mode allows the visualization of the spatial distribution of cyanobacteria and associated microalgae, bacteria and archea in phototrohic biofilms as well as the distribution of EPS components by collecting series of optical sections at the appropriate excitation and emission wavelengths (Figure 3). The different channels map individual biofilm components, detecting differences in the biofilm-forming phototrophic cells thanks to their specific autofluorescence, due to their intrinsic content in chlorophylls and phycobiliproteins absorbing in different wavelenghts. The superimposition of optical sections results in 2D and 3D images that show the cellular and sub-cellular heterogeneous distribution along the biofilm. Since the CLSM techniques guarantee the structural integrity of biofilm communities, it is possible to evaluate the distribution of the different exopolymers that constitute the matrix by using different fluorochromes to bind glycoconjugates, proteins and nucleic acids. We used different fluorochromes on monospecific cyanobacterial biofilms at the initial stage of development, and the CLSM observations have shown neutral exopolysaccharides specifically deposited within the envelope around the cells, especially where filaments attach to the substratum. Our results, based on CLSM observation, highlights how the diverse compositions of exopolysaccharides surrounding vegetative cells reflect the different roles of polymers at different positions (Figure 3).

## Conclusion

Bacterial successional changes can be described by applying the CARD-FISH protocol to intact biofilms, thereby avoiding biofilm detachment or manipulations. Our approach, in combination with an appropriate spatial analysis, could contribute to elucidate how specific bacterial clusters participate in the development of the complex biofilm structures and the mechanisms that regulate community composition dynamic and cell dispersion in aquatic environments. Moreover, thanks to the intrinsic content in pigments of phototrophic cells and the use of fluorochromes, EPS-binding is possible to obtain information on spatial distribution of cyanobacterial, algal and exopolymeric components of phototrophic biofilms. These technologies help to understand the first steps of the biofilm development process for micro-and macro-fouling control and prevention.

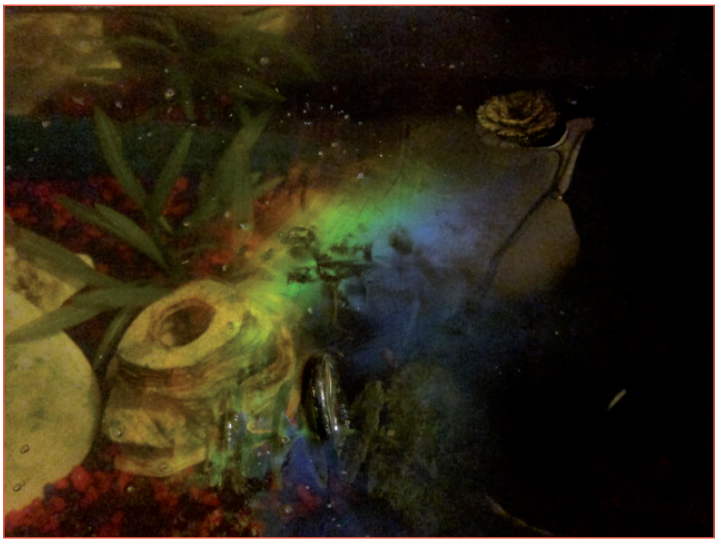
references


1. D.G. Allison (2003), Molecular architecture of the biofilmmatrix, in: Biofilms in medicine, industry, and environment technology, p. 81–90, Lens P. editor, IWA Publishing, London.
2. R. Amann, H. Lemmer & M. Wagner (1998), Monitoring the community structure of wastewater treatment plants: a comparison of old and new techniques, FEMS Microbio Ecol 25:205-215.
3. L.C. Antunes, R.B. Ferreira, M.M. Buckner & B.B. Finlay (2010), Quorum sensing in bacterial virulence, Microbiology 156:2271–2282.
4. T.J. Battin, W.T. Sloan, S. Kjelleberg, H. Daims, I.M. Head, T.P. Curtis & L. Eberl (2007), Microbial landscape: new paths to biofilm research, Nature 5:76–81.
5. T.J. Battin, L.A. Kaplan, J.D. Newbold & C.M.E. Hansen (2003), Contributions of microbial biofilms developing under various external factors, Eur J Phycol 40:1-8.
6. K. Besemer, H. Peter, J.B. Logue, S. Langenheder, E.S. Lindtröm, L.J. Tranvik & T.J. Battin (2012), Unraveling assembly of stream biofilm communities, The ISME J 6:1459-1468.
7. K. Besemer, G. Singer, R. Limberger, A.K. Chlup, G. Hochedlinger, I. Hodl, C. Baranyi & T.J. Battin (2007), Biophysical controls on community succession in stream biofilms, Appl Environ Microbiol 73(15):4966-4974.
8. B. Braun, I. Richert & U. Szewzyk (2009), Detection of iron-depositing Pedomicrobium species in native biofilms from the Odertal National Park by a new, specific FISH probe, J Microbiol Methods 79:37-43.
9. J.F. Briand, I. Djeridi, D. Jamet, S. Coupé, C. Bressy, M. Molmeret, B. Le Berre, F. Rimet, A. Bouchez & Y. Blache (2012), Pioneer marine biofilms on artificial surfaces including anti-fouling coatings immersed in two contrasting FrenchMediterranean coast sites, Biofouling 28:453–463.
10. J.A. Callow & M.E. Callow (2006), Biofilms in antifouling compounds, in: Progress in molecular and subcellular biology: marine molecular bio-technology. Fusetani N. and Clare A.S. editors, p. 141–169, Springer-Verlag, Berlin.
11. F. Casse' & G.W. Swain (2006), The development of microfouling on four commercial antifouling coatings under static and dynamic immersion, Int Biodeterior Biodegr 57:179–185.
12. L.D. Chambers, K.R. Stokes, F.C. Walsh & R.J.K. Wood (2006), Modern approaches to marine antifouling coatings, Surf Coat Technol 201:3642–3652.
13. H.C. Chung, O.O. Lee, Y.L. Huang, S.Y. Mok, R. Kolter & P.Y. Qian (2010), Bacterial community succession and chemical profiles of subtidal biofilms in relation to larval settlement of the polychaete Hydroides elegans, ISME J 4:817–828.
14. J.W. Costerton, P.S. Stewart & E.P. Greenberg (1999), Bacterial biofilms: a common cause of persistent infections, Science 284:1318–1322.
15. H. Daims & M. Wagner (2007), Quantification of uncultured microorganisms by fluorescence microscopy and digital image analysis, Appl Microbiol Biotechnol 75:237-248.
16. H. Dang & C.R. Lovell (2000), Bacterial primary colonization and early succession on surfaces in marine waters as determined by amplified rRNA gene restriction analysis and sequence analysis of 16S rRNA genes, Appl Environ Microbiol 66:467–475.
17. A.W. Decho (2000), Microbial biofilms in intertidal systems: an overview, Cont Shelf Res 20:1257-1273.
18. R. Delatolla, N. Tufenkji, Y. Comeau, D. Lamarre, A. Gadbois & D. Berk (2009), In situ characterization of nitrifying biofilm: Minimizing biomass loss and preserving perspective, Water Res 43:1775-1787.
19. F. Di Pippo, N. Ellwood, A. Gismondi, L. Bruno, F. Rossi, P. Magni et al. (2013) Characterization of exopolysaccharides produced by seven biofilm-forming cyanobacterial strains for biotechnological applications, J Appl Phycol 25:1697-1708.
20. S. Dobretsov & J.C. Thomason (2011), The development of marine biofilms on two commercial non-biocidal coatings: a comparison between silicone and fluoropolymer technologies, Biofouling 27:869–880.
21. S. Dobretsov, M. Teplitski & V. Paul (2009), Mini-review: quorum sensing in the marine environment and its relationship to biofouling, Biofouling 25:413–427.
22. S.V. Dobretsov & P.Y. Qian (2002), Effect of bacteria associated with the green alga Ulva reticulate on marine micro- and macro-fouling, Biofouling 18:217–228.
23. R.M. Donlan (2002), Biofilms: Microbial Life on Surfaces, Emerg Infect Dis 8(9):881-890.
24. S. Fazi, S. Amalfitano, J. Pernthaler & A. Puddu (2005), Bacterial communities associated with benthic organic matter in headwater stream microhabitats, Environ Microbiol 7(10):1633-1640.
25. S. Fazi, S. Amalfitano, I. Pizzetti & J. Pernthaler (2007), Efficiency of fluorescence in situ hybridization for the identification of bacterial cells in river sediments with contrasting water content, Syst Appl Microbiol 30:463-470.
26. S. Fazi, S. Amalfitano, C. Piccini, A. Zoppini, A. Puddu & J. Pernthaler (2008), Colonization of the overlying water by bacteria from dry river sediments, Environ Microbiol 10:2760–2772.
27. H.C. Flemming & J. Wingender (2010), The biofilm matrix, Nature Rev Microbiol 8: 623-633.
28. H.C. Flemming, T.R. Neu & D.J. Wozniak (2007), The EPS matrix: the "house of biofilm cells", J Bacteriol 189:7945-7947.
29. J.A. Flood & N.J. Ashbolt (1998), Phylogenetic identification and in situ detection of individual microbial cells without cultivation, Nature Rev Microbiol 209:734-736.
30. A. Gieseke, L. Bjerrum, M. Wagner & R. Amann (2003), Structure and activity of multiple nitrifying bacterial populations co-existing in a biofilm, Environ Microbiol 5:355–369.
31. M.G. Hadfield (2011), Biofilms and marine invertebrate larvae: what bacteria produce that larvae use to choose settlementsites?, Annu Rev Mar Sci 3:453–470.
32. M.J. Huggett, B.T. Nedved & M.G. Hadfield (2009), Effects of initial surface wettability on biofilm formation and subsequent settlement of Hydroides elegans, Biofouling 25:387–399.
33. D. Inbakandan, P.S. Murthy, R. Venkatesan & S.A. Khan (2010), 16S rDNA sequence analysis of culturable marine biofilm forming bacteria from a ship's hull, Biofouling 26:893–899.
34. T. Ito, S. Okabe, H. Satoh & Y. Watanabe (2002), Successional development of sulfate-reducing bacterial populations and their activities in a wastewater biofilm growing under microaerophilic conditions, Appl Environ Microbiol 68:1392-1402.
35. A. Jain & N.B. Bhosle (2009), Biochemical composition of the marine conditioning film: implications for bacterial adhesion, Biofouling 25:13–19.
36. P. Jones, M. Cottrell, D. Kirchman & S. Dexter (2007), Bacterial community structure of biofilms on artificial surfaces in an estuary, Microb Ecol 53:153–162.
37. E. Karatan & P. Watnik (2009), Signals, regulatory networks, and materials that build and break bacterial biofilms, Microbiol Mol Biol Rev 73:310–347.
38. M.A. Lock (1993), Attached microbial communities in rivers, in: Aquat. Microb., p. 113-138, Ford T.E. editor, Blackwell, Cambridge.
39. G. Lupini, L. Proia, M. Di Maio, S. Amalfitano & S. Fazi (2011), CARD–FISH and confocal laser scanner microscopy to assess successional changes ofthe bacterial community in freshwater biofilms, J Microbiol Meth 86:248-251.
40. C.M. Magin, S.P. Cooper & A.B. Brennan (2010), Non-toxic antifouling strategies, Mater Today 13:36–44.

Rd

references


41. W. Manz, K. Wendt-Potthoff, T.R. Neu, U. Szewzyk & J.R. Lawrence (1999), Phylogenetic composition, spatial structure, and dynamics of lotic bacterial biofilms investigated by fluorescent in situ hybridization and confocal laser scanning microscopy, Microb Ecol 37:225-237.
42. S. Matsumoto, A. Terada, Y. Aoi, S. Tsuneda, E. Alpkvist, C. Picioreanu & M.C.M. van Loosdrecht (2007), Experimental and simulation analysis of community structure of nitrifying bacteria in a membrane-aerated biofilm, Water Sci Technol 55:283-290.
43. C. Mayer, R. Moritz, C. Kirschner, W. Borchard, R. Maibaum, J. Wingender & H.C. Flemming (1999), The role of intermolecular interactions: studies on model systems for bacterial biofilms, Int J Biol Macromol 26:3-16.
44. S. Mieszkin, M.E. Callow & J.A. Callow (2013), Interactions between microbial biofilms and marine fouling algae: a mini review, Biofouling 29(9):1097-1113.
45. S. Mieszkin, P. Martin-Tanchereau, M.E. Callow & J.A. Callow (2012), Effect of bacterial bio
films formed on fouling release coatings from natural seawater and Cobetia marina, on the
adhesion of two marine algae, Biofouling 28:953–968.
46. P.J. Molino, E. Campbell & R. Wetherbee (2009a), Development of the initial diatom microfouling layer on antifouling and fouling-release surfaces in temperate and tropical Australia, Biofouling 25:685–694.
47. P.J. Molino, S. Childs, M.R.E. Hubbard, J.M. Carey, M.A. Burgman & R. Wetherbee (2009b), Development of the primary bacterial microfouling layer on antifouling and fouling release coatings in temperate and tropical environments in Eastern Australia, Biofouling 25:149–162.
48. L.N. Mueller, J.F. de Brouwer, J.S. Almeida, L.J. Stal & J.B. Xavier (2006), Analysis of a marine phototrophic biofilm by confocal laser scanning microscopy using the new image quantification software PHLIP, BMC Ecol 6:1.
49. O.A. Olapade & L.G. Leff (2005), Seasonal response of stream biofilm communities to dissolved organic matter and nutrient enrichments, Appl Environ Microbiol 71:2278-2287.
50. H.W. Paerl, J.L. Pinckney & T.F. Steppe (2000), Cyanobacterial-mat consortia: examining the functional unit of microbial survival and growth in extreme environments, Environ Microbiol 2: 11–26.
51. M. Pasmore & J.W. Costerton (2003), Biofilms, bacterial signaling, and their ties to marine biology, J Ind Microbiol Biotechnol 30:407–413.
52. A.I. Railkin (2004), Marine biofouling: colonization processes and defences,303 pp., Boca Raton (FL), CRC Press.
53. A. Rosenhahn, S. Schilp, H.J. Kreuzer & M. Grunze (2010), The role of inert surface chemistry in marine biofouling prevention, Phys Chem Chem Phys 12:4275-4286.
54. M. Salta, J.A. Wharton, Y. Blache, K.R. Stokes & J.F. Briand (2013), Marine biofilms on artificial surfaces: structure and dynamics, Environ Microbiol 15(11):2879–2893.
55. N.J. Shikuma & M.G. Hadfield (2010), Marine biofilms on submerged surfaces are a reservoir for Escherichia coli and Vibrio cholera, Biofouling 26:39–46.
56. F. Shiraishi, B. Zippel, T.R. Neu & G. Arp (2008), In situ detection of bacteria in calcified biofilms using FISH and CARD–FISH, J Microbiol Methods 75:103-108.
57. N.A. Tujula, C. Holmström, M. Mußmann, R. Amann, S. Kjelleberg & G.R. Crocetti (2006), A CARD–FISH protocol for the identification and enumeration of epiphytic bacteria on marine algae, J. Microbiol Methods 65: 604-607.
58. B. Vu, M. Chen, R.J. Crawford & E.P. Ivanova (2009), Bacterial extracellular polysaccharides involved in biofilm formation, Molecules 14:2535–255.
59. M. Wahl (1989), Marine epibiosis 1. Fouling and antifouling:some basic aspects, Mar Ecol Prog Ser 58:175–189.
60. C.M. Waters & B.L. Bassler (2005), Quorum sensing: cell-to-cell communication in bacteria, Annu Rev Cell Dev Biol 21:319–346.
61. G. Wolf, C. Picioreanu & M.C.M. Van Loosdrecht (2007), Kinetic modeling of phototrophic biofilms: The PHOBIA model, Biotechnol Bioeng 97:1064-1079.
62. D.M. Yebra, S. Kiil & K. Dam-Johansen (2004), Antifouling technology–past, present and future steps towards efficient and environmentally friendly antifouling coatings, Prog Org Coat 53:256–275.
63. K.A. Zargiel, J.S. Coogan & G.W. Swain (2011), Diatom community structure on commercially available ship hull coatings, Biofouling 27:955–965.
64. C.E. Zobell & E.C. Allen (1935), The significance of marine bacteria in the fouling of submerged surfaces, J Bacteriol 29: 239–251.

(BIO)FOULING AND ANTIFOULING MEASURES

# Biocides based antifoulings: industrial outlook

Marine biofouling is a natural process with unwanted consequences on surfaces immersed into the seawater. A ship hull covered by fouling faces an increment in both drag and fuel consumption up to 40%, compared to a smooth and cleaned hull surface.
The aim of industry is to manufacture high performance antifouling paints ensuring a high level of protection for both human and animal health and the environment, in compliance with the enforced global legislation

*DOI: 10.12910/EAI2014-41*

*Cristina Bocca, Mauro Legrottaglie*

## Introduction

Surfaces immersed in the seawater rapidly get covered with marine organisms, such as algae and barnacles. Their accumulation increases the ship's drag, reduces the flux in water cooling pipes and destroys protection and the equipment used in aquaculture. To protect surfaces, antifouling paints have been applied. Modern antifouling paints erode upon contact with marine water and the biocide is consequently released to the surrounding water [1]. Recent human and environmental concerns have led to legislation measures also in the European Union.

## Biofouling and antifouling systems

Marine biological fouling, often called biofouling, is a natural process with unwanted consequences on manmade surfaces, which consists of the accumulation of microorganisms, plants and animals on artificial surfaces immersed in the seawater. Biofouling can be summarized as a sequence of regular steps, from the absorption of various organic compounds to the settlement of different organisms [2], as shown in Figure 1.
In the case of ships, the adverse effects caused by biofouling are well known [3]:

- High frictional resistance, which leads to an increase in weight and subsequent potential speed reduction and loss of maneuverability. Thus, the fuel consumption increases and higher emissions of harmful compounds take place [4, 5]. In the case of a ship hull covered by soft fouling (bacterial and microalgae based film), the drag force increases up to 3-10% [6]. The increase in fuel consumption can be up to 40% for ship hulls covered by hard fouling (macro algae or calcareous organism such as barnacles), if compared with a cleaned and smooth hull surface [7]. It also causes an increase in voyage overall costs of as much as 77%.
- An increase in the frequency of dry-docking operations, which leads to a large amount of toxic wastes generated during this process.
- Deterioration of the coating so that corrosion, discoloration, and alteration of the electrical conductivity of the material are favored [8].

**Cristina Bocca, Mauro Legrottaglie**
*Boero Bartolomeo SpA - Centro Ricerca & Sviluppo Riccardo Cavalleroni - Genoa*

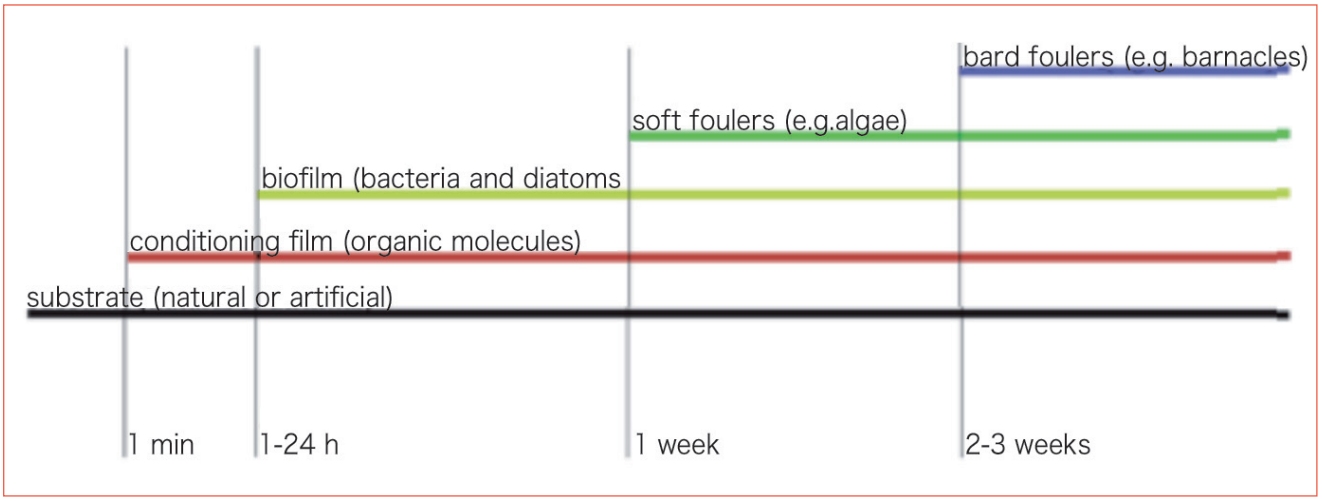



**FIGURE 1** Simplified temporal succession of biofouling process
*Source: E. Pinori thesis [2]*

- Introduction of species into environments where they were not naturally present [9, 10].

Examples of fouled hull and settlement of artificial surfaces are shown in Figures 2 and 3.

Among all the different solutions proposed throughout the history of navigation in the second half of the 20th century, an organo-tin compound, tributyltin (TBT), has been the best solution in terms of antifouling efficacy and economic profile. But, unfortunately, the TBT-SPC systems have shown unwanted environmental consequences [11]. As an example, it has been shown that extremely low concentrations of tributyltin cause defective shell growth in the oyster *Crassostrea gigas* (20 ng/l) and imposex, development of male characteri-

**FIGURE 2** Example of fouled ship hull
*Source: Boero Bartolomeo field tests*

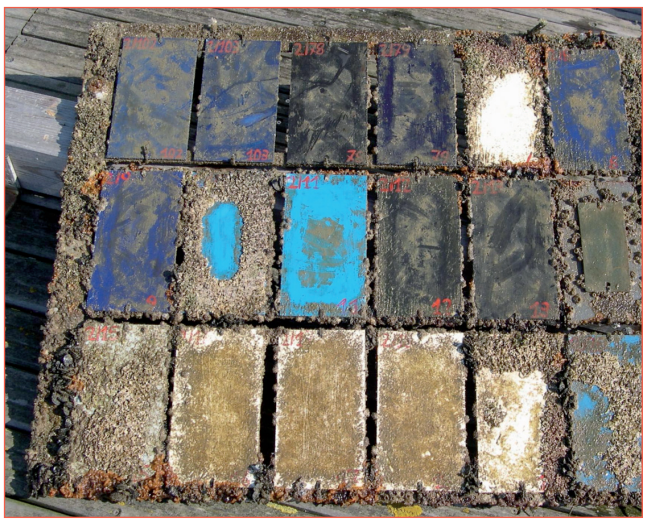


**FIGURE 3** Example of settled artificial surfaces
*Source: Boero Bartolomeo raft tests*

stics in female genitalia, in the dog-whelk Nucella sp. (1 ng/l) [12, 13]. Malformations have been observed in many other species and also accumulation in mammals has been reported by the International Maritime Organization (IMO). These facts determined the development of national regulations in countries all over the world and TBT-containing coatings have been globally banned since 2008 after a long debate [14, 15, 16]. Thus, the paint industry has been urged to replace the TBT-based products with TBT-free ones and, in the meantime, to obtain the same economic benefits and environmentally-friendly antifouling systems, in order to have less harmful effects on the environment. Nowadays, tin-free antifouling paints are the most adopted solutions. They are paints containing copper oxide and other co-biocides, also called booster biocides, in soluble paint matrix.

## Antifouling paints technology

Following the ban of TBT-based antifouling paints, a lot of improvements have been done and good results have been achieved by using antifouling systems containing copper compounds, and toxins or active ingredients will hereby called biocides to harmonize our terminology to the newly introduced regulations. Some regulatory aspects for the modern antifoulings will be described in the following.

The general principle of antifouling paints is to create a protective layer around the ship hull, working as control delivery system for biocides. In order to reach good performance and to be environmentally-friendly, an antifouling paint should have the following basic features: anticorrosion properties, efficacy, environmental compatibility, long-life properties, economic feasibility, compliance with the enforced legislation, abrasion and biodegradation resistance, no surface roughness, very low environmental toxicity, very low environmental persistence, low costs, chemical stability. To achieve this goal, several components are demanded in the paint formulation, in order to control and maintain the release rate of biocides. These components are: binder (that defines the matrix type), pigments, extenders, additives, solvents, and biocides. Biocides have to be active to both hard and soft fouling (typically barnacles and algae, respectively). The released biocides have to be bioavailable to the target organisms at the surface. The release rate of biocides from the paint matrix, called leaching rate, has to be kept above a limit threshold in order to reach and maintain a minimum inhibition concentration of the biocide at the exposed surface [17, 18]. The leaching rate is usually expressed in micrograms per square centimeter per day [19].

Different types of antifouling paints have been developed in the second half of the 20th century. These paint products, systematically based on the dispersion of toxicants in different types of polymeric binders, have become differentiated over recent decades according to the mechanisms they use to release the toxicants in the sea water. These mechanisms determine the application, behavior and duration of the antifouling coatings obtained. In the following, the main types of antifouling paints are described according to their behavior mechanisms and to the release rate of their toxicants over time [20].

Soluble matrix paints, with binders based on rosins and their derivatives and incorporating biocide such as copper, started to be developed in the 1950s. They are soluble in the sea water, present poor mechanical strength, and only allow the inclusion of low concentrations of biosoluble materials and the application of relatively fine films [21, 22]. Their leaching rate decreases with time quickly and they do not assure protection for more than 12–15 months (see Figure 4).

Insoluble matrix paints use high molecular mass binders, which are insoluble in the sea water. As the biocide particles are deeper in the paint film, the leaching rate gradually decreases in time, and the protection afforded becomes increasingly less efficient [23]. The lifetime of these paints is between 12 and 24 months, depending on the exposure conditions, which limits their application on some types of ships [24].

TBT self-polishing paints are based on an acrylic copolymer with TBT groups bonded to the main polymer chain by ester bonds [25, 26], in which the polymer is soluble in the seawater. Since this dissolution can be controlled at molecular level, it is possible to obtain a well-known self-polishing effect in these paints. Unlike insoluble matrix paints, in these type of products, the water is prevented from penetrating the film [27]. Thus,

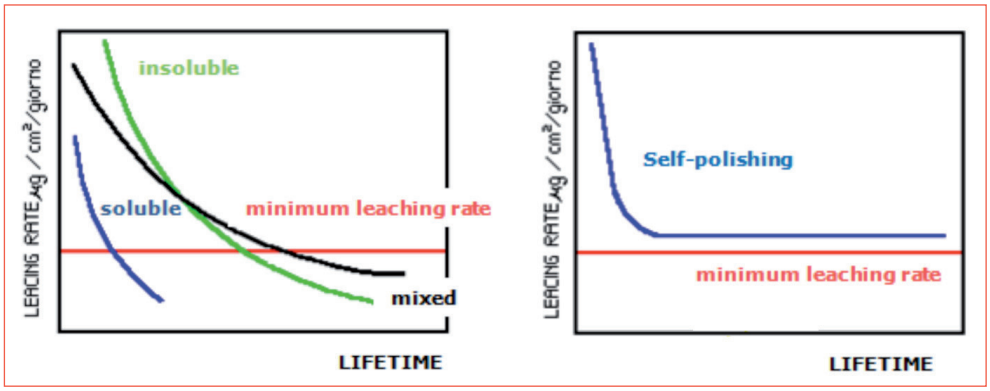



**FIGURE 4** Biocide release rates of traditional insoluble and soluble matrix paints and self-polishing ones. "Minimum leaching rate" indicates the limit for efficient protection against fouling (dependent on the fouling conditions)

the sea water barely manages to fill the pores created by the dissolution of the soluble pigment particles, as represented in Figure 5.

As previously mentioned, due to the environmentally harmful action of the well known, efficient and versatile TBT self-polishing paints, and the consequent total worldwide

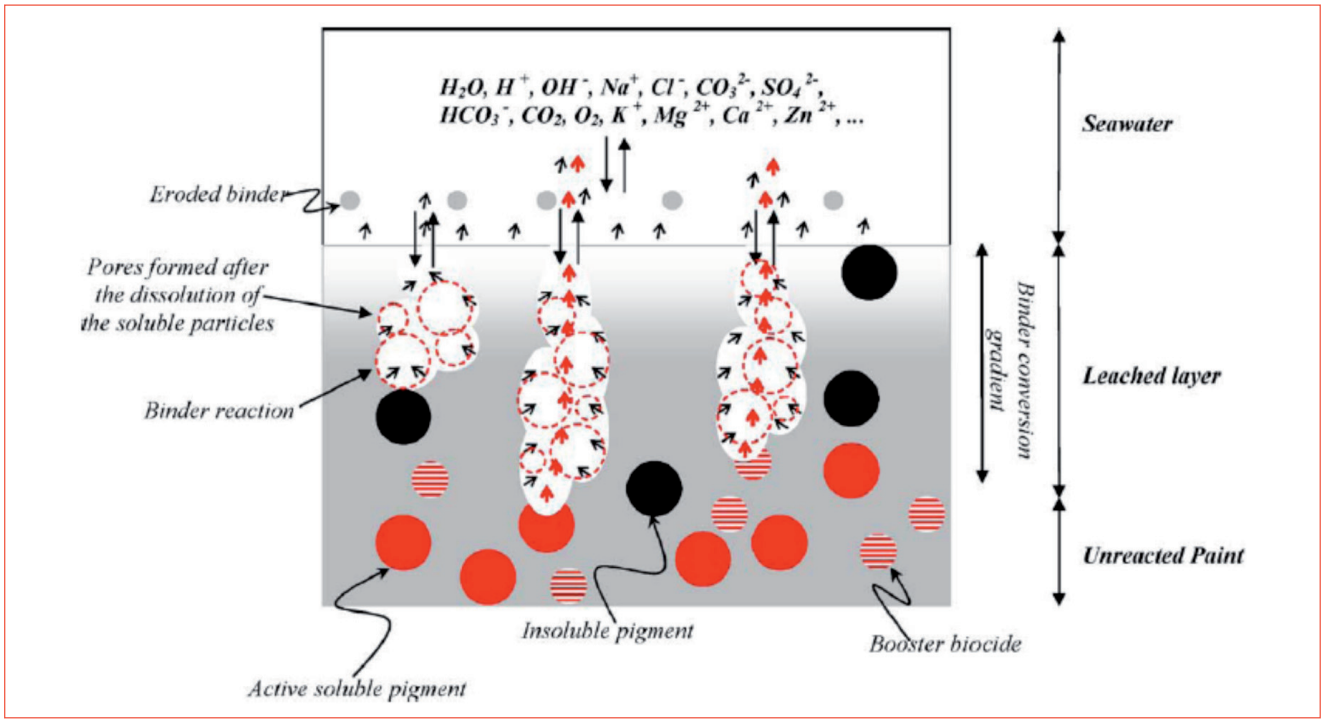



**FIGURE 5** Schematic illustration of the behavior of a biocide-based antifouling system exposed to the sea water
*Source: [3]*

prohibition of their application and presence on ship surfaces following 1st January, 2008 [28, 29], paint manufacturers have been forced to urgently study and develop new more environmentally-friendly antifouling paints.
Among the products with biocides that have recently been marketed for this purpose, tin-free, biocide-containing, self-polishing paints (TF-SPCs) are very common. In this type of paints, products are integrated in an acrylic matrix to which different pendent groups of the main chain are added, however without tin. Like in self-polishing paints containing tin, the pendent groups are considered to be released when in contact with the sea water. Nevertheless, and despite the high number of patents registered in this domain until 1996, these groups are in no case as effective as TBT [30]. These polymers interact with the sea water, and their self-polishing effect is seen with the controlled release of biocides [31]. Due to their relatively high polishing rate, the maximum service life of this type of paint is normally around 3 years, although in some cases 5-year service lives have been reported [32, 33, 34]. However, according to various authors, they do not achieve the same level of efficiency as TBT-based self-polishing paints.

## Regulations and industrial developments

The active ingredients in antifouling paints are regulated under the Biocide Products Regulation (BPR, Regulation EU 528/2012 – formerly the Biocides Products Directive, 98/8/EC).
This Regulation concerns the making available on the market and use of biocidal products and its purpose is to improve the functioning of the internal market through the harmonization of its rules, whilst ensuring a high level of protection of both human and animal health and the environment. It aims to improve the EU market by the harmonization of the various local legislation, breaking down barriers of trade between countries. It covers 22 very different product types; biocides for antifouling paints belong to the Product-type 21 – "Antifouling product: Products used to control the growth and settlement of fouling organisms (microbes and higher forms of plant or animal species) on vessels, aquaculture equipment or other structures used in water".
The approvals of active ingredients have to be based on scientific risk assessments and best practice, products do not pose any unacceptable risks to humans, animals and the environment and safe use must be demonstrated. In the meantime, as the products work as claimed, efficacy must be demonstrated. Among the many data requirements in order to obtain the approval of an active ingredient belonging to a specific product type, the main ones are: physical, chemical, technical properties (e.g., storage stability), toxicological and eco-toxicolo-

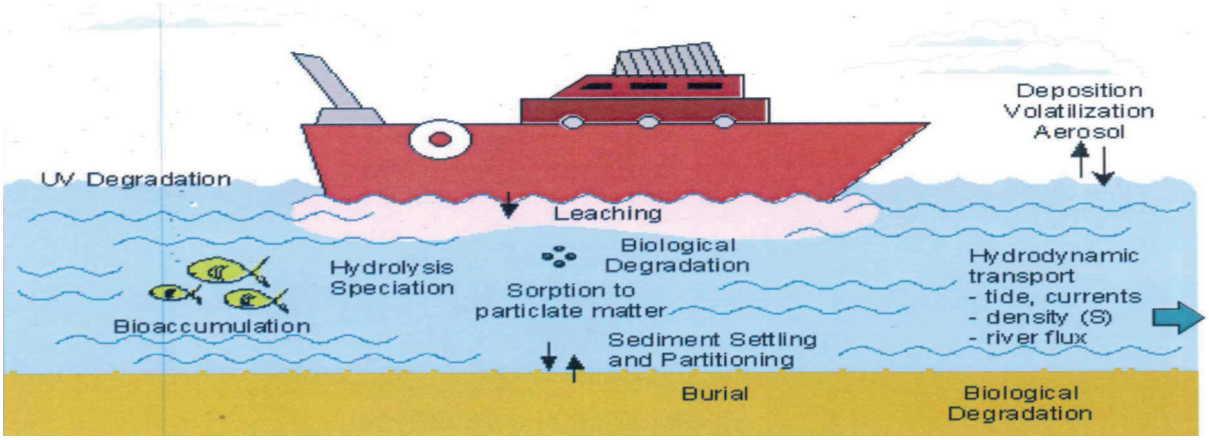



**FIGURE 6** Environmental fate of a marine antifoulant
*Source: A. Jacobson [35]*

gical profile, and effectiveness of the products, which have to combine the label claim with the efficacy, environmental fate and behavior. Regarding the last one, it is very interesting because the environmental fate of a marine antifoulant involves very different processes. Many complex and interacting processes that can be of a biological, chemical or physical nature determine the chemical fate of contaminants in the marine environment. Some of the major transport and transformation processes have been summarized in Figure 6.

Especially in energy-rich marine environments the hydrodynamic transport and mixing processes of water masses tend to have a major impact for most compounds. For compounds with a high affinity to particulate matter or sediment, sediment transport phenomena will be of dominant importance. Stable dissolved compounds are likely to be affected most by river discharges or tidal currents. In specific marine environments with low exchange rates or pseudo stagnant conditions the chemical and biological processes will become more important. The relative importance of each of these processes is highly compound- and habitat-specific and may vary between seasons. Biodegradation processes are highly temperature dependent and may be the dominant removal process in tropical water, while in temperate or polar zones this may be less. Photolysis may have a prominent role in the open sea even at greater depths in warm and transparent waters, while in turbid estuarine environment in temperate zones this only may be of importance in the upper water layers [36].

Stringent environmental regulations is pushing the innovation developments. As above mentioned, in UE the coatings industry is heavily regulated and hence it is strictly controlled in many ways. Also in the US there are a series of Regulations governing the substances that can be used in the marine sector together with rules for VOC's and biocides. In addition, new countries are regulating in these areas such as Far East. Due to the global impact of the regulatory drivers, the coating industry is investing in developing eco-friendly products such as metal-free, anti-fouling coatings or silicone- or fluororesin-based, foul-release products at worldwide level. New biocides issues are added day by day to the already treated articles obliging companies to invest

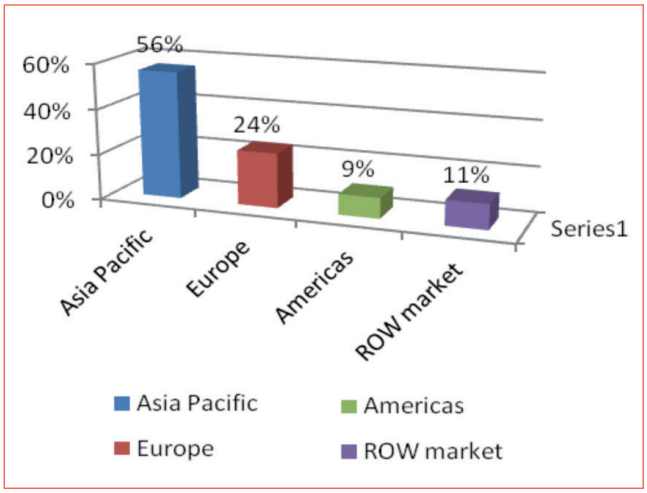



FIGURE 7 Marine coating market distribution

on and develop new eco-friendly and less environmental impacting solutions to keep their marine business and their leadership in the specific market segments.

## Marine coatings market

The Marine Coatings Global market size was about $ 4.8 billion in 2012. The end markets are new-building, repair and maintenance of deep sea, coastal and navy vessels. In the past the market especially depended on new-building activity, while maintenance and repair were a less cyclical business. On the contrary, in the last few years the new ship building market continues to be in decline and the market for marine coatings benefitted from an increase in ship repair and maintenance.

It seems that in 2013 the market for marine coatings has continued to show signs of improvement and this trend can go on beyond, especially as Asia Pacific continues to lead the way in new shipbuilding and dry-docking. As a consequence the Asia Pacific region remains the most important area for marine coatings manufacturers, with China, South Korea and Japan representing nearly 80% of world's new building capacity, and China now leading in the number of dry docks and dry dockings, Asia is growing two or three times faster than any other region. Concerning the European region, tank coating

work in Europe will be the main segment (Figure 7). Within the total marine coatings market value, the dimension of the market for antifouling paints in 2012 was around $ 1.4 billion, of which 80% of the market is ocean-going ships, 20% leisure boats & offshore structures.

## Conclusion

Since remote times Man has been fighting a never-ending battle against the fixing of marine organisms on surfaces immersed in the sea water in general, and on ship hulls in particular. Even when the problem seemed to have been solved, thanks to the boom in the development of TBT-based antifouling paints, with their well-known technology in which, by suitably controlling the molecular composition of the binder, it was practically possible to tailor-make antifouling paints to meet the needs of each particular type of ship, it was soon to become an issue once again. Its harmful effect for marine organisms has lead to the total ban of TBT-based antifouling paints after 1st January, 2008. Meanwhile, the numerous alternative techniques to antifouling painting which have been tested over time have either not proven to be sufficiently efficient nor are so expensive and/or difficult to apply on ship hulls, so that they have not been applied with the hoped-for success. Thus, in a first attempt to address the problem, antifouling paint manufacturers replaced TBT in self-polishing polymers with other chemical ligands of their main chains, such as copper, and reinforced the biocidal effect of copper with artificial biocides, such as certain known herbicides and pesticides. However, many of the latter have also proven to be highly harmful to the environment, and the long-term effect of many others has not been fully clarified yet. Moreover, the implementation of the European legislation on biocide, EU Regulation 528/2012, imposes certain requirements for the acceptance and registration of new biocide products, which encourage the abandonment of this type of products in the sea water. In these conditions, antifouling paint manufacturers have no alternative but to intensify their research in the quest for biocide-free products that prevent the attachment of marine organisms. For the purpose of both efficacy and safety for human health and the environment, the future developments of the antifouling paint systems are designed to use biocides at very low concentrations and very high and quick biodegradation, Smart technology, green Chemistry, nanotechnologies.

references


1. E. Pinori et al. “Distribution of Organic Biocide in Antifouling Paint as Determined using Time of Flight Secondary Ion Mass Spectroscopy (ToF-SIMS)”, (In Manuscript).
2. E. Pinori, “Low Biocide Emission Antifoulig Based on a Novel Route of Barnacles Intoxication”, PhD Thesys, University of Gothenburg.
3. D. M. Yebra et al., “Antifouling technology-past, present and future steps towards efficient and environmentally friendly antifouling coatings”, Progress in Organic Coatings 50 (2004) 75–104.
4. A.Abbott, P.D. Abel, D.W. Arnold, A. Milne, Sci. Total Environ., 258 (2000) 5–19.
5. A.M. Rouhi, Chem. Eng. News 27 (1998) 41–42.
6. M.A. Champ, “A review of organotin regulatory strategies, pending actions, related costs and benefits”, Science of the total environment, 2000. 258(1-2): p. 21-71.
7. Schultz, M.P., et al., “Economic impact of biofouling on a naval surface ship.”, Biofouling, 2010. 27(1): p. 87-98.
8. J.J. Cooney, R.J. Tang, Meth. Enzymol. 310 (1999) 637–645.
9. M.S. Brancato, OCEANS’99 MTS/IEEE, Riding the Crest into the 21st Century, vol. 2, no. 2, 1999, p. 676.
10. K. Reise, S. Gollasch, W.J. Wolff, Helgoländer Meeresunters 52 (1999) 219–234.
11. C. Alzieu, “Environmental problems caused by TBT in France: Assessment, regulations, prospects”, Marine Environmental Research, 1991. 32(1–4): p. 7-17.
12. G. Swain, “Proceedings of the International Symposium on Sea water Drag Reduction”, The Naval Undersea Warfare Center, Newport, 1998, pp. 155–161.
13. S.M. Evans, T. Leksono, P.D. McKinnel, Mar. Pollut. Bull. 30 (1) (1995) 14–21.
14. L. Gipperth, “The legal design of the international and European Union ban on tributyltin antifouling paint: Direct and indirect effects.”, Journal of Environmental Management, 2009. 90: p. S86-S95.
15. S. M. Evans et al., “The TBT Ban: Out of the Frying Pan into the Fire?”, Marine Pollution Bulletin, 2000. 40(3): p. 204-211.
16. A. Abbott, et al., “Cost-benefit analysis of the use of TBT: the case for a treatment approach.”, Science of the Total Environment, 2000. 258(1-2): p. 5-19.
17. M. Thouvenin, et al., “A study of the biocide release from antifouling paints”, Progress in Organic Coatings, 2002. 44(2): p. 75-83.
18. D. M. Yebra, et al., “Reaction rate estimation of controlled release antifouling paint binders: Rosin-based systems.”, Progress in Organic Coatings, 2005. 53(4): p. 256-275.
19. Assessment of antifouling agents in coastal environments. [Final Scientific and technical Report] 2002; Final Scientific and Technical Report for EU-MAST project].
20. E. Almeida et al., “Marine paints: The particular case of antifouling paints.”, Progress in Organic Coatings 59 (2007) 2–20.

Rd

references


21. C.A. Giudice, et al., J. Coat. Technol. 58 (733) (1986) 45.
22. C.A. Giudice, B. del Amo, EuroCoat 1–2 (1993) 16.
23. F. Marson, J. Appl. Chem. 19 (1969) 93.
24. CEPE Antifouling Working Groupe, Final Report, EC Project No.961559/3040/DEB/E2, 1999.
25. C.D. Anderson, Self-polishing antifoulings: a scientific perspective, Proc. Ship Repair Conv. 93 (1993).
26. A.B. Samui, U.R. Hand, P.C. Deb, J. Coat Technol. 69 (867) (1997), 67.
27. A.R. Fernandez Alba, et al., Anal. Chim. Acta 456 (2002) 303.
28. Y. Honda, "Technology after ban TBT in Japan, in: Proceedings of the Emerging Non-metallic Materials in Marine Environment", Honolulu, HI, USA, March, 1997, p. 18.
29. IMO-MEPC 38 (1996) (v), "Terms of reference for a corresponding group on the reduction of harmful effects of the use of antifouling paints for ships", IMO-MEPC Paper MEPC 38/WP.6, 1996.
30. D.M. Yebra, S. Kiil, K.D. Johansen, POC 50 (2004) 75.
31. D. Crisp,"The role of the biologist in antifouling research", in:R.F. Acker et al., Proc. of the Third I.C.M.C.F., Northwestern University Press, Evanston, IL, 1973, p. 88.
32. Y. Honda, "Technology after ban TBT in Japan", in: Proceedings of the Emerging Non-metallic Materials in Marine Environment, Honolulu, HI, USA, March, 1997, p. 18.
33. Anon., "Review of current and future marine antifouling coatings", Marine Safety Agency, UK, Report 93/TIPEE/4787, 1993.
34. P. Kuo, et al., "Interface-crashed polishing type of tin-free antifouling coatings", in: Proceedings of the Emerging Nonmetallic Materials for the Marine Environment, Honolulu, HI, USA, March, 1997.
35. A. Jacobson et.al, "Modelling of marine antifoulants", Rohm and Haas Company, European Laboratories, Valbonne (France) and Research Laboratories, Spring House (PA, USA), (1996), p. 1-12 (manuscript).
36. B. van Hattum et al. , "Computer model to generate predicted environmental *concentrations (PECs) for antifouling products in the marine environment", 2nd Edition,* Report number E-02-04 / Z 3117, August 2002.


(BIO)FOULING AND ANTIFOULING MEASURES

# Biofouling and antifouling: new approaches to the development of sustainable protection technologies

The development of antifouling systems has a long history but the last decade has seen an increase in the focus on environmentally acceptable alternatives. This paper highlights the latest research strategies dedicated to the development of new non-toxic antifouling technologies

*DOI: 10.12910/EAI2014-42*

*Marco Faimali*

## Biofouling as a technological problem

Biological fouling, usually termed biofouling, can be defined as the undesirable accumulation of micro- and macro-organisms on artificial surfaces immersed in water.
Biofouling has been described as a four-step sequential ecological process. The first two steps, which produce a microbial biofilm, occur similarly whether on a surface in the sea or on a catheter in a hospital room. The following two steps are unique to aquatic habitats and involve the attachment of unicellular and multicellular eukaryotes to an inorganic or living surface. The multi-step process results from the web of interactions in the initial biofilm and subsequent community of colonizers, culminating in the establishment of a mature community composed of prokaryotes, fungi, protists and adult invertebrates.
Biofouling assemblages on artificial substrates are a complex phenomenon resulting from several processes, the rate and extent of which are influenced by numerous physical, chemical and biological factors in the immediate proximity of the surface, and cannot be defined as distinct and univocal entities (Figure 1).
From the initial adsorption of organic molecules, to the colonisation by microorganisms, to the development of complex and diverse sessile assemblages, biofouling affects most man-made surfaces, resulting in significant economic costs.
Fouled ships, for instance, need 40% more fuel in order to maintain the same speed. This leads to a global cost of about $ 7.5 billion per year and to related environmental issues due to 20 million tons of $CO_2$ more, that are emitted annually. The US Office of Naval Research estimated that the periodically cleaning and restoring of ship hulls cost to the US Navy about $1000 million per year [2].
The costs of biofouling are clearly not limited to ship hulls nor to the marine environment. Control of fouling in water intakes, piping systems and desalinisations plants (Figure 2) cost over $15 billion per year [3]. In food industry, the formation of fouling layer within food processing equipment for pasteurization and sterilization costs to the US industrial community about $10 billion per year [4]. Biofilm-associated infections extend hospital stays of an average of about three days and it is estimated that up to 65% of nosocomial infections are biofilm-based

**Marco Faimali**
*Institute of Marine Sciences – National Research Council (ISMAR-CNR)*

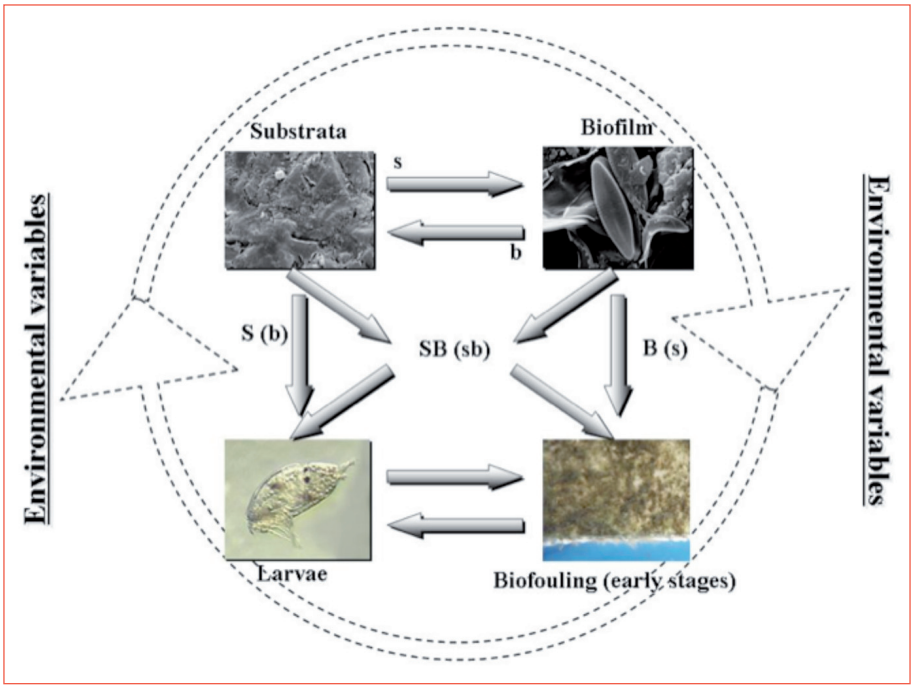



**FIGURE 1** Preliminary model of interactions between larvae, biofilm and substratum during settlement process. The role of subtratum and biofilm on settlement S, B is indirectly modulated by their mutual relationships (s, b). In natural conditions, these interactions can be changed by other chemical, physical and biological (environmental) variables [1]

with an associated treatment cost in excess of $1 billion per year. Up to 82% of nosocomial bacteremias are the result of bacterial contamination of intravascular catheterizations [5]. AF technologies are necessary in order to avoid the colonisation of surfaces by biofoulers and consequently the high costs relative to transport delays, hull repairs, cleaning of desalination units and biocorrosion (estimated at 150 billion USD per year) [6].

## Biocide-based antifouling coatings: the past

During the '60s, the chemical industry developed efficient AF paints using organotin compounds as biocides: tributyltin (TBT) and triphenyltin (TPT). During the late 1970s, the AF research and development efforts were mainly focused on the successful TBT-based, self-polishing, copolymer systems. Unfortunately, these biocides were highly toxic for many aquatic organisms

**FIGURE 2** Biofouling colonization residual inside cooling water system [1]

FIGURE 3 The hull of a ship protected with biocide-based antifouling coatings *(Photo of M. Faimali)*

and have been proven to contaminate the food chain and to be persistent in the environment.

TBT has been described as one of the most dangerous substances ever deliberately introduced into the marine environment. As a consequence of different environmental diseases observed by researchers between the late '70s and the beginning of the '80s, the use of self-polishing coatings containing organotins compounds has been restricted by European Community since December, 1989. The total ban on the presence of TBT-based antifoulings on ships hulls in EU ports came into effect on 1st January, 2008. As a consequence of the ban, in the last few decades a great deal of attention has been devoted to find alternative antifouling technologies [7].

Following the ban of TBT-based products in AF paints, alternatives containing high amounts of copper (Cu)-based compounds were developed. As it is about ten times less toxic than TBT, cobiocides, also called boosters, were used to enhance the AF performance of copper-based coatings [8].

All these compounds vary in terms of their mode of action, environmental persistence, and toxicological properties. Several reviews have been published presenting an overview of the biocides used in AF paints and their specific fate and effects in the environment [9,10-14].

As a consequence of the growing investigations on its toxicity, the release rate of Cu-based soluble species from AF paints has been regulated in several areas, for example, Sweden and the U.S. States of Washington and California [7].

Copper and many of the so-called "booster biocides" have come under increasing scrutiny and local legislation and restriction in as much as the same way and to the same degree than TBT did.

The key property of a good AF biocide with respect to the environment is that it is effective in preventing fouling of the painted surface without persisting at concentrations greater than those that can cause detrimental environmental effects [12].

In order to identify potential candidates able to possess these characteristics in recent years, using a biomimetic approach, the possibility of exploiting marine natural product antifoulants (NPAs) utilized by marine organisms (e.g., sponges, corals, and macroalgae) to prevent them from colonization by other marine organisms has been investigated [15-17].

To date, purification of active products has yielded ca. 200 molecules with some degree of AF activity against a wide range of marine fouling organisms, assayed mainly through laboratory tests [17].

The challenge of finding a natural product which fulfills the required criteria of low toxicity, broad spectrum activity, and ease of production has yet to be realized, and is the main reason why they have not been so far successfully commercialized.

Also the idea of using enzymes, catalytically active proteins omnipresent in nature, for developing new enzyme-based coatings has received increased interest in recent years [18,19].

Enzymes can degrade the fouling organism or its bio-

adhesive, or produce other biocidal compounds. Direct enzymatic AF covers the application of "biocidal" or adhesive-degrading enzymes, whereas indirect enzymatic AF is based on enzymatic generation of biocides from substrates present in the seawater or coating-ingredients [20]. In several cases, concepts as well as short-term AF activity in coatings have been proven, but long-term efficiency toward all fouling organisms remains to be reported.

## Changes of strategy in the development of antifouling technologies

Furthermore, the definitive failure of the "chemically active strategy" in Europe has been catalyzed by the fact that the predisposition of biocidal compounds (synthetic and/or natural origin) to cause environmental adverse effects has received in recent years, a greater attention, and biocide containing AF paints are currently regulated and require approval.

In the European Union and its member states, the EU Biocidal Products Directive (BPD) regulates all biocide products that are placed on the market. The BPD sets the stage for all businesses selling biocidal products, and each of these businesses will have to deal with the BPD's requirements for documentation. From 1st September, 2013, the Biocidal Products Regulation (BPR) will replace the BPD and henceforth regulate all biocidal products in the European Union. The BPR will introduce new procedures for all EU countries and authorities now require testing of new active substance prior to marketing authorization [21].

The total costs have to be taken into account, for example, not only by preparing agreed protocols and placing studies but also by monitoring studies, analysis of the results, risk assessments based on exposure scenarios, dossier preparation, registration costs, task force participations, legal fees, etc., as well as management activities of the directive and associated registration.

For the development of new biocides, the estimated costs are as follows: toxicity studies on active substances: € 1–3M, environmental studies & ecotoxicity: € 0.6–4M, formulation studies: > € 1M, risk assessments/ exposure scenarios expertise needed > € 1M, dossier preparation: € 0.1–0.25M, registration fees: € 0.1–0.2M, task forces: € 0.05–0.2M [22].

The very high costs and long times for the registration process almost totally limit the development of new biocides, regardless of their potential AF efficacy and environmental compatibility.

The awakening of the global environmental awareness in the form of legislative measures has completely changed the way AF research is conducted nowadays.

| Author(s) [Ref] | Title | Year |
|---|---|---|
| Yebra, DM; Kiil, S; Dam-Johansen, K [23] | Antifouling technology – past, present and future steps towards efficient and environmentally friendly antifouling coatings | 2004 |
| Chambers LD et al. [24] | Modern approaches to marine antifouling coatings | 2006 |
| Almeida, E, Diamantino, TC, De Sousa, O [25] | Marine paints: The particular case of antifouling paints | 2007 |
| Maréchal JP, Hellio C [22] | Challenges for the development of new non-toxic antifouling solutions | 2009 |
| Grozea, CM, Walker, GC [26] | Approaches in designing non-toxic polymer surfaces to deter marine biofouling | 2009 |
| Magin CM, Cooper SP, Brennan AB [27] | Non-toxic antifouling strategies | 2010 |
| Cao S et al. [28] | Progress of marine biofouling and antifouling technologies | 2011 |
| Callow JA, Callow ME [29] | Trends in the development of environmentally friendly fouling-resistant marine coatings | 2011 |
| Kirschner CM, Brennan AB [30] | Bio-Inspired Antifouling Strategies | 2012 |
| Lejars M, Margaillan A, Bressy C [7] | Fouling Release Coatings: A Nontoxic Alternative to Biocidal Antifouling Coatings | 2012 |

**TABLE 1** Selection of scientific papers related to the new trends of antifouling technology

An overview of the main papers that in recent years have addressed the changes in the strategy of research in the field of antifouling technologies are summarized in Table 1.

## Non-toxic antifouling coatings: the future

Within the context of worldwide pressure for legislation limiting the use of biocides, and ever-increasing fuel prices, there is now a real need for the continuous development of new non-toxic AF formulations and an interesting and promising line of research is inspired by biomimetic solutions.

Nature provides examples of antifouling surfaces that emphasize the importance of both chemical and physical concepts. Physical cues, such as surface roughness and fluid hydrodynamics, can act singularly or in concert with surface chemistry to enhance or inhibit the attachment of organisms to a surface. Chemical cues, especially surface energy, influence not only the ability of an organism to initially attach to a surface, but also the degree of fouling-release from the surface once adhesion has been established.

They are many examples from natural fouling-resistant organisms, which can serve as a basis for new scientific investigations but two general (non-exclusive) strategies are typically followed in the design of novel, non-biocidal, non-fouling surfaces and are now considered to be the most promising environmentally-friendly, antifouling technology [23].

- *Engineered Microtopographical Surfaces,* in which the objective is to deter the recruitment stages of fouling organisms from attaching in the first place.
- *Fouling Release Coatings (FRC)*, which do not prevent organisms from attaching, but the interfacial bond is weakened so that attached organisms are more easily removed by the hydrodynamic shear forces.

These two general approaches are not mutually exclusive and in fact the distinction is overly simplistic. In both cases the objective is to achieve the desired re-

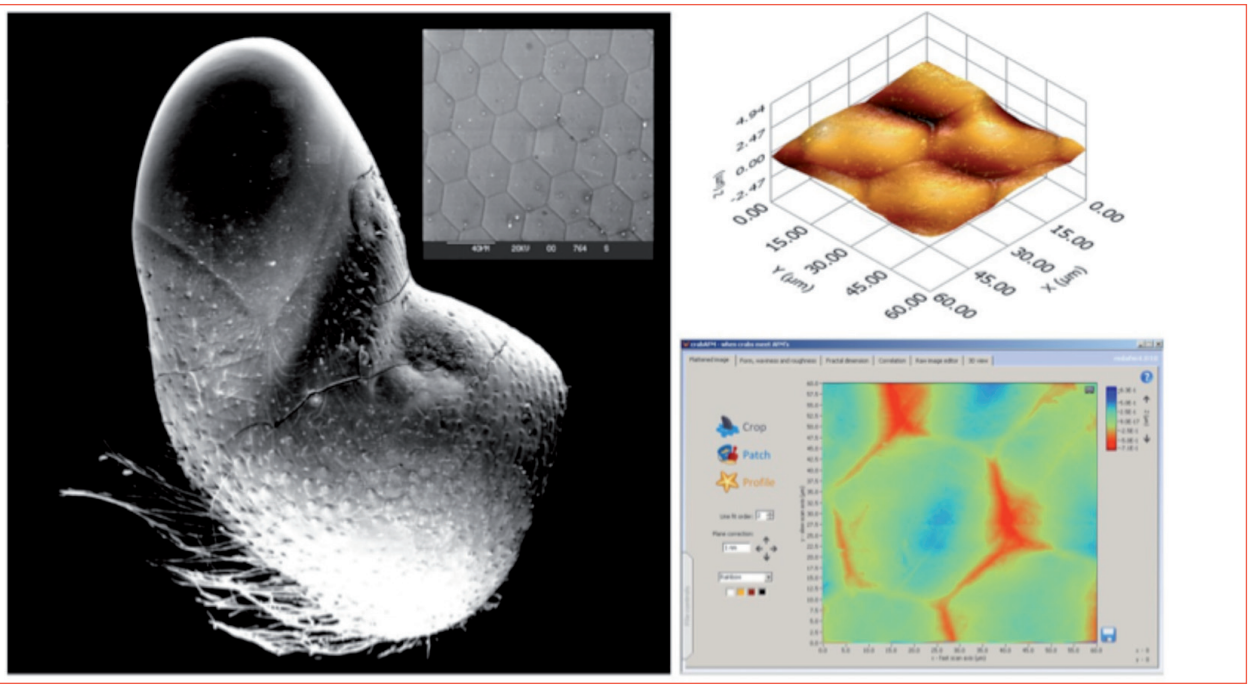



**FIGURE 4** Microtopography of the eye surface of the crab Carcinus maenas
*Source: SEM image and elaboration: G. Greco - ISMAR-CNR, [30])*

sult through the manipulation of the physicochemical properties of coating materials (for example, elastic modulus, frictional coefficient) [29].

Some of the most promising strategies that define a new era of antifouling technology have been inspired by nature and can be summarized in two main approaches [31]:

- *Bio-inspired chemical/physical strategies:* antifouling surface material and topography inspired by natural antifouling surface (eg., shells of mollusks and crabs and skin of marine mammals and sharks).
- *Bio-inspired stimuli-responsive strategies:* surface self-cleaning mechanism inspired by the skin of marine mammals and fishes that have the capability to respond to stimuli in the environment.

At this point, no single technology has been demonstrated to be universally effective and one way forward will be to design 'multifunctional smart coatings' combining chemical, physical, and stimuli-responsive strategies in order to develop the best non-toxic antifouling solutions.

references


1. Terlizzi A, Faimali M (2010). Biofouling Processes in Industry - Fouling on artificial substrata. In: Dürr S, Thomason JC (eds). Biofouling. Blackwell Publishing. pp. 170-184.
2. Alberte, R.S., Snyder. S., Zahuranec, B.J. & Whetstone, M. (1992). Biofouling research needs for the United States Navy: program history and goals. Biofouling, 6, 91-95.
3. Meesters K.P.H., Groenestijin J.W. and J. Gerritse (2003). Biofouling reduction in recirculating cooling system through biofiltration of process water, Wat. Res., 37 pp. 525–532.
4. Jun S & Puri VM, (2005). 3D milk-fouling model plate heat exchangers using computational fluids dynamics. Int. J. Dairy Thecnol., 58(4): 214-224.
5. Archibald, L. K., and R. P. Gaynes (1997). Hospital acquired infections in the United States: the importance of interhospital comparisons. Nosocomial Inf., 11(2):245-255.
6. Schultz, M.P. (2007). Effects of coating roughness and biofouling on ship resistance and powering. Biofouling, 23, 331–341.
7. Lejars M., Margaillan A., Bressy C. (2012). Fouling Release Coatings: A Nontoxic Alternative to Biocidal Antifouling Coatings. Chem. Rev., 112 (8): 4347–4390.
8. Swain, G. W. (1998). Proceedings of the International Symsposium on Seawater Drag Reduction; Newport, RI; p 155.
9. Omae I. (2003). General aspects of tin-free antifouling paints. Chem Rev. 2003 Sep;103(9):3431-48.
10. Arai, T.; Harino, H.; Ohji, M.; Langston, W. J. (Eds.), 2009. Ecotoxicology of Antifouling Biocides, 1st ed.; Springer.
11. Thomas, K. V. (2009). Advances in Marine Antifouling Coatings and Technologies; Woodshead Publishing: Cambridge, U.K.,; p 522.
12. Thomas, K. V.; Brooks, S. (2010) The environmental fate and effects of antifouling paint biocides. Biofouling, 26, 73.
13. Voulvoulis, N.; Scrimshaw, M. D.; Lester, J. N. (1999) Alternative antifouling biocides. Appl. Organomet. Chem., 1999, 13, 135.
14. Konstantinou, I. K.; Albanis, T. A. (2004). Worldwide occurrence and effects of antifouling paint booster biocides in the aquatic environment: a review, Environ. Int. 30(2):235-48.
15. Clare, AS. (1998) Towards nontoxic antifouling. J. Mar. Biotechnol., 6, 3. 10:181–202.
16. Rittschof D (2000). Natural product antifoulants: one perspective on the challenges related to coatings development. Biofouling, 15: 119–127.
17. Hellio, C.; Maréchal, J.-P.; Da Gama, B. A.; Pereira, M.; Clare, A. S. (2009). Natural marine products with antifouling activities. In Advances in Marine Antifouling Coatings and Technologies; Hellio C., Yebra D.M.Y. (Eds) Woodshead Publishing: Cambridge, UK. pp. 572-622.
18. Olsen, S.M., Pedersen, L.T., Laursen, M.H., Kiil, S., Dam-Johansen, K. (2007). Enzyme-based antifouling coatings: A review. Biofouling, 23, 369—383.
19. Kristensen JB, Meyer RL, Laursen BS, Shipovskov S, Besenbacher F, Poulsen CH. (2008). Antifouling enzymes and the biochemistry of marine settlement. Biotechnol Adv., 26(5):471-81.
20. Olsen, S. M.; Pedersen, L. T.; Hermann, M. H.; Kiil, S.; Dam-. Johansen, K. Advances in Marine Antifouling Coatings and Technologies, Woodshead Publishing: Cambridge, U.K., 2009; p 623.
21. Hellio C, Yebra DMY. Introduction. In: Hellio C, Yebra DMY, editors. Advances in Marine Antifouling Coatings and Technologies. Woodshead Publishing; Cambridge, UK: 2009. pp. 1–15.
22. Maréchal JP. and Hellio C. (2009). Challenges for the development of new non-toxic antifouling solutions. Int. J. Mol. Sci., 10, 4623-4637.
23. Yebra, D.M.; Kiil, S.; Dam-Johansen, K. (2004). Antifouling technology—past, present and future steps towards efficient and environmentally friendly antifouling coatings. Progress in Organic Coatings, 50(2): 75–104.
24. Chambers LD, Stokes KR, Walsh FC, Wood RJK. (2006). Modern approaches to marine antifouling coatings. Surface and Coatings Technology, 201:3642-3652.
25. Almeida, E; Diamantino, T.C.; De Sousa, O. (2007). Marine paints: The particular case of antifouling paints, Progress in Organic Coatings, 59 (1): 2–20.
26. Grozea, C. M.; Walker, G. C. (2009). Approaches in designing non-toxic polymer surfaces to deter marine biofouling. Soft Matter, 5:4088-4100.
27. Magin CM, Cooper SP, Brennan AB. (2010). Non-toxic antifouling strategies. Mater. Today, 13:36–44.
28. Cao Shan, JiaDao Wang, HaoSheng Chen, DaRong Chen (2011). Progress of marine biofouling and antifouling technologies, Chinese Science Bulletin, 56(7): 598-612.
29. Callow JA, Callow ME. (2011). Trends in the development of environmentally friendly fouling-resistant marine coatings. Nat Commun., 2:244.
30. G Greco, TS Lanero, S Torrassa, R Young, M Faimali, J Davenport (2013). Microtopography of the eye surface of the crab Carcinus maenas: an atomic force microscope study suggesting a possible antifouling potential. J. R. Soc. Interface, 10(84):20130122.
31. Kirschner C. M. and Brennan A.B. (2012). Bio-Inspired Antifouling Strategies. Annual Review of Materials Research, 42: 211-229.

(BIO)FOULING AND ANTIFOULING MEASURES

# Environmental compatibility evaluation of classical and innovative antifouling paints

Nowadays, anti-fouling paints are generally formulated with toxic copper or other biocides. In order to contain the possible adverse effect upon non-target biota, industries are developing new paints with limited sloughing of toxic metals or hard ones that release biocides slowly. Furthermore, there are very promising innovative “biocide-free” antifouling coatings, too. The potential toxicity of several types of paints through the application of biological assays in accordance with standardized bio-assay protocols has been evaluated

*DOI: 10.12910/EAI2014-43*

*Marco Francese, Paola Frisenda, Lucio Panizza, Elena Martelli, Alejandro Hochkoeppler*

## Introduction

Shoreline is a cooperative company with a primary focus on Environmental Protection and a twenty-five-year long experience in providing management services to the Miramare Marine Protected Area (WWF) and consultancy in the Mediterranean basin. Shoreline performs research on and monitors marine coastal environments, basing its activities on chemical-physical parameters, eco-toxicological analyses and ecological survey. It also offers aquaculture and fishery consultancy services, from sustainable management to EMAS certification. Finally, Shoreline offers organizational services in Environmental Education and Eco-tourism at the international level as well as highly interactive, nature-themed museum projects.

In this field of work the company has often encountered the problem of the need for anti-fouling paints. In particular, leisure boating and marine infrastructures, as well as boats and equipment for fishery and fish farming, and equipment used in coastal-marine protected areas, such as buoys or beacons, have this requirement. In fact bio-fouling is a limiting factor not only for the navigation or the floating equipment, but also for cooling systems and water distribution in fish farming plants.

Nowadays, anti-fouling paints are formulated with toxic copper or other biocides-special chemicals, in order to prevent the growth of sessile marine organisms. These compounds are entrapped in a releasing matrix or in an ablative paint, where the active ingredient is constantly leaching out. Following this approach, nowadays industries are trying to develop new synthetic biocides, paints with limited sloughing of toxic metals or hard antifouling paints, which create a porous film

**Marco Francese, Paola Frisenda**
*Shoreline. società cooperativa AREA Science Park, Trieste*

**Lucio Panizza, Elena Martelli**
*Archimede R&D Srl, Bologna*

**Alejandro Hochkoeppler**
*University of Bologna, Department of Pharmacy and Biotechnology*

on the surface where biocides are held and released slowly.

But there are also very promising alternative ways for innovation. Non-toxic silicon coatings produce slick surfaces where fouling growth cannot attach, but the critical issue is that they do not last long. Other innovative antifouling coatings use fluoropolymers or biodegradable polymers, or are made of a biocide-free epoxy resin. High-tech antifouling coatings are the amphiphilic/hybrid systems or the surface created with micro-topography.

However, considering the environmental impact of traditional antimicrobials, alternative antifouling strategies were recently considered [1]. In particular, the addition of antimicrobial nanoparticles or enzymes to paints has also been investigated. In our case study the active component, which was the subject of the experimentation carried out by Shoreline, was a paint-entrapped enzyme (also defined active component), without chemical bonds with the resin. Active component does not contain any heavy metals, nor other substances which, according to the current definition, are listed in the Biocides Directive (Directive 98/8/EC). The biocomponent-based antifouling paint can alter physical and chemical natural parameters all around the hull, taking advantage of substances present in the environment, according to the principle: produce in situ what you need and when you need it. The researcher has indeed considered that a micro-effervescence produced by a treated surface would physically (mechanically) and chemically (indirectly) inhibit the colonization by microorganisms.

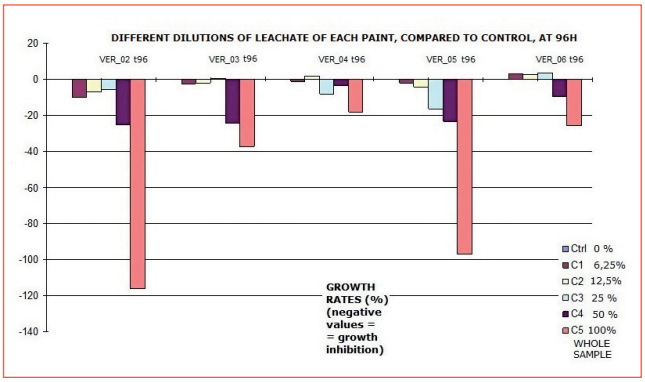
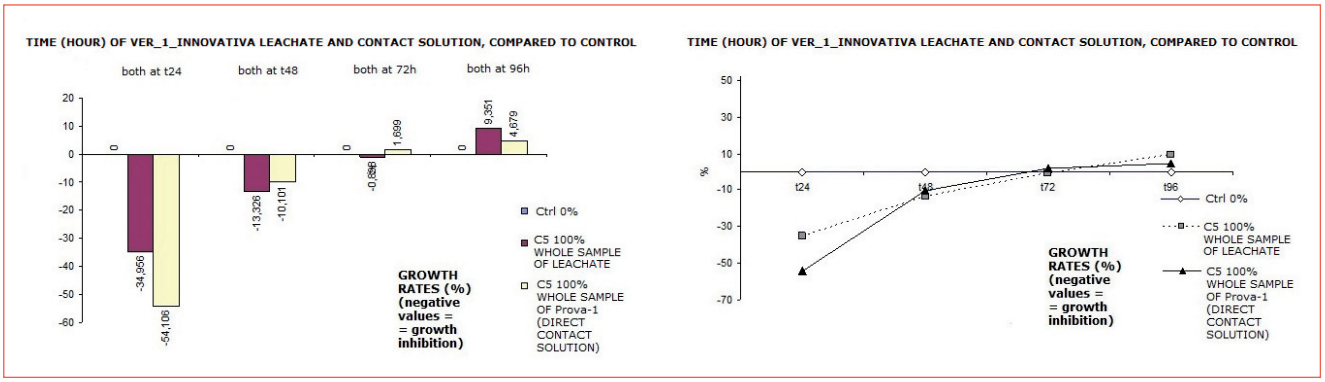



**FIGURE 1** Results (expressed as growth rate %) of the algal bioassays on various classical anti-fouling paints by leaching protocol

In recent years, only some environmentally-friendly materials, such as wood [2] and concrete for urban building [3] and road maintenance [4-5], have been tested for the possible direct effects on the environment. With regard to the "biocide-free" anti-fouling paints, a lot of research and experimental procedures have been carried out to demonstrate whether they are toxic or not [6-10], but no unanimous protocols have been developed yet, in order to assess whether they are completely non-hazardous for the environment [11].

The main purpose of this preliminary work was to evaluate the potential toxicity of several types of paints through the application of biological assays in accordance with standardized bio-assay protocols.




**FIGURES 2 AND 3** Results day by day (expressed as growth rate %) of the algal bioassays on innovative paints by leaching protocol and by direct contact protocol

## Materials and methods

The study was carried out both on paintings that could be defined as classic and on innovative antifouling paintings. The containers used for the tests were 500 mL glass beckers.

The media used for antifouling paints were fibreglass slides 5 cm long and 5 cm wide, with a specific primer for each paint. The specimens are described as follows:

- VER_01 INNOVATIVA (acrylic resin matrix, transparent)
- VER_02 (hard matrix, high percentage in copper oxide, water-based, coloured)
- VER_03 (ablative, low percentage in zinc oxide, coloured)
- VER_04 (hard matrix, high percentage in zinc oxide, coloured)
- VER_05 (ablative, medium percentage in copper oxide and zinc oxide, coloured)
- VER_06 (hard matrix, medium percentage in copper oxide and low percentage in zinc oxide, coloured)

The leaching protocol included an extraction time of 24 hours on an orbital shaker, at 20 ± 2°C (Ecotox Ecotherm 80), and the leaching solution (natural seawater filtered 0.45 microns (Millipore)) was not renewed. In the case of VER_01 INNOVATIVA paint, in addition to the leaching protocol, during the 96h algal test the sample was left inside a becker and submerged in a solution where unicellular seaweeds were inoculated, following a direct contact protocol (Prova-1).

The eco-toxicological assays that were applied are (i) the 96h growth inhibition using Phaeodactylum tricornutum Bohlin 1897 *(Bacillariophyceae, Naviculales)* (ISO 10253:2006), and (ii) the 48h mortality rate using *Artemia franciscana Kellogg 1906 (Crustacea, Branchiopoda),* as 2nd and 3rd nauplius stages (APAT-IRSA-CNR 2003 n. 8060). The target species and protocols chosen for the biological assays, were not the ones generally used to evaluate the effectiveness of antifouling paints, but they were the standard ones used for the evaluation of the pollutants toxicity in the natural environment. In this trial soft changes to the protocol were adopted since no toxicity of contaminated solutions was evaluated, but the physical

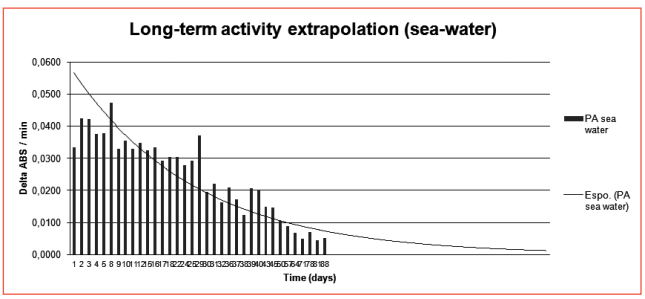
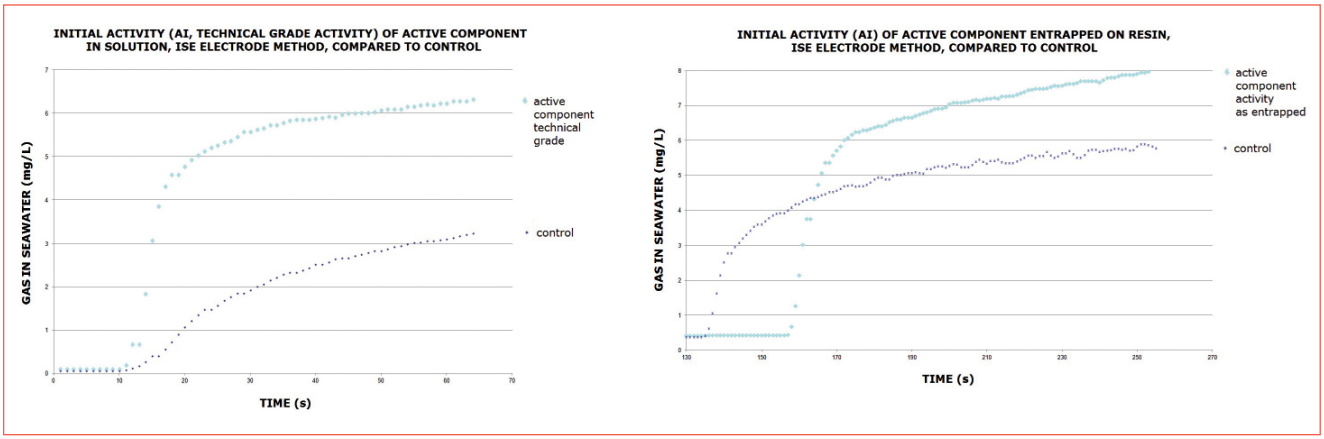



**FIGURE 4** Long-term activity extrapolation of active component activity in a seawater solution




**FIGURES 5 AND 6** Activity of active component (ISE electrode method) both in solution and entrapped on resin, compared to control

(mechanical) and chemical (indirectly) effects of the active component.

In addition, the active component stability and activity were tested both in a seawater solution and, after entrapment, in a water-based contaminant-free polymeric resin (Crilat 4816-Vinavil) spread on fiberglass slides. The analyses have been carried out using spectrophotometric techniques (Uvikon 923 UV/VIS) and gas sensitive electrodes (CyberScan pH 2100 BenchMeter - Eutech Instruments, with ionoselective membrane Mettler Toledo).

## Results

First of all, the comparison of the 2 bioassays that we chose to perform on classical anti-fouling paints has highlighted that Artemia did not show any differential effect, being non-toxic in all cases, whereas unicellular seaweeds were more sensitive, showing a differential toxic effect. Different growth inhibitions were observed, related to concentration of leachate: strong inhibition with whole sample (C5 100%) (Figure 1).

Conversely, in the innovative paint sample only an initial inhibition of algal growth rates was observed, but the final growth rates were subsequently comparable in the experimental and the control slides. During the experiment the algae were exposed both to leaching sample (Leaching) and to direct contact sample (Prova-1) over a period of 96 hours. Indeed, focusing the discussion on the rate of growth related to the whole sample (C5 100%) in the *P. tricornutum* assay (Figures 2 and 3), growth inhibition was observed during the

first 48h of exposure. In particular, after 24h there was a 35% decrease in the leaching sample (Leaching) and a 54% decrease if observing the results of direct contact protocol experiment (Prova-1), both compared to the control. After 48h, a decrease of about 10% was observed in both cases. After 72h and 96h, growth rates were similar to the control or even higher, with differences of about 5-10%.

Such results could not be associated to an active component inactivation, since data on active component activity were also gathered for a seawater solution and a long-term activity. By the data extrapolation carried out, the activity duration was estimated to be of about 6 months (Figure 4).

In addition, it was shown that after entrapment in resin the active component remained active and generated micro-effervescence, whereas this effect was observed as a lower rate in the control (Figures 5 and 6).

## Conclusions

According to these results, we can conclude that the use of bioassays to test non-releasing and non-toxic innovative paints is nonetheless valid, although some changes in the exposition protocols, as well as complementary data for result interpretation, are required. However, since the *A. franciscana* assay - which is commonly used to test the efficacy of anti-fouling paints [12] – appeared to be definitely less sensitive than the algal assay with *P. tricornutum*, we plan to carry out further experiments using different types of assays on target and non-target species. With regard to the enzyme-based paints, we can conclude that this first set of experiments have shown that the new product, which is active and stable for a considerably long time, does not induce sub-chronic toxicity. Further and innovative efficacy and resistance tests will be developed.



references


1. Faimali M., Piazza V., Geraci S. (2001), "Natural antifouling products: new technologies, old problems", *European Coatings,* num. 17, vol. 77: 59-64.
2. Libralato G., Marchetto D., Losso C., Arizzi Novelli A., Tagliapietra D.e Volpi Ghirardini A. (2006), "I lisciviati del legno: valutazione dei potenziali impatti ecotossicologici sulle acque marine costiere", *Atti del XV Congresso Nazionale della Società Italiana di Ecologia (Torino, 12-14 settembre 2005),* S3.11 Ecologia, a cura di Comoglio C. & Comino E. & Bona F. [online] URL: http://www.xvcongresso.societaitalianaecologia.org/articles/Libralato-144.pdf
3. Van der Sloot, H.A. e J.J. Dijkstra (2004). "Development of horizontally standardized leaching tests for construction materials: a material based or released based approach? Identical leaching mechanisms for different materials", ECN-C-04-060 (project nr. 2003.06.089): 1-56, Dutch Ministry of Housing, Spatial Planning and the Environment (VROM), Soil, Water and Rural Environment Directorate.
4. Nelson P.O., Huber W.C., Eldin N.N., Williamson K.J., Lundy J. R., Azizian M.F., Thayumanavan P., Quigley M.M., Hesse E.T., Frey K.M. e Leahy R.B. (2001). "Environmental Impact of Construction and Repair Materials on Surface and Ground Waters Summary of Methodology, Laboratory Results, and Model Development Transportation research board", *NCHRP Project 25-9 Report 448 National Research Council,* vol unico: 1-129, National Academy Press Washington d.c..
5. Herrington, P., Kvatch I., O'Halloran K. (2004). "Assessing the environmental effects of new and recycled materials in road construction: proposed guidelines", *Transfund New Zealand Research Report,* vol. 306: 1-70, Herrington & Kvatch (Opus Central Laboratories) & O'Halloran (Landcare Research Ltd).
6. Piazza V., Ferioli A., Giacco E., Melchiorre N., Valenti A., Del Prete F., Biandolino F., Dentone L., Frisenda P., Faimali M. (2012), "A standardization of *Amphibalanus (Balanus)* amphitrite *(Crustacea, Cirripedia)* larval bioassay for ecotoxicological studies", *Ecotoxicol Environ Saf.*, ed. 2012, vol. 79: 134–138.
7. Faimali M., Garaventa F., Cozzolino D., Romairone V. (2002a), "Efficacy and toxicity of experimental antifouling paints; a multi-bioassays screening (part one)", *European Coatings,* num. 10, vol. 78: 7-14.
8. Faimali M., Garaventa F., Cozzolino D., Romairone V. (2002b), "Efficacy and toxicity of experimental antifouling paints; a multi-bioassays screening (part two)", *European Coatings,* num. 16, vol. 78: 7-11.
9. Frisenda P., Francese M. (2012), "Protocollo sperimentale per il controllo della compatibilità ambientale di materiali innovativi", *Atti delle Giornate di Studio 5° edizione Livorno 7-9 novembre 2012,* Atti 2013: 235-241, ISPRA.
10. Mesariča T., Sepčiča K., Piazza V., Gambardella C., Garaventa F., Drobnea D. and M. Faimali. (2013), "Effects of nano carbon black and single-layer graphene oxide on settlement, survival and swimming behaviour of *Amphibalanus amphitrite larvae", Chemistry and Ecology 2013,* ISSN 1029-0370 (Online), vol. unico: 1-10, Taylor & Francis.
11. Löschau, M. e R. Krätke (2005). "Efficacy and toxicity of self-polishing biocide-free antifouling paints", *Environmental Pollution,* vol. 138: 260-267.
12. Persoone, G. e Castritsi-Catharios J. (1988). "A simple bioassay with Artemia larvae to determine the acute toxicity of antifouling paints", *Water research,* vol. 23: 893-897.

ENVIRONMENTAL OCCURRENCE AND CONCERNS OF ANTIFOULING BIOCIDES

# Ecological risk assessment and potential adverse effects posed by antifouling biocides to saltwater environments

Among human activities, the use of the antifouling paints in order to protect the ship's hull or submerged static structures from the colonization of aquatic organisms (fouling) represents a dangerous source of chemical contamination for coastal aquatic ecosystems worldwide. In recent years, the estimation of potential negative effects of biocides contained in antifouling paints upon the organisms and the aquatic ecosystems became an issue of great interest. To this aim, many ecological risk assessment (ERA) studies were conducted

*DOI: 10.12910/EAI2014-44*

*Luisa Parrella*

## Introduction

Fouling is the successive development of a community of bacteria, protozoa, algae and invertebrates on the surfaces exposed to water. The fouling formed on the boat hulls and submerged static structures is an undesirable process with economic and environmental negative consequences; for example, boat hull fouling causes an increase in water resistance during navigation and a consequent increase in fuel demand and pollution generated by the products of fuel combustion. In order to control and minimize the progressive biofouling on submerged surfaces, antifouling paints formulated to slowly release potent biocides are usually applied. Organotin biocides, especially tributyltin (TBT), were the most used additives in antifouling paints, but the International Maritime Organization (IMO) banned the use of TBT and similar compounds starting from 2003 worldwide, due to the high toxic effects posed to various non-target aquatic species. Consequently, paint manufacturers have developed new "TBT-free" formulations; the most common being the copper-based antifouling paints, in which a herbicidal booster biocide is added to enhance the antifouling effect. Active ingredients commonly incorporated as booster biocides in antifouling paints are Irgarol 1051, Diuron, Sea-nine 211, Chlorothalonil, Zinc pyrithione, and Dichlofluanid [1].

The extensive use of these biocides in antifouling paints may be responsible of the contamination of the costal aquatic environment worldwide [1]. Chemical contamination of coastal water and sediment may constitute an important hazard for non-target aquatic species and equilibrium of ecosystems. So, the quantitative estimation of occurring biocides in the environment and the evaluation of their potentially adverse effect on the aquatic ecosystems became a question of concern

**Luisa Parrella**
*University of Naples "Federico II"*

from both, ecological and economic point of view. For the diverse and complex nature of ecosystems, a quantitative estimation of the negative consequences is often difficult and far-reaching. In this context, the Ecological Risk Assessment provides an adequate interdisciplinary approach to estimate the potential effect associated to the occurrence of biocides in the environment.

## Ecological risk assessment

Ecological Risk Assessment (ERA) is defined as a process that evaluates the likelihood that adverse ecological effects may occur or are occurring as a result of exposure to one or more stressors [2]. A stressor can be any chemical, physical, or biological entity able to determine an adverse ecological effect; that is, changes that are considered undesirable because they alter important structural or functional characteristics, or components of ecological systems.

The process is used for systematically evaluating and organizing data, information, assumptions, and uncertainties, in order to understand and predict the relationships between stressors and ecological effects. There are two main advantages of ERA [3]: it comprises a framework that supports the environmental decision making, and it considers the natural high variability of ecosystems, or rather the aleatory uncertainties (which can never be fully eliminated), in estimating the adverse effects of stressors.

The final outcome of a risk assessment may range from qualitative judgments to a quantitative estimate of the possible risk associated to a stressor.

ERA can be used both in assessing whether effects are caused by past exposure to stressors (retrospective assessment) and in predicting the likelihood of future adverse effects (prospective assessment). The evaluation of the risk linked to the historic contamination of coastal seawaters from TBT, provides an excellent example of retrospective ERA while the evaluation of the risk posed by the new biocides formulation carried out before releasing in the environment is a typical case of prospective assessment.

The most common approach is described in the Guidelines for ERA from USEPA; it is worked out again in a compatible way in the ASTM (American Society for Testing and Materials) standard guide E 2205-02 for Eco-RBCA (Risk-Based Corrective Action for protection of Ecological resources), and consists in a three-stage methodology (Figure 1): 1) problem formulation 2) analysis 3) risk characterization. The process is more often iterative than linear, in fact one or more phases of the risk assessment can be revaluated integrating new data and new information. In the following paragraphs, the three phases of the procedure will be analyzed and the key issues related to ERA of biocides used in antifouling paints will be summarized.

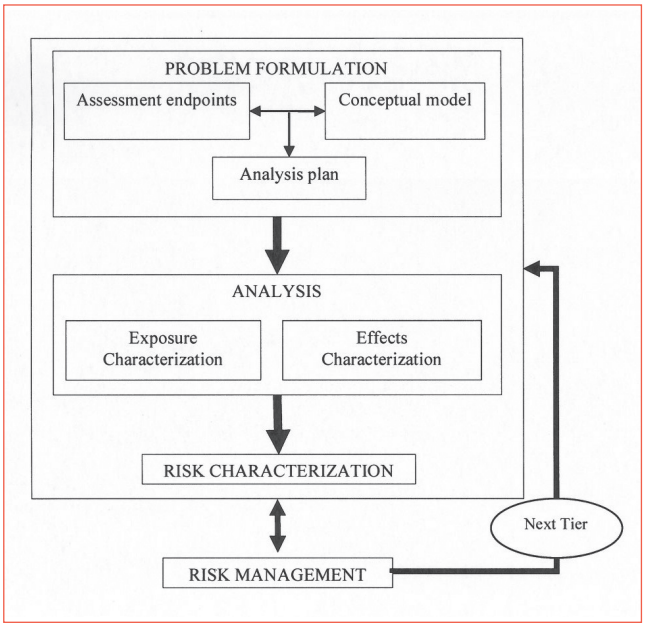



**FIGURE 1** Framework of ecological risk assessment

## Problem formulation

In the problem formulation, the goals that have to be addressed in the risk evaluation phase are identified; to this end all the available information on sources, stressor, effects and the ecosystem are collected; then, from the integration of this information, assessment endpoints are selected, and the conceptual model is prepared. The selection of appropriate assessment endpoints is a basic element of the risk evaluation process. Assessment endpoints are "explicit expression of the environmental value that is to be protected,

operationally defined by an ecological entity and its attributes" [2]. An ecological entity can be considered as a very important ecological receptor. It may include, for example, species or communities protected or rare, recreational, or commercial, or cultural important resources, specific valued habitat, species or communities that are important in maintaining the integrity and biodiversity of the environment [4]. Once the potential entity of concern has been identified, it is necessary to define what are the priority measurable attributes (i.e., survival, growth or reproduction endpoints) to be protected and potentially at risk. Generally the appropriate measures that have to be used in assessment endpoints are identified during the conceptual model development. The conceptual model is defined on the basis of the preliminary information about the ecosystem at risk, stressor characteristics, exposure pathways and ecological effects on assessment endpoints. The goal consists in defining the working hypothesis and developing an exposure diagram that describes the possible exposure and effect scenarios (Figure 2).

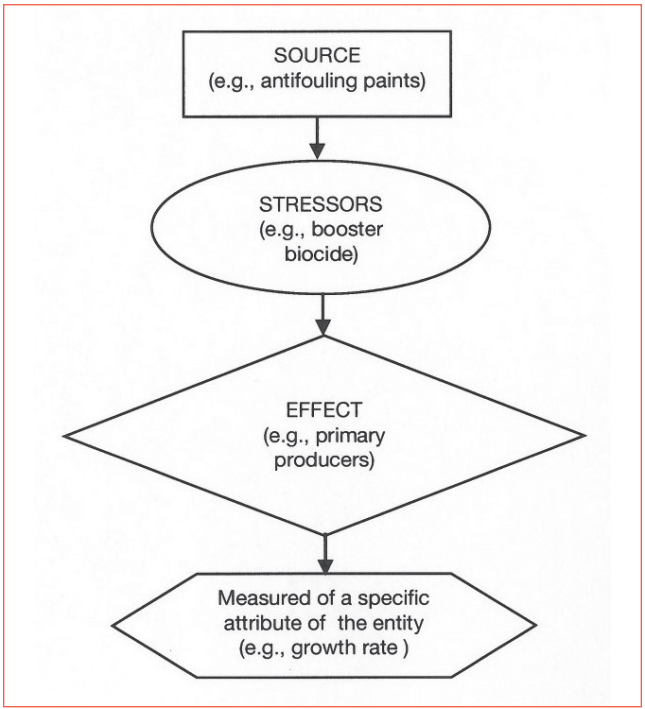



FIGURE 2 Flow diagram of the conceptual model

In the estimation of risk associated to the occurrence of antifouling biocides in aquatic ecosystem, the conceptual model can be based on the hypothesis that the use of antivegetative paint (i.e., source) on the submerged structures has contributed to the environment contamination through the release of these active substances; so, the booster biocides can be identified as primary chemical stressors. In addition, albeit banned from many years, also the tributyltin (TBT) represents an hazardous chemical stressor; in fact various studies showed that TBT contamination is still an actual problem for the environment, since its degradation in sediment (ranging from months to years) is much slower than in water (that is on the order of days), and sediments may then continue to be a source for the water column exposure [5].

Hence, the assessment consists in determining how these chemical stressors might have adverse effects towards the specific assessment endpoint. Some studies show that in the aquatic environment the most susceptible organisms to these substances, used as algaecide, are the plant species which may be directly affected rather than animal species, which may be affected indirectly. Consequently, to ensure a conservative approach the appropriate endpoint of concern is generally identified among non-target aquatic species of primary producers (Phytoplancton and Macrophite species), with the aim of evaluating the long-term viability of aquatic communities (plant, fish, invertebrates, etc.) and the integrity of the ecosystem's structures and functions [6-10]. Just in a few cases, marine invertebrate species [11] or both, aquatic plants and animals (i.e., Phytoplancton, Zooplancton, benthic and fish species) were considered. This is the case of TBT, since it has a significant tissue burden in many taxa with the highest bioaccumulation factor into the mollusks (minimal metabolic potential) [5].

Finally, the problem formulation step ends with the production of an investigation plan that has to be developed in the following "analysis" phase of ERA.

## Analysis

The analysis phase includes the exposure and effect characterization. This step is aimed at determining how

exposure to stressors may occur (i.e., exposure characterization) and what are the possible adverse ecological effects that may occur under exposure to this stressor themselves (i.e., effects characterization).
The objective of the exposure characterization is to produce an exposure profile that identifies the receptor (i.e., the exposed ecological entity), describes the paths of stressors from the source(s) to receptors (i.e., the exposure pathway), and evaluates – in terms of intensity, space and time – the stressors-receptors contact, or the co-occurrence of both. Estimation of exposure concentrations may be determined by using measured environmental concentration (MEC), obtained from monitoring studies, or predicted environmental concentration (PEC), obtained from computer simulations. Single exposure data can be used for a deterministic ERA, or to develop the distribution of P/MEC used in the probabilistic approach.
A synthesis of literature data related to exposure characterization as maximum environmental concentration of worldwide marinas, and the 90$^{th}$ percentile used as exposure benchmarks for different biocides were summarized in Table 1.

Literature data used in the exposure characterization of the most common booster biocides showed that, as expected, in open water areas the biocide concentrations were low or non-detected, while in enclosed or semi-enclosed marinas areas, higher biocide concentrations were found.
To complete the analysis phase, it is necessary to produce an accurate effect characterization. To this aim, the relationship between stressor levels and ecological effects, together with the plausibility that effects may occur, or are occurring as a result of exposure to stressors have to be examined [2]. Finally, these results were summarized in a stressor dose-response profile.
Identifying the appropriate ecotoxicological benchmark is another important step into effect characterization. The ecotoxicological benchmark is defined as the concentration of a chemical that is not likely to pose unacceptable adverse risks to the exposed biota [4]. In other words, it is the concentration value for which the ecosystem may be considered protected.
The reference value can be obtained by applying an assessment factor (AF) to ecotoxicological data, or also by the statistical extrapolation method, based on

| Stressor | Maximum values (ng l$^{-1}$) | 90$^{th}$ percentile (ng l$^{-1}$) | Site investigated | Years | References |
|---|---|---|---|---|---|
| Irgarol | 4000 | - | - | - | [11] |
| | 173 | 61 | Gulf of Napoli, Italy | 2005-2006 | [9] |
| | 1693 | 133 | European countries | 1992-1997 | [6] |
| | 1816 | 745 | Chesapeake Bay, U.S. | 2003 | [14] |
| | 182 | 64 | Southeast Florida, U.S. | 1999-2001 | [7] |
| | 85 | 48 | Carolinian Province, U.S. | 2004 | [14] |
| | 2427 | - | East Anglia, UK | - | [8] |
| | 186 | - | Brittany, France | - | [10] |
| | 410 | - | Pearl Harbour Estuary | - | [15] |
| | 620 | - | Hong Kong Waters | - | [15] |
| Diuron | 3050 | - | Japanese waters | - | [16] |
| | 430 | - | Dutch waters | - | [17] |
| | 1380 | 741 | Gulf of Napoli, Italy | 2005-2006 | [9] |
| | 249 | - | East Anglia, UK | - | [8] |
| | 268 | - | Brittany, France | - | [10] |
| Chlorothalonil | 1400 | - | - | - | [11] |
| Sea-Nine | 3700 | - | - | - | [11] |
| Dichofluanid | 5800 | - | - | - | [11] |
| TBT | 1801 | 387 | Chesapeake Bay U.S. | 1985-1996 | [5] |

**TABLE 1** Maximum Environmental concentration and 90$^{th}$ percentile exposure benchmarks for biocide stressors

sensitivity species distribution (SSD). For example the Predicted Non Effect Concentration (PNEC) can be obtained from measured or extrapolated effects concentration, such as the L/EC50 (lethal/effective median concentration), or NOEC (no-observed effect concentration), divided by an AF that ranges from 10 to 1000.

| Stressor | Organisms | Data type - Water type | Toxicity benchmarks (ng l-1) – (method) | | References |
|---|---|---|---|---|---|
| Ingarol | Plant species | L/EC50 - SW+FW | 251 | 10th Percentile | [7] |
| | Plant species | L/EC50 - SW+FW | 297 | 10th Percentile | [9] |
| | Plant species | EC50 - FW | 40.9 | 10th Percentile | [15] |
| | | EC50 - SW | 346.9 | | |
| | | NOEC - SW | 43.9 | | |
| | Invertebrate species | EC10[a] - SW | 80000 | PNEC | [11] |
| | | EC10[b] – SW | 290000 | | |
| | | EC10[c] - SW | 92000 | | |
| | Plant species | EC50 - FW | 130 | 5th Percentile | [10] |
| | | NOEC - FW | 5 | | |
| | | EC50 - SW | 110 | | |
| | | NOEC - SW | 4 | | |
| | | EC50 - SW+FW | 108 | | |
| | | NOEC - SW+FW | 3.7 | | |
| Diuron | Plant species | L/EC50 - SW+FW | 4846 | 10th Percentile | [9] |
| | Plant species | EC50 - FW | 2000 | 5th Percentile | [10] |
| | | EC50 - SW | 2900 | | |
| | | NOEC - SW | 260 | | |
| | | EC50 - SW+FW | 2300 | | |
| | | NOEC - FW+SW | 55 | | |
| Chlorothalonil | Invertebrate species | EC10[a] - SW | 450 | PNEC | [11] |
| | | EC10[b] – SW | 430 | | |
| | | EC10[c] - SW | 1200 | | |
| Sea-Nine | Invertebrate species | EC10[a] - SW | 710 | PNEC | [11] |
| | | EC10[b] – SW | 590 | | |
| | | EC10[c] - SW | 5800 | | |
| Dichlofluanid | | EC10[a] - SW | 5200 | PNEC | [11] |
| | | EC10[b] – SW | 28000 | | |
| | | EC10[c] - SW | 22000 | | |
| TBT | Plant+Animal species | L/EC50 - SW | 320 | 10th Percentile | [5] |
| | | L/EC50 – FW | 103 | | |
| | Invertebrate species | L/EC50 - SW | 5 | | |
| | | L/EC50 – FW | 102 | | |

[a] EC10 – *M. edulis,* [b] EC10 – *P. lividus,* [c] EC10 – *C. intestinalis*

**TABLE 2** Different toxicity benchmarks estimated for freshwater (FW) and saltwater (SW) organisms from different types of toxicity data

Toxicity Benchmarks corresponding to protection different levels as 95% (5$^{th}$ percentile), or 90% (10$^{th}$ percentile) of the species that composes the investigated ecosystem, can be obtained based on the effects concentration distribution derived from point estimates of acute or chronic toxicity values [12].
In Table 2, literature toxicity benchmarks are reported for the most commonly used biocides. Toxicity characterization studies highlight that autotrophic groups of species (i.e., macroalgae, microalgae, or cyanobacteria) are much more sensitive to Irgarol (43.9 ng $l^{-1}$) than the other biocides. In particular, results show that Irgarol 1051 is generally more toxic to the microalgae than to macroalgae, while the toxic response of Cyanobacteria to irgarol is still largely unknown [15], even if they are important primary producers in marine ecosystems and serve as essential food for many herbivores. By looking at Table 2, we can observe that very high values of sensitivity are presented by invertebrate saltwater species toward the TBT (5 ng $l^{-1}$).
Finally, based on the PNEC values, the considered biocides may be ranked in the following order from the highest to lowest toxicity: Chlorothalonil, Sea-Nine, dichlofounid and Irgarol.

## Risk characterization

Risk characterization is the final phase of ERA. During this step, the information obtained from all of the previous phases are integrated and presented in a comprehensive way for non-specialists to make the communication of key information possible for supporting decision-makers. The information contents should include a description of the nature, the risk magnitude for ecological resources, and also a qualitative and quantitative characterization of uncertainty [2].
Two specific methods are generally used to evaluate the adverse ecological effects of pollutants to organisms and ecosystem: (1) the hazard quotient calculation and (2) the probabilistic approach [2]. Numerical hazard quotient (HQ), or deterministic method is defined as the ratio of the MEC or PEC of the stressor, divided by a toxicant reference value as PNEC. If the resulting value is higher than one, a potential negative effects towards ecological receptors may be expected.
Main advantages of the quotient method are the easiness and velocity of use, and that risk assessors and managers are familiar with its application. In addition it provides an efficient, inexpensive tool for identifying high- or low-risk situations even if it may result useless when quantification of risk is needed. Moreover, in most cases, the quotient method does not explicitly consider the uncertainty. Therefore, in recent literature the use of probabilistic analysis has become preferable [7, 13].
The probabilistic analysis is a quantitative approach based on the comparison between exposure distribution for chemical stressors and a point estimate of effects or a distribution of effects. So, the full range of variability in the exposure and in the effect data is adequately represented.
Figure 3 shows that the likelihood that a certain percentage of species may be adversely affected, is indicated: in case (1), by the proportion of exposure distribution where concentration values exceed the effect levels of concern; instead in case (2), by the degree of overlapping of the curves of effect and exposure distribution (i.e., % of probability exceedence).
Results of the probabilistic ERA on antifouling biocides are reported for different water areas of the world. In European waters, the probability of exceedence of plant 10$^{th}$ percentile for Irgarol 1051 is evaluated in Cote d'Azur (France) with a maximum of 40% of exceedence. As expected, the highest value of exceedence occurred in marinas (24%) more than in the estuaries (1%) and in the coastal type stations (<1%) [6]. Viceversa, the ecological risk from exposure to Irgarol can be considered in the low risk range (0.1%-4%) for various marinas, ports, rivers, bay/embayments, open ocean and channel areas in the United States' surface waters (Chesapeake Bay, southeast Florida and Carolinian Province) [7, 14].
In this area, an exception occurs in Port Annapolis's marina (Chesapeake Bay), where in two different studies significant risk levels for both contaminants, Irgarol and TBT, are found. The analysis results suggest that TBT may pose a risk to aquatic biota with a 12% exceedence [5], whereas the annual probability of exceedence for Irgarol 1051 is extremely high, 99% in 2003 and 82% in 2004, even if additional measures of various

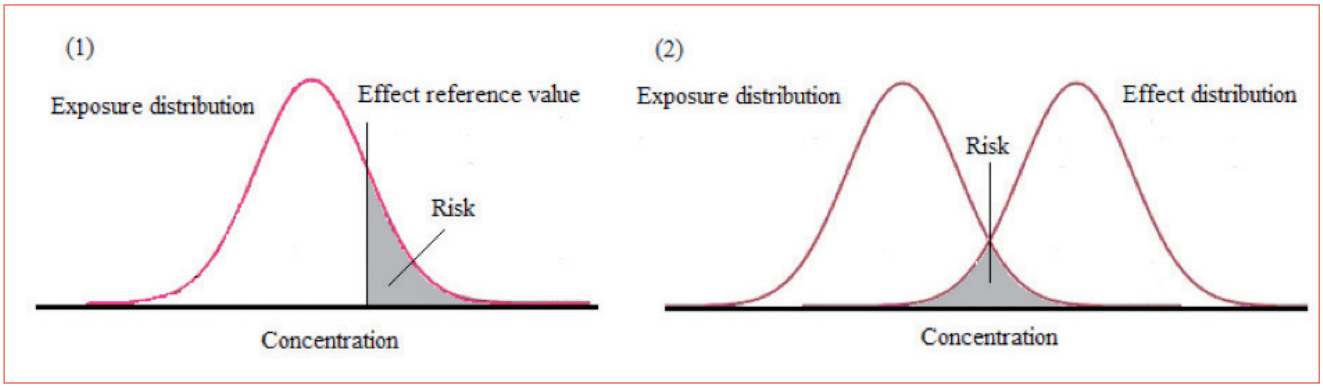



**FIGURE 3** Comparison of effect distribution with a single effect value and an effect distribution

functional and structural properties of resident phytoplankton communities in this areas do not support this severe evaluation [14].

The risk posed by Irgarol 1051 and Diuron considered as single contaminants was evaluated in the bay of Vilaine area (Brittany, France) [10], and in harbours and marinas in the Gulf of Napoli (Italy) [9]. In the first study, for the examined area high risk levels were observed for both contaminants, whereas in the second one, the risk levels posed by Irgarol and Diuron were estimated as negligible (<0.001%-5.5%) or low (<0.001%-13%), respectively.

The results obtained from the computation of HQ values allowed to conclude that in 2001 the freshwater of East Anglia (UK) contained Irgarol and Diuron at levels that induce stress and reduce the growth rate in the macrophyte populations [8]. Finally, from the HQ values of more commonly used booster biocides, chlorotahalonil, Sea-Nine 211 and dichlofluanid levels in marinas are found to possibly cause deleterious effects on the marine invertebrate population exposed (1.1<HQ<26), whilst Irgarol 1051 showed no toxic effects on the exposed organisms (HQ<1) [11].

The result of risk characterization can be used by risk managers to decide on a scientific basis whether the risks are acceptable or unacceptable for the environment, and to consider whether further activities are required. Risk managers may decide on risk mitigation measures, and then develop a monitoring plan to determine whether the procedures were efficient or whether ecological recovery is occurring. Managers may also elect to conduct another planned tier or iteration of the risk assessment, if needed, to support a management decision [2].

## Main considerations about antifouling biocides risk assessment

Potential ecological risk from exposure to the most common antifouling biocide was observed in many aquatic systems in Europe, the United States and other countries. However, to refine the risks conclusion and to improve the process of estimation of the potential impacts and of the level of protection for the aquatic species exposed, some critical aspects have to be much more investigated. They can be highlighted from the analysis of risk evaluation studies existing in literature.

The first critical aspect is to determine the role of marinas and of their endemic species. In fact these aquatic systems, due to their generally limited water exchange and intense yachting activity, represent the most sensitive areas where the worst case scenarios for biocides maybe applied. Hence, a key issue is to determine if the contaminated marinas systems serve as a nursery or as a refuge area for aquatic organisms and if, among potentially affected organisms, keystone species of high ecological, recreational or commercial value are included.

The second critical aspect is related to the need for determining the status of aquatic resources in marinas,

also taking into account that numerous stressors coexist in these environments. Therefore, a greater effort is demanded to improve our understanding on the site-specific ecotoxicological status, which is hardly available.
In spite of these limitations, the probabilistic risk assessment remains an attractive approach that allows to focus on the more significant problems related to chemical contamination of ecological systems, and to provide a basis for comparing, ranking and prioritizing risks.
Last but not least, with the aim of better exploiting economic resources, the risk assessment results can also be used in a cost-benefit analysis, which offers an additional interpretation of the effects of an alternative management option [2].

references


1. Konstantinou I. K. and Albanis T. A. (2004), Worldwide occurrence and effects of antifouling paint booster biocides in the aquatic environment: a review, *Environment International,* 30, 235-248.
2. U.S. Environmental Protection Agency (1992), Framework for ecological risk assessment, Washington, DC: Risk Assessment Forum, U.S. Environmental Protection Agency, EPA/630/R-92/001.
3. Chapman P. M., Wang F., (2000), Issues in ecological risk assessment of inorganic metals and metalloids, *Human and Ecological Risk Assessment,* 6, 965-988.
4. E 2205-02, Risk-Based Corrective Action for protection of Ecological resources, Annual Book of ASTM standard guide (2005), vol. 11.04, 1340-1400, West Conshohocken, PA: American Society for Testing and Materials.
5. Hall Jr. L. W., Scott M. C., Killen W. D., Unger M. A., (2000), A probabilistic ecological risk assessment of Tributyltin in surface waters of the Chesapeake Bay whatershed, *Human and Ecological Risk Assessment,* 6, 141-179.
6. Hall Jr. L. W., Giddings J. M., Solomon K. R., Balcomb R., (1999), An ecological risk assessment for the use of Irgarol 1051 as an algaecide for antifoulant paints, *Critical Reviews in Toxicology,* 29, 367-437.
7. Hall Jr. L. W. and Gardinali P. (2004), Ecological risk assessment for Irgarol 1051 and its major metabolite in united states surface waters, *Human and Ecological Risk Assessment,* 10, 525-542.
8. Lambert S. J., Thomas K. V., Davy A. J., (2006), Assessment of the risk posed by antifouling booster biocides Irgarol 1051 and Diuron to freshwater macrophytes, *Chemosphere,* 63, 734-743.
9. Di Landa G., Parrella L., Avagliano S., Ansanelli G., Macello E., Cremisini C., (2009), Assessment of the potential ecological risk posed by antifouling booster biocide to the marine ecosystem of the Gulf of Napoli (Italy), *Water Air Soil Pollut,* 200, 305-321.
10. Caquet Th., Roucaute M., Mazzella N., Delmas F., Madigou C., Farcy E., Bourgeot Th., Allenou J. P., Gabellec R., (2013), Risk assessment of herbiceds and booster biocide along estuarine continuums in the bay of Vilaine area (Brittany, France), *Environ Sci Pollut Res,* 20, 651-666.
11. Bellas J., (2006), Comparative toxicity of alternative antifouling biocides on embryos and larvae of marine invertebrates, *Science of the Total Environment,* 367, 573-585.
12. Solomon K. R., Baker D. B., Richards R. P., Dixon K. R., Klaine S. J., La Point T. W., Kendall R. J., Weisskope C. P., Giddings M. J., Giesy J. P., Hall Jr. L. W., Williams W. M., (1996) Ecological risk assessment of atrazine in north American surface waters, Annual Review, *Environmental Toxicology and Chemistry,* 15, 31-76.
13. Regan H. M., Akcakaya H. R., Ferson S., Root K. V., Carroll S., Ginzburg L. R., (2003) Treatments of uncertainty and variability in ecological risk assessment of single-species populations, *Human and Ecological Risk Assessment,* 9, 889-906.
14. Hall Jr. L. W., Killen W. D., Anderson R. D., Gardinali P., Balcomb R. (2005), Monitoring of Irgarol 1051 concentrations with concurrent phytoplankton evaluations in east coast areas of the United States, *Marine Pollution Bulletin,* 50, 668-681.
15. Zhang A. Q., Leung K. M. Y., Kwok K. W. H., Bao V. W. W., Lam M. H. W., (2008), Toxicities of antifouling biocide Irgarol 1051 and its major degraded product to marine primary producers, *Marine Pollution Bulletin,* 57, 575-586.
16. Okamura H., Ayoama I., Ono Y., Nishida T., (2003), Antifouling herbicides in the coastal waters of western Japan, *Marine Pollution Bulletin,* 47, 59-67.
17. Lamoree M. H., Swart C. P., van der Horst A., van Hattum B., (2002), Determination of Diuron and antifouling paint biocide Irgarol 1051 in Dutch marinas and coastal waters, *Journal of Chromatography A.,* 970, 183-190.

ENVIRONMENTAL OCCURRENCE AND CONCERNS OF ANTIFOULING BIOCIDES

# Biocides in antifouling paints: environmental concentration levels and distribution

Antifouling (AF) paints prevent the settlement, adhesion and growth of organisms on a painted surface by the action of biocides, which are the active ingredients. Many chemicals were and are used as biocides, which have very different physico-chemical properties and therefore differing environmental effects. Copper and organotin compounds have raised concern worldwide: extensive research exists to understand their bioavailability and toxicity. For longstanding biocides, for example Irgarol 1051 and Diuron, there is a large amount of environmental data allowing the assessment of their impact. For other biocides such as dichlofluanid, SeaNine 211 or zinc/copper pyrithione, fate and effects are clear, but only few monitoring studies have been performed

*DOI: 10.12910/EAI2014-45*

*Salvatore Chiavarini, Carla Ubaldi, Sigfrido Cannarsa*

## Introduction

Colonisation by fouling organisms is a problem for any structure placed in the aquatic environment and can be controlled through both chemical biocides and non-biocidal technologies. In spite of the work on diverse non-biocidal technologies and an increase in the commercial use of fouling-release coatings, the majority of vessels are still protected by antifouling (AF) paints containing biocides.

The key property of a good AF biocide should be its effectiveness in preventing the fouling of the painted surface without persistence at concentrations that can cause detrimental environmental effects. This can be achieved through rapid transformation following release from the surface or possibly a very specific mode of action.

Physico-chemical data pertinent to the environmental fate of AF compounds are, e.g., octanol–water partition coefficient $K_{OW}$, degradation half-life ($t_{½}$) and principal degradation mechanisms, as well as their known primary metabolites. Such data are readily available from literature and have been summarized in many excellent reviews [1-4] and therefore will not be discussed or reported here.

Release rates (or leaching rates) are crucial factors to model environmental concentrations. Over the past years, very few studies have been reported on the release of AF biocides from painted surfaces. Standard protocols (ASTM, ISO) can be applied, however published release rate data are scarce and there is concern that standard laboratory methods do not replicate what occurs in the environment and, therefore, are not representative [5].

**Salvatore Chiavarini, Carla Ubaldi, Sigfrido Cannarsa**
*ENEA, Technical Unit for Environmental Characterization, Prevention, and Remediation*

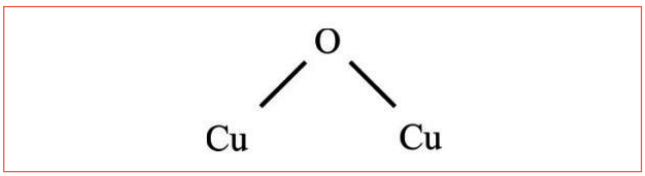
**Sigfrido Cannarsa**
*ENEA, Technical Unit for Marine Environment and Sustainable Development – Santa Teresa Research Centre*

## Environmental fate and occurrence

Processes controlling persistence and sinks are very important because biocides are deliberately released into the water column. Key processes important in understanding the fate of AF biocides are degradation, partition onto sediments and uptake into organisms. Compounds with a half-life >50 days are considered to be persistent, whereas those with a log $K_{OW}$ >3 are considered as bioaccumulating. The sediment-specific equilibrium sorption constant, $K_d$, describes the distribution of a compound between sediment and water. The values range from $10^5$, for compounds such as DDT and PCBs that bind strongly to sediments, to <1 for compounds that are weakly sorbed and soluble in water.

## Copper oxide

Copper oxide leaches from the boat surface and enters the water as a free copper ion ($Cu^+$) and is immediately oxidized to $Cu^{2+}$, forming complexes with inorganic and organic ligands. The process is thought to occur within the first few micrometers of the painted surface. The presence of a biofilm on the vessel surface can act as a source of DOC, which can bind the free copper. In the dissolved phase, the speciation varies greatly with respect to water properties, such as DOC, pH, hardness and salinity. Copper easily adsorbs to suspended particulate matter (SPM), settling and accumulating in the sediment. As a result, copper concentrations are often two to three orders of magnitude higher in the sediment than in the water column [6]. In aerobic sediments, copper is mainly bound to metal oxides and high molecular weight organic matter while in anaerobic sediments, copper is bound strongly to sulphides reducing bioavailability. However, for both aerobic and anaerobic conditions, sediment disturbance events, such as dredging and storms can significantly increase the copper input into the water column from the underlying sediment.



Copper oxide

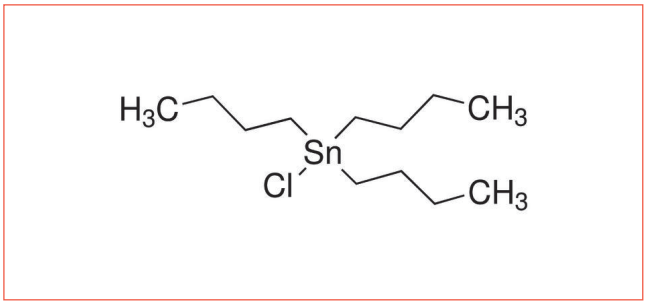
Copper obviously occurs naturally in the environment. Natural background concentrations of copper within estuarine and coastal seawater typically range between 0.5 and 3 μg $L^{-1}$. Copper concentrations can potentially rise in the marine environment in enclosed harbours and marinas with little water exchange and high boat densities, but despite the projected problem in these high risk areas, very little monitoring work has been carried out [1].

Most monitoring studies reporting on copper concentrations in the marine environment measure total dissolved copper concentration, which fails to provide information on the bioavailability of the metal. The speciation of copper is fundamental to its bioavailability and toxicity, with the free ion considered as the most toxic form. In a recent survey of UK coastal waters [6], total dissolved copper ranged from 0.30 to 6.68 μg $L^{-1}$, with only one concentration above the EQS of 5 μg $L^{-1}$. Also in this case, elevated concentrations were found in an enclosed marina with little to no water exchange. The labile copper concentrations for the same water samples ranged from 0.02 to 2.69 μg $L^{-1}$, with labile copper contributing 10–30% of the total dissolved copper concentration. Despite the elevated concentrations of total dissolved copper at some marinas, the labile copper concentration remained stable.

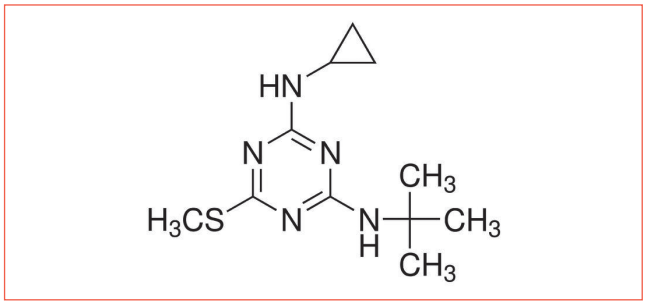
Being a strongly regulated metal in biota, bioconcentration is not an issue for copper as BCF (bioconcentration factor) is a meaningless parameter in this case.

## Tributyltin (TBT)

TBT compounds are organic derivatives of tin ($Sn^{4+}$), having the general formula $(n\text{-}C_4H_9)_3Sn\text{-}X$, where X is an anion or a group linked covalently through a hetero-atom. X influences the physicochemical properties (water solubility, vapour pressure, etc.). Generally, the toxicity of the organotin is more influenced by the alkyl substitutes than by the anionic substitutes. Tributyltin oxide (TBTO) and tributyltin chloride




Tributyltin (TBT)




Irgarol

have been normally used in laboratory experiments to investigate organotin toxicity. In the aquatic environment, TBT is quickly removed from the water column to bed sediments: TBT has a high specific gravity (about 1.2 kg $L^{-1}$ at 20 °C), low solubility (<10 mg $L^{-1}$ at 20 °C and pH 7.0), and log $K_{ow}$ values near 4.4 at pH 8.

Additionally, TBT is ionisable having pKa=6.25. Therefore, TBT sorption/desorption from natural sediment can be strongly influenced by changes in pH and salinity, similarly to what has been reported for other ionisable hydrophobic organic contaminants in sandy sediments or soils. The reversible adsorption of TBT makes contaminated sediments a long-term source of dissolved-phase contamination to water column. Adsorption to sediments is positively correlated to the extent of substitution on the Sn atom (monobutyltin (MBT)<dibutyltin (DBT)<TBT).

Many studies have involved surveys of TBT distribution in the water column, sediments, and biota [4]. Given its strong affinity, benthic sediments are the major sink for TBT in the environment. Measurements taken prior to restrictions on TBT use in antifouling paints have shown levels higher than 500 ng $L^{-1}$ in North American and European marinas. In recent investigations, it has been reported that TBT concentrations in water, sediment and biota have generally declined, rarely exceeding 100 ng $L^{-1}$. This testifies that past measures against pollution caused by organotin compounds have been at least partly successful [2]. Nevertheless, this decline might be called into question, as several monitoring campaigns did not reach conclusive results. Exceptions to this general decline of TBT in bottom sediments have been reported as hot spots associated with ship channels, ports, harbours, and marinas [7,8]. Other exceptions to this general decline of TBT pollution have been observed in newly industrialising countries [9].

## Irgarol

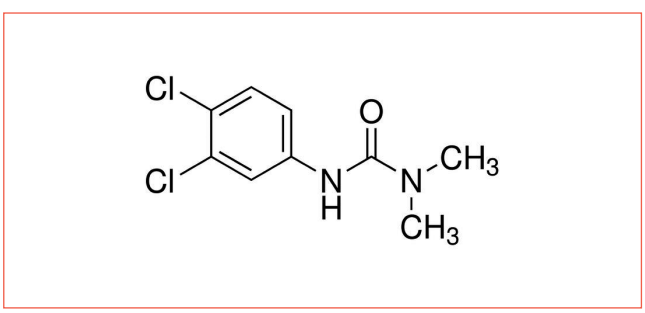
Irgarol 1051 does not easily degrade in water, which may explain its persistence once released from painted surfaces. In natural seawater, Irgarol 1051 has a half-life of between 100 and 350 days whilst being very persistent in anaerobic sediments. 2-methylthio-4-tert-butylamino-6-amino-s-triazine (M1) is the main transformation product of Irgarol 1051, produced through n-dealkylation following biodegradation, photodegradation or chemical hydrolysis. Other metabolites (M2, M3) have been shown to occur in the environment. The persistence of the metabolites is largely unknown, but M1 is relatively stable in water (t½ >200 days) and sediment (t½ >260 days).

Water-sediment partitioning coefficients suggest that Irgarol 1051 will be mainly associated with the dissolved phase and estimations suggest that around 4% of Irgarol 1051 in marina waters will partition to sediments. Indeed, Irgarol 1051 occurs in sediments with numerous reported campaigns [1,10]. A few studies have also reported the occurrence of M1 in sediments, at lower concentrations than Irgarol 1051. Irgarol 1051 is persistent in sediments whether adsorbed to sediment particles or associated with paint particles. Little is known about the bioavailabilty of Irgarol 1051 present in sediments. It has been shown

that the resuspension of sediments contaminated with Irgarol 1051 can result in release of bioavailable Irgarol 1051 into the water column.
Irgarol 1051 is the most studied of the non-organotin AF biocides with a large number of reports on its environmental occurrence. Irgarol 1051 was first reported as an aquatic contaminant in 1993 in the Mediterranean, with its occurrence subsequently reported in Europe, the US, Caribbean, Asia and Australia in areas where there are boats coated with AF paints containing Irgarol 1051. Freshwater environments where intensive boating activity, combined with limited water exchange, are present can have very high aqueous concentrations of Irgarol 1051.

## Diuron

Diuron also persists in seawater, but is less persistent in marine sediments with a half-life of 14 days. The aerobic degradation of Diuron results in the transformation of Diuron to 1-(3,4-dichlorophenyl)-3-methylurea (DCPMU) and 1-(3,4-dichlorophenyl)urea (DCPU). Anaerobic degradation in sediments results in the formation of 1-(3-chlorophenyl)-3,1-dimethylurea (CPDU). Diuron is relatively soluble in water (35 mg $L^{-1}$) and has a reported log $K_{OW}$=2.8, suggesting that it will predominantly be found in the dissolved phase and only weakly sorbed to sediments, which is in line with reported environmental concentrations. In fact, although Diuron can be present at high concentrations in marina surface waters, it has only been detected at low concentrations in sediments [11,12]. Concentrations as high as 1.4 μg $g^{-1}$ have been reported in sediments collected from an enclosed marinas in the UK. However, these high concentrations are likely to be due to the contamination of marina sediments with AF paint particles that are washed into the water, following shore-side scrubbing of boat hulls on hard standings [5]. Albeit relatively persistent in seawater, Diuron is thought to undergo degradation under anaerobic conditions to form CPDU (t½ >14 days). When associated with AF paint particles, this transformation is significantly reduced, with very little degradation seen over 42 days.



Diuron

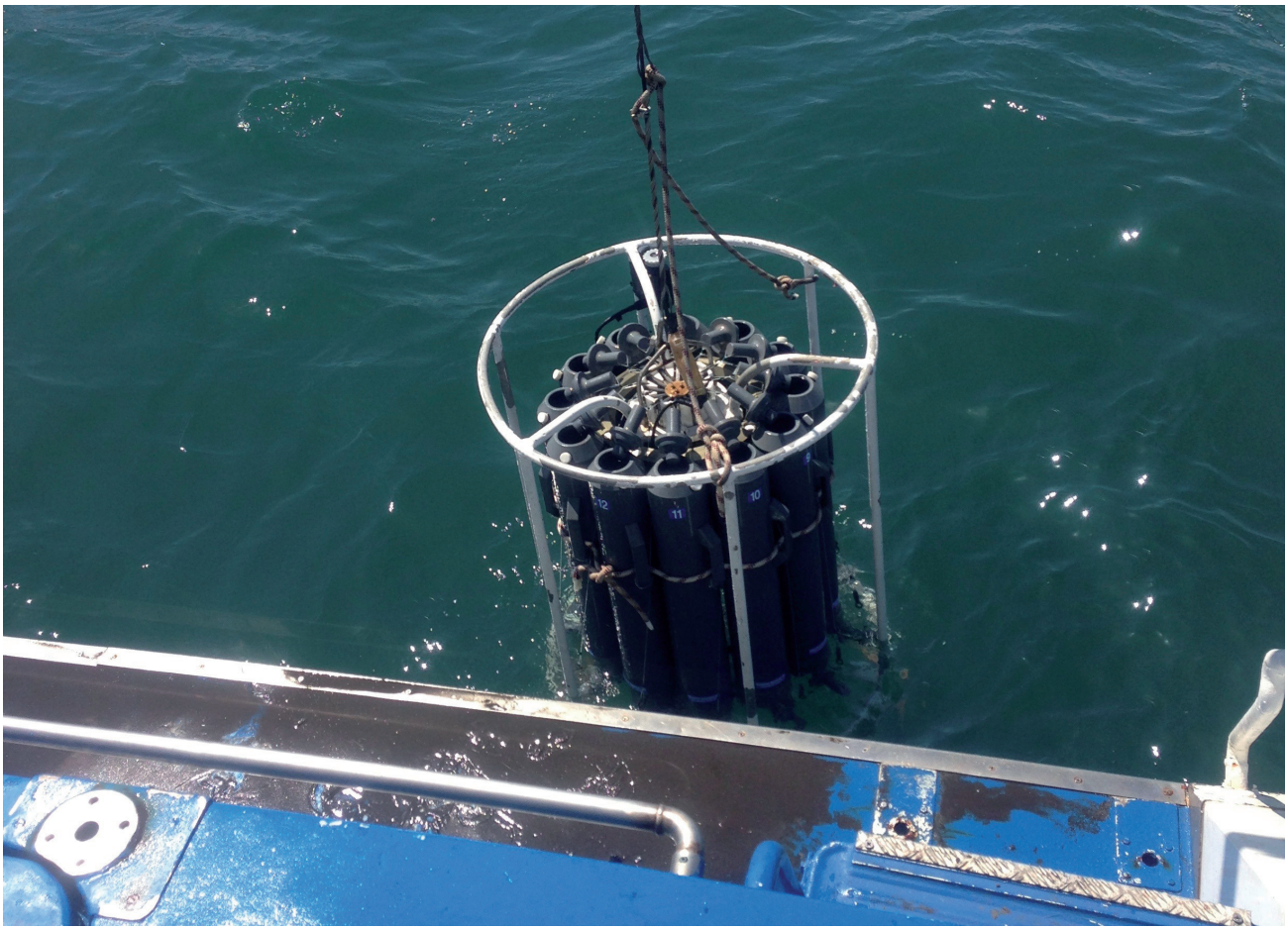
## Conclusions

Extensive monitoring of biocides concentration in water, sediment, and biota is needed to support concerted actions to ban or regulate the use of booster biocides. Enough data are available for the biocides most commonly used in Europe, North America and Japan (Irgarol 1051, Diuron, SeaNine 211), whilst few or no data are available for other biocides, or devoloping countries. Few data are also available on the occurrence of degradation products that are mainly referred to Irgarol 1051 and Diuron metabolites. For new or candidate biocides (triphenylborane pyridine, capsaicin, etc.) very scarce information seems to be available at the moment.
Monitoring, behaviour and toxicity of degradation products should be emphasized. The need for further research in several vitally important areas, such as occurrence, fate and effects of booster biocides, is well established by the scientific community, in order to underpin risk assessments and protect the environments close to moored vessels. Although the concentration levels of some biocides were not high enough to have acute toxic effects directly on higher species, their chronic effects at low concentrations are unknown and difficult to determine. Gaps in the available data make the evaluation of their impact on the aquatic environment difficult. The precautionary principle provides a good basis on which to formulate policies to the marine environment, and should be invoked when it comes to the use of booster biocides.

Rd

references


1. Thomas, K. V & Brooks, S. The environmental fate and effects of antifouling paint biocides. *Biofouling* 26, 73–88 (2010).
2. Matthiessen, P. Detection, monitoring, and control of tributyltin--an almost complete success story. *Environ. Toxicol. Chem.* 32, 487–9 (2013).
3. Sousa, A. C. A., Pastorinho, M. R., Takahashi, S. & Tanabe, S. History on organotin compounds, from snails to humans. *Environ. Chem. Lett.* 12, 117–137 (2014).
4. Antizar-Ladislao, B. Environmental levels, toxicity and human exposure to tributyltin (TBT)-contaminated marine environment. A review. *Environ. Int.* 34, 292–308 (2008).
5. Thomas, K. V, Fileman, T. I. M. W., Readman, J. W. & Waldock, M. J. Antifouling Paint Booster Biocides in the UK Coastal Environment and Potential Risks of Biological Effects. *Mar. Pollut. Bull.* 42, 677–688 (2001).
6. Jones, B. & Bolam, T. Copper speciation survey from UK marinas, harbours and estuaries. *Mar. Pollut. Bull.* 54, 1127–38 (2007).
7. Harino, H. Occurrence and degradation of representative TBT free-antifouling biocides in aquatic environment. *Coast. Mar. Sci.* 29, 28–39 (2004).
8. Chiavarini, S., Massanisso, P., Nicolai, P., Nobili, C. & Morabito, R. Butyltins concentration levels and imposex occurrence in snails from the Sicilian coasts (Italy). *Chemosphere* 50, 311–319 (2003).
9. Jadhav, S., Bhosle, N. B., Massanisso, P. & Morabito, R. Organotins in the sediments of the Zuari estuary, west coast of India. *J. Environ. Manage.* 90 Suppl 1, S4–7 (2009).
10. Konstantinou, I. K. & Albanis, T. a. Worldwide occurrence and effects of antifouling paint booster biocides in the aquatic environment: a review. *Environ. Int.* 30, 235–48 (2004).
11. Thomas, K. V, McHugh, M. & Waldock, M. Antifouling paint booster biocides in UK coastal waters: inputs, occurrence and environmental fate. *Sci. Total Environ.* 293, 117–27 (2002).
12. Landa, G. et al. Assessment of the Potential Ecological Risks Posed by Antifouling Booster Biocides to the Marine Ecosystem of the Gulf of Napoli (Italy). *Water. Air. Soil Pollut.* 200, 305–321 (2008).

ENVIRONMENTAL OCCURRENCE AND CONCERNS OF ANTIFOULING BIOCIDES

# An overview of the analytical methods to determine the main antifouling paint biocides in marine samples

This paper offers a general overview of the analytical techniques and instruments employed in trace analysis of common booster biocides from antifouling (AF) paints, in seawater and sediment samples. Due to low concentrations and matrix effects, a suitable sample preparation step is usually performed prior to analysis. To identify and quantify AF compounds, gas or liquid chromatography is typically used, with either a selective detector that exploits analyte properties, or a mass spectrometer that allows the analysis of a broader range of compounds

*DOI: 10.12910/EAI2014-46*

*Giuliana Ansanelli, Giuseppe Di Landa*

## Introduction

As the International Maritime Organization (IMO) prohibited the presence of highly toxic tributyltin (TBT) on ship and boat hulls, paint manufacturers have developed copper-based antifouling (AF) paints; however these alternative AF paints have to be supplemented with specific organic compounds, the so-called 'booster biocides', in order to achieve protection against copper-resistant fouling organisms. Main booster biocides used in AF paints are Irgarol 1051, Diuron, dichlofluanid, chlorothalonil, Sea-Nine 211, TCMTB (2-(thiocyanatomethylthio)benzothiazole), zinc pyrithione (ZnPT), dithiocarbamates (including maneb, thiram, zineb and ziram), and TCMS-pyridine (2,3,5,6-tetrachloro-4-(methylsulphonyl)pyridine).

Some AF biocides are also used as herbicides and fungicides in agriculture. A large number of studies has been performed on the adverse effects of these active compounds to non-target marine organisms, and they showed toxic action at the µg/L and ng/L levels. Due to the harmful behaviour and in some cases environmental persistence, AF biocides raised concern as environmental contaminants, and this has encouraged the development of reliable and sensitive analytical methods able to monitor their occurrence in the marine environment.

Environmental samples are usually characterized by trace levels of organic pollutants, but also a large number of matrix components which may disrupt the analysis. To overcome these problems, the analytical methodologies usually involve a pre-concentration/clean-up step prior to the determination by gas or liquid chromatography (GC or LC). A further step (derivatization) may be required for some AF compounds (e.g., Diuron) not directly amenable to GC analysis. Sample pretreatments are usually labor-intensive and time-consuming tasks, and often constitute the bottleneck of the analytical procedures since they account for more than 75% of the analysis time.

**Giuliana Ansanelli, Giuseppe Di Landa**
*ENEA, Technical Unit for Technologies Development - Portici Research Centre*

In this paper we will focus on the main methods aimed at the extraction and analysis of booster biocides in both seawater and sediment matrix, which are reported in the scientific literature. These analytical methodologies are summarized in Table 1.
A recent trend in determining AF biocides is towards the development of multiresidue analytical methods that allow the simultaneous determination of several analytes in a single analysis, thus reducing time and costs. This approach is not feasible for the determination of ZnPT and specific methods have been reported [1, 2].

| Compound | Matrix | Extraction method | Analytical system | % Recovery (R.S.D.[a]) | LOD (ng/L in seawater, ng/g dw in sediment) | Reference |
|---|---|---|---|---|---|---|
| Chlorotalonil | Seawater | LLE (DCM) | GC-EI-MS | 90-92 (4-6) | 20.0 | 3 [b] |
| | Seawater | SPE (C18) | HPLC–DAD | 92 (5.9) | 10.0 | 6 [b] |
| | Seawater | LLE (toluene) | GC-MS | 120.3 (4.9) | 5.5 | 4 [b] |
| | Seawater | SME (toluene, xylene) | GC-ECD | 94 (3.4) | 2.5 | 14 [b] |
| | Seawater | SPE (C18) | GC-ECD | 93 (3-12) | 5.0 | 13 [b] |
| | Seawater | SPME (PDMS 100 um) | GC-ECD | 103 (5-15) | 5.0 | 13 [b] |
| | Seawater | SBSE (PDMS) | GC-MS | 81-120 (6.4) | 10.0 | 15 [b] |
| | | | | | | |
| | Sediment | Shaking (acetone, DCM)/LLE | GC-EI-MS | 81-82 (8-12) | 50.0 | 3 [b] |
| | Sediment | sonication (acetone)/SPME (PDMS) | GC-MS | 74 (11) | 6.0 | 19 [b] |
| Dichlofluanid | River water | LLE (DCM) | GC-EI-MS | 90-91 (5-7) | 20.0 | 3 [b] |
| | Seawater | SPE (C18) | HPLC–DAD | 68 (10.8) | 415.0 | 6 [b] |
| | Seawater | LLE (toluene) | GC-MS | 93.8 (2.3) | 1.8 | 4 [b] |
| | Seawater | SME (toluene, xylene) | GC-ECD | 88 (4.6) | 3.0 | 14 [b] |
| | Seawater | On line SPE (LiChrolut EN) | HPLC-APCI-MS | 87-89 (1-8) | 5.0 | 7 [b] |
| | Seawater | SPE (EACDc) | GC-ECD | 95 (3-12) | 9.0 | 13 [b] |
| | Seawater | SPME (PDMS 100 um) | GC-ECD | 103 (5-15) | 2.0 | 13 [b] |
| | Seawater | SBSE (PDMS) | GC-MS | 76-119 (6.6) | 30.0 | 15 [b] |
| | Seawater | On line SPE (PLRP-S) | GC-MS | 67 (5-19) | 20.0 | 24 [b] |
| | Seawater | SPE (Envirelut Pesticide) | HPLC–ESI/MS/MS | >72 (<10) | 0.1-0.2 | 31 [b] |
| | | | | | | |
| | Sediment | Shaking (acetone, DCM)/LLE | GC-EI-MS | 81-83 (4-7) | 50.0 | 3 [b] |
| | Sediment | sonication (acetone)/SPME (PDMS) | GC-MS | 84 (7) | 1.0 | 19 [b] |
| | Sediment | MAE (MeOH) +SPE (Envirelut Pesticide) | HPLC-MS/MS | 76.2 (4.4) | 0.3 | 22 [b] |
| Diuron | River water | LLE (DCM) | GC-EI-MS | 92-93 (2-4) | 20.0 | 3 [b] |
| | Seawater | SPE (C18) | HPLC–DAD | 101 (3.5) | 38.0 | 6 [b] |
| | Seawater | LLE (DCM) | HPLC–ESI/MS/MS | 98 (5.2) | 0.7 | 5 [b] |
| | Seawater | On line SPE (LiChrolut EN) | HPLC–APCI-MS | 97-99 (1-8) | 10.0 | 7 [b] |
| | Seawater | SPE (Excelpak SPE-GLF) | HPLC–ESI/MS/MS | 93 (11) | 0.7 | 8 [b] |
| | Seawater | SPE (C18) | HPLC–APCI-MS | 100.3 (12.1) | 1.0 | 9 [b] |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Seawater | SPE (C18) | HPLC-MS/MS | 127 (10) | 2.0 | 21 [b] |
| | Seawater | SPE (Envirelut Pesticide) | HPLC–ESI/MS/MS | >72 (<10) | 0.1-0.2 | 31 [b] |
| | Sediment | Shaking (acetone,DCM)/LLE | GC-EI-MS | 84-85 (4-7) | 50.0 | 3 [b] |
| | Sediment | Shaking (acetone,DCM) | HPLC–ESI/MS/MS | 96 (8) | 0.08 | 5 [b] |
| | Sediment | Shaking (ACN)+SPE (Excelpak SPE-GLF) | HPLC–ESI/MS/MS | 94 (7.5) | 0.08 | 8 [b] |
| | Sediment | ASE (DCM) | HPLC-MS/MS | 91 (13) | 0.3 | 21 [b] |
| | Sediment | MAE+SPE (Envirelut Pesticide) | HPLC-MS/MS | 92.9 (5.1) | 0.2 | 22 [b] |
| Folpet | Seawater | On line SPE (LiChrolut EN) | HPLC-APCI-MS | 85-90 (1-8) | 200.0 | 7 b |
| | Seawater | SPE (C18) | GC-ECD | 82 (3-12) | 5.0 | 13 b |
| | Seawater | SPME (PDMS 100 um) | GC-ECD | 99 (5-15) | 10.0 | 13 b |
| Irgarol 1051 | Seawater | SPE (C18) | HPLC–DAD | 93 (3.8) | 31.0 | 6 [b] |
| | Seawater | LLE (toluene) | GC-MS | 73.5 (1.6) | 7.7 | 4 [b] |
| | Seawater | LLE (DCM) | HPLC–ESI-MS/MS | 90 (6.5) | 0.8 | 5 [b] |
| | Seawater | On line SPE (LiChrolut EN) | HPLC-APCI-MS | 91-95 (1-8) | 5.0 | 7 [b] |
| | Seawater | SPE (Excelpak SPE-GLF) | HPLC–ESI-MS/MS | 97(6.5) | 0.8 | 8 [b] |
| | Seawater | SPE (C18) | GC-ECD | 96 (3-12) | 2.0 | 13 [b] |
| | Seawater | SPME (PDMS 100 um) | GC-FTD, GC-MS | 101 (5-15) | 5.0 | 13 b |
| | Seawater | HS-SPME (PDMS–DVB 65 um) | GC-FTD | 118 (5-15) | 8.0 | 16 b |
| | Seawater | SBSE (PDMS) | GC-MS | 97-116 (7.3) | 5.0 | 15 b |
| | Seawater | SFE-IAC | GC-NPD | 87 (8.5) | 3.0 | 17 |
| | Seawater | SPE (C18) | HPLC-MS/MS | 102 (18) | 1.0 | 21 b |
| | Seawater | On line SPE (PLRP-S) | GC-MS | 84 (5-19) | 10.0 | 24 b |
| | River water | SPE (SDB) | GC-MS (ion trap) | 101 (8.7) | 0.1 | 25 |
| | Seawater | SPE (Isolute ENV+) | GC-MS (ion trap) | 94.6-116 (2.5) | 3.1 | 26 |
| | Seawater | SPE (Envirelut Pesticide) | HPLC–ESI/MS/MS | >72 (<10) | 0.1-0.2 | 31 b |
| | Sediment | MAE (water)+SPE (C18) | GC-MS | 94.1(7.1) | 1.7 | 23 |
| | Sediment | Shaking (acetone,DCM) | HPLC–ESI/MS/MS | 85 (7) | 0.08 | 5 b |
| | Sediment | Shaking (ACN)+SPE (Excelpak SPE-GLF) | HPLC–ESI/MS/MS | 80 (10) | 0.048 | 8 b |
| | Sediment | sonication (acetone)/SPME (PDMS) | GC-MS | 91 (4) | 0.5 | 19 b |
| | Sediment | Soxhlet acetone/SPE(C18)/GPC | GC-AFID, GC-MS | 61 (13) | 0.55 | 20 |
| | Sediment | ASE (DCM) | HPLC-MS/MS | 89 (16) | 0.3 | 21 b |
| | Sediment | MAE+SPE (Envirelut Pesticide) | HPLC-MS/MS | 91.1 (2.5) | 0.1 | 22 b |
| | Sediment | MAE (water) | GC-MS (ion trap) | >85 (2.5) | 1.7 | 26 |
| M1 | Seawater | LLE (DCM) | HPLC–ESI/MS/MS | 90 (8.9) | 1.9 | 5 b |
| | Seawater | SPE (Excelpak SPE-GLF) | HPLC–ESI/MS/MS | 83 (13) | 1.9 | 8 b |
| | Seawater | SPE (C18) | HPLC-MS/MS | 109 (15) | 1.0 | 21 b |
| | Seawater | SPE (Isolute ENV+) | GC-MS(ion trap) | 82-96.4 (2.5) | 0.5 | 26 |
| | Sediment | Shaking (acetone, DCM) | HPLC–ESI-MS/MS | 95 (9) | 0.18 | 5 b |
| | Sediment | Shaking (ACN)+ SPE(Excelpak SPE-GLF) | HPLC–ESI/MS/MS | 103 (8.5) | 0.18 | 8 b |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Sediment | ASE (DCM) | HPLC-MS/MS | 99 (18) | 0.3 | 21 b |
| | Sediment | MAE (water) +SPE (C18) | GC-MS | 93.1 (2.7) | 0.9 | 23 |
| | Sediment | MAE (water) | GC-MS (ion trap) | >85 (2.5) | 0.9 | 26 |
| Sea-nine 211 | Seawater | SME (toluene, xylene) | GC-ECD | 91 (9.1) | 2.5 | 14 b |
| | Seawater | LLE (DCM) | HPLC–ESI-MS/MS | 85 (10) | 0.3 | 5 b |
| | Seawater | SPE (C18) | GC-ECD | 94 (3-12) | 5.0 | 13 b |
| | Seawater | SPME (PA 85 um) | GC-ECD | 92 (5-15) | 1.0 | 13 b |
| | Seawater | SPE (Excelpak SPE-GLF) | HPLC–ESI/MS/MS | 75 (12) | 0.3 | 8 b |
| | Seawater | SPE (C18) | HPLC–APCI-MS | 100.4 (10) | 1.0 | 9 b |
| | Seawater | HS-SPME (PDMS–DVB 65 um) | GC-FTD | 96 (5-15) | 7.0 | 16 b |
| | Seawater | SBSE (PDMS) | GC-MS | 72-106 (7.2) | 8.0 | 15 b |
| | Sediment | Shaking (acetone,DCM) | HPLC–ESI/MS/MS | 80 (11) | 0.04 | 5 b |
| | Sediment | Shaking(ACN)+SPE (Excelpak SPE-GLF) | HPLC–ESI/MS/MS | 75 (10) | 0.04 | 8 b |
| | Sediment | sonication (acetone)/SPME (PDMS) | GC-MS | 88 (6) | 1.5 | 19 [b] |
| TCMS pyridine | Seawater | SPE (C18) | HPLC–APCI-MS | 113.1(18.4) | 5.0 | 9 [b] |
| TCMTB | Seawater | SPE (C18) | HPLC–DAD | 85 (4.7) | 7.0 | 6 b |
| | Seawater | SPE (C18) | HPLC-APCI-MS | 91.2 (20.1) | 1.0 | 9 b |
| | Seawater | SBSE (PDMS) | GC-MS | 79-125 (11) | 900 | 15 b |
| | Seawater | SPE (Envirelut Pesticide) | HPLC–ESI/MS/MS | >72 (<10) | 0.1-0.2 | 31 b |
| | | | | | | |
| | Sediment | MAE+SPE (Envirelut Pesticide) | HPLC-MS/MS | 78.1 (3.3) | 0.3 | 22 [b] |
| Thiram | Seawater | SPE (C18) | HPLC–DAD | 96 (6.6) | 22.0 | 6 [b] |
| ZnPT, PT | River water | SAX- SPE (monolithic C18) | HPLC–APCI-MS | 72 (27) | 18.0 | 1 |
| | Seawater | LLE (DCM) | HPLC–APCI-MS | 77 (17) | 20.0 | 2 |
| | Seawater | LLE (DCM) | HPLC–ESI-MS/MS | 83 (13) | 80.0 | 5 b |
| | | | | | | |
| | Sediment | Shaking (acetone, DCM) | HPLC–ESI-MS | 90 (13) | 8.0 | 5 [b] |

a) Relative Standard Deviation;
b) multiresidue method;
c) EACD: Empore-activated carbon disks;
d) M1: Irgarol 1051 degradation product (2-methylthio-4-t-butylamino-6-amino-s-triazine)

**TABLE 1** Methods for the extraction and analysis of common AF biocides in water and sediment matrices

## Sample preparation

### *Seawater*

Extraction of booster biocides from aqueous samples can be performed with different techniques.

The traditional Liquid-Liquid Extraction (LLE) has been extensively reported in less recent studies, but it is a simple and popular procedure still used today [3, 4, 5]. LLE involves the use of a water immiscible solvent, such as dichloromethane (DCM), toluene and hexane, to partition AF compounds from seawater into the organic solvent. Despite its low cost and satisfactory recoveries, this technique has severe limitations, namely the use of

large volumes of solvents and being a time-consuming and labor-intensive procedure. These drawbacks have led to the development and spread of faster methods, with the possibility of easy automation and where lower solvent volumes are employed.

In the last decades LLE has been largely replaced by Solid Phase Extraction (SPE). With this approach, the target analytes are removed from the liquid sample due to retentive interactions with a sorbent phase and, subsequently, are selectively eluted with an appropriate solvent. A large variety of sorbent materials – such as octadecylsilane (C18 bonded silica), graphitized carbon black (GCB) and polymeric materials (poly(N-vinylpyrrolidone-divinylbenzene) copolymer, PVP-DVB; polystyrene-divinylbenzene copolymer, PS-DVB; hydroxylated polystyrene-divinylbenzene copolymer, PS-DVB-OH) – is commercially available, and applications to real samples have been described [6, 7, 8, 9, 10]. Main drawbacks for SPE are the use of specific glassware, namely SPE vacuum manifold to simultaneously process many samples, and the need of preventive filtration of seawater so as to avoid the frits of SPE columns can be blocked by particulate matter. Gatidou et al. [11] carried out a study where they compared PS-DVB/ PS-DVB-OH polymeric materials with C18 bonded silica for the extraction of Diuron, Irgarol 1051, and some of their metabolites. For polymer-based SPE columns, a smaller sorbent mass is usually required to achieve extraction than C18-based (200 versus 500-1000 mg). In addition, higher recoveries for polar compounds such as the metabolites were observed due to further interaction mechanisms with target analytes ($\pi$–$\pi$ and dipole-dipole interaction, hydrogen bonds). However satisfactory recoveries (>70%) were obtained for both solid phases with all analytes except 3,4-dichloroaniline (<35%). GCB materials are suitable for the SPE of six common booster biocides (dichlofluanid, chlorothalonil, Diuron, TCMTB, Irgarol 1051 and Sea nine 211) and some degradation products of Diuron and Irgarol 1051 from seawater, but due to the great adsorption power, elution of the analytes is troublesome, demanding the use of 18 mL dichloromethane-methanol (8:2) mixture, followed by 2 mL methanol [12]. Poor batch-to-batch reproducibility is another issue for this material.

Evaporation of the SPE eluate or LLE organic extract to obtain a final extract with an adequate concentration factor is usually a critical step, and procedural loss for some biocides could be observed unless a careful control of key parameters (temperature, very gentle stream of $N_2$) is realized. Some specific cartridges with low polymeric mass (Envirelut Pesticide) allow to skip this step as elution of the analytes can be carried out with small volume (1 mL) of an organic solvent (methanol) compatible with HPLC analysis [6].

Solvent-free approaches such as Solid-Phase MicroExtraction (SPME) [13], Solvent Micro Extraction (SME) [14], and Stir Bar Sorptive Extraction (SBSE) [15] have also been applied for the determination of AF biocides in coastal waters. SPME is based on an equilibrium process that involves partitioning of analytes from a liquid phase into the polymeric phase according to their distribution coefficients, $K_d$. A very small amount of polymeric material is used as a fused silica fiber coating, so that SPME process could be considered as a miniaturized, albeit non-exhaustive, extraction. Poly(dimethylsiloxane) (PDMS), polyacrylate (PA), poly(dimethylsiloxane)–divinylbenzene (PDMS–DVB), and Carbowax–DVB are typical examples of SPME coating materials with different ranges of polarity and thickness. SPME is a simple and quick technique and in most cases it is carried out by direct dipping of the coated fiber into the aqueous sample [13]. In Headspace SPME, the fiber is exposed to the headspace of the sample solution that is heated and stirred to increase the volatility of analytes [16]. The main parameters affecting $K_d$ for AF Biocides (i.e., pH, salt additives, stirring rate, and adsorption-time profile) should be carefully optimized during the method development.

With SME, a microdrop of solvent is suspended from the tip of a syringe needle, and then immersed in the sample under investigation for a predefined time. The microdrop is then withdrawn into the syringe to be analysed [14].

In the SBSE enrichment method, the target analytes are absorbed onto a thick film of stationary phase (PDMS) coating a glass magnetic stir bar during its immersion in the aqueous sample. The relatively large volume of PDMS (50 µl) increases absorption capacity so SBSE has shown a greater sensitivity than SPME [15].

Immunoaffinity chromatography (IAC) exploits the specific antibody–antigen interaction for purification

and pre-concentration of target analytes from the sample, and can be considered as a tailored SPE. Specific immunosorbents for selective extraction of Irgarol 1051 were prepared and IAC procedure was applied for the determination in real seawater samples [17].
On the other hand, passive samplers are an example of modern sampling strategy that combines sampling, analyte isolation and preconcentration in a single step. These tools are used to measure time-averaged environmental contamination of surrounding waters, not affected by short-term fluctuations in analyte concentrations, and this avoids some drawbacks of the grab sampling. Recently a type of passive sampler well suited for deployment of polar pollutants (Polar Organic Contaminants Integrative Sampler, POCIS) has been used for a monitoring study of AF biocides among others, in the marine environment [18]. Moreover, the passive sampling technique is the focus of another paper of this Special Issue [37].

*Sediment*

Booster biocides, mainly those compounds with a log $K_{ow} \geq 3.0$ and half-life > 50 days, should be considered as persistent and bioaccumulative pollutants; they tend to partition onto sediments, where they may be a source of ongoing contamination, thus representing a potential threat to the marine ecosystem. On the occasion of dredging in harbours or other events disturbing the sediment, the trapped biocides can be once more released in the marine environment. Therefore, an investigation of their presence in the sediment is required.
The conventional approach to sample preparation of solid matrices is a labor-intensive procedure that involves a liquid-solid extraction usually ultrasound assisted (USE), or combined with mechanical shaking. Organic solvents most frequently used are acetone, DCM, acetonitrile (ACN), methanol (MeOH), or proper mixtures (e.g., acetone with DCM or *n*-hexane). Raw extracts obtained from sediments are not directly amenable to LC or GC analysis and an additional clean-up step, based on LLE [3], SPE [8] or alternative SPME [19] technique, is often carried out to remove matrix interferences. On the other hand, Biselli et al [20] employed the traditional Soxhlet apparatus to extract Irgarol 1051 from marine sediment, but the recovery was low (61%).
Some new methodologies, with possibility of automation, allow to minimise the solvent usage and extraction time with respect to conventional ones. The systems for Pressurized Liquid Extraction (PLE) operate under high pressure: this allows to perform the extraction of the analytes from the solid matrix at temperatures above the boiling points of conventional organic solvents. At elevated temperature analyte desorption from matrix is faster, and so is the transfer of AF biocides from marine sediment to the bulk of organic solvent. PLE with DCM was used for fast extraction of Irgarol 1051, its major metabolite and Diuron from marine sediment [21].
Likewise, the Supercritical Fluid Extraction (SFE) employs $CO_2$, at temperature and pressure near or above the critical point, and mixes it with a low percentage of organic solvent (MeOH) to further enhance the solvent power of the supercritical fluid. SFE was employed for the determination of Irgarol 1051 in marine sediments, with a recovery up to 87% [17].
A very promising technique is the Microwave-Assisted Extraction (MAE). It allows to accomplish an extraction of several samples simultaneously, in a few minutes, with reduced amounts of organic solvent, a great reproducibility and high recovery rates. Extraction solvent absorbs the microwave energy and reaches a temperature near the boiling point in a closed vessel. This promotes the diffusion of the target compounds from the sediment into the solvent. Due to the mild temperature conditions achieved, MeOH has been chosen as solvent in the MAE procedure for thermally labile constituents, such as Diuron and dichlofluanid, and good recoveries (> 75%) have been obtained [22]. A drawback of MAE is the co-extraction of interferences, so an additional clean-up step such as SPE is needed. For Irgarol 1051 and its main degradation product, water can be an optimal extraction solvent, making the MAE technique even more convenient and environmentally friendly [23]. The solvent evaporation and/or dilution step is avoided, and the aqueous extract can be directly loaded on the SPE cartridge.

## Chromatographic determination

*General remarks*

The identification and quantification of AF biocides in environmental samples are generally based on the appli-

cation of chromatographic methods, such as Gas Chromatography or Liquid Chromatography, both coupled to mass spectrometer detection (GC–MS or LC–MS), which have been widely used because of their inherent selectivity and sensitivity. In the last decade LC–MS has been effectively applied to the determination of AF biocides, and many analytical methodologies based on this technique were developed. Some recent published LC–MS methods rely on the use of tandem mass spectrometry detection (MS/MS). The MS/MS fragmentation pattern is a powerful tool for obtaining confidence in compound identification as well as structural elucidation. In addition, the use of MS/MS detection allows a great gain in the limits of detection of these micropollutants and quantification to ultra trace level, especially when triple quadrupole mass analyzers are used.

*Gas chromatography*

Gas chromatography is a suitable technique for the separation and determination of all booster biocides with a GC amenable molecular structure. This includes chlorothalonil, dichlofluanid, Irgarol 1051 and its stable degradation product M1, Sea nine 211 and TCMTB. Diuron is a compound with poor thermal stability and decomposes during GC injection, although it can also be determined using GC after a derivatization procedure, but results are often unsatisfactory.

The chromatographic separation of these compounds can usually be achieved with common GC capillary columns filled with nonpolar stationary phases, such as methylpolysiloxane or phenyl-methylpolysiloxane, and increasing GC oven temperatures from 60-80 °C up to 280-320 °C. Splitless injection mode is a well-established approach because of its robustness, but injection volume is limited to sample volumes as low as 1-2 µl, since band broadening and peak deformation are usually observed when large amount of solvent enters the capillary column. In order to increase sensitivity but avoiding the drawback, GC with large volume injection was developed, where bulk of solvent is separated from analytes before chromatography starts. Some authors used cool on-column interface with partially concurrent solvent evaporation using a solvent vapor exit accessory, and were able to inject 100 µl ethyl acetate SPE extract. This made the development of an online SPE-GC-MS method for the determination of Irgarol 1051 and dichlofluanid [24] feasible. The alternative technique of programmed temperature vaporization (PTV) injection in the solvent vent mode, improved the analytical procedure for the determination of Irgarol 1051 in estuarine samples. A 40 µl sample of the 200 µl final extract could be injected in the capillary GC column with this PTV injector [25].

Conventional GC detection systems, such as electron capture detector (ECD), flame thermionic detector (FTD), flame ionization detector (FID), alkali flame ionization detector (AFID) and nitrogen phosphorous detector (NPD), have been used for the determination of booster biocides. Specifically, ECD is a selective detector for halogenated compounds (i.e., chlorothalonil, dichlofluanid) in environmental samples that offers high sensitivity and good reproducibility. However, interference can be frequently observed and, due to the low identification capability of conventional GC detectors, false positives could be detected. On the other hand, MS detectors provide unambiguous component identification due to the availability of library spectra. Hence GC-MS methods are most frequently used to determine the concentration of these compounds in seawater and marine sediments, and they are progressively replacing classic GC detectors.

A remarkable increase in sensitivity of MS systems can be obtained with selected ion monitoring (SIM) and tandem (MS/MS) operation modes. Sub-to-low ng/L levels (0.1–1 ppt) were the reported detection limits using an ion trap mass spectrometer in MS/MS mode, combined with large volume injection GC [25].

The single quadrupole analyzer with electron impact ion source (EI) is a very common analytical approach to the determination of AF biocides, giving optimal sensitivity especially when the SIM mode is used [26, 27, 28]. An alternative ionization technique, such as chemical ionization (CI) with methane as the reagent gas, has been evaluated in some papers. Negative chemical ionization (NCI) is also suitable for the analysis of chlorinated biocides (chlorothalonil, dichlofluanid, Sea nine 211) as it offers higher sensitivity than EI. However NCI is not the ideal ionization technique for Irgarol 1051 since a great loss in sensitivity with respect to EI was observed, which is a serious limitation to the development of multi-residue methods [29]. The absence of spectral libraries as

well as poor fragmentation are also drawbacks for CI. In this sense, considering the identification power offered by the EI spectrum on the basis of the number of fragment ions and relative abundance, EI has been used by most authors.

*Liquid chromatography*

Despite the traditional use of GC for booster biocide determination, LC is able to separate these compounds (including Diuron ) effectively without tedious derivatization processes, hence several LC methodologies have been developed. Reversed phase high performance liquid chromatography (RP-HPLC) is commonly used for the separation of AF biocides with an octadecyl silica stationary phase (C18), although octyl columns (C8) have also been used [30]. HPLC columns are usually packed with 5 μm particles, whilst in more recent papers the use of smaller particle size (2.4 and 3 μm) have been reported [31, 32]. The mobile phase used for elution consists of either methanol or acetonitrile mixed with water. Some modifiers (e.g., ammonium acetate, formic acid or ammonium formate) are commonly added to HPLC eluents in order to enhance ion production and improve the sensitivity of MS detection. All these buffers are volatile and thus suitable for atmospheric pressure ionization (API) MS techniques. Binary gradients starting from a low percentage of organic solvent and increasing linearly to high percentage are usually adequate to separate mixtures of AF compounds and degradation products, but also isocratic conditions were reported [31]. Injection volumes are typically increased up to 50 μL in order to improve the detectability of the target analytes, but this requires the evaporation to dryness of sample extract and reconstitution in a suitable elution solvent mixture [32].

A more affordable choice than MS is the absorbance detection using a diode array detector (DAD), which has traditionally been employed for analysis of phenylurea pesticides. In some studies, DAD has been used for the simultaneous determination of Irgarol 1051, Diuron and their main degradation products [11]. The identification of analytes by LC-DAD is accomplished by comparing the retention time and UV spectrum obtained for detected peaks in the sample with those of the target compounds in a standard solution. A limited identification capability can be achieved by this detector.

Over the last decade liquid chromatography-mass spectrometry (LC-MS) has advanced dramatically in sensitivity, specificity and reliability; this allowed it to gain acceptance as a routine analytical technique and led to its widespread application in environmental analysis, also for the determination of AF biocides in marine samples. The use of MS detectors coupled to LC enabled a more discriminatory identification of analytes and the obtainment of high quality data on the occurrence of organic contaminants in the environment at very low concentration levels. Moreover LC-MS allows the determination of practically all of AF biocides (except zinc pyrithione) in a single analysis, and this means the development of true multi-residue analytical methods with reduction of time and costs.

The mass analyzers that have been commonly used are single quadrupole [32, 12], triple quadrupole [33, 34] and, more recently, hybrid instruments such as triple stage quadrupole/linear ion trap [35]. Different ionization techniques are usually available in LC-MS: electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). Both negative ionization (NI) and positive ionization (PI) modes have been evaluated. Better sensitivity is achieved for chlorothalonil, dichlofluanid and TCMTB using NI mode whereas Irgarol 1051, Diuron and Sea nine 211 are commonly determined using PI [12]. In recent papers, the ionization of molecules of AF biocides is obtained by ESI [31, 32, 33, 34, 35] and this preference was confirmed by performing a comparison of ESI and APCI which showed that the best ionization technique for Irgarol 1051, Diuron and their main degradation products was ESI with PI mode [36]. Ionization of chlorothalonil is only possible using APCI and not by ESI.

One of the limitations of LC-MS is the susceptibility of interfaces to coelution with matrix components of the sample that can result in the suppression or, less frequently, in the enhancement of the analyte signal. However, these matrix effects can be minimized by good sample preparation and improved chromatographic separation, or can be compensated for with the use of isotopically labelled internal standard.

When a single quadrupole analyzer is used, structural information about a particular molecule is produced by increasing cone voltage, which affects the transmission and fragmentation of the molecular ion $MH^+$. Thus,

with a high voltage, more fragmentation occurs and an in-source collision induced dissociation (CID) of $MH^+$ is obtained. Determination of target analytes has been usually carried out with selected ion monitoring (SIM) mode in order to increase sensitivity.

In environmental analysis, the confirmation of positive findings should be based on the use of identification points (IPs) proposed by the European Commission Guidelines (EU Commission Decision 2002/657/EC) for the identification and quantification of organic residues and contaminants. The decision proposes a system of IPs, where at least three IPs are required to confirm a positive finding. In addition, the deviation of the relative intensity of the recorded ions must not exceed ±20% with respect to that observed in the reference standard, and the retention time must not deviate more than 2.5%. This means we should acquire at least three ions in single-mass spectrometry instruments (3 IPs), but this is not viable for analytes with poor fragmentation and unequivocal identification is compromised.

The MS/MS fragmentation is a more powerful tool for obtaining confidence in compound identification. This is based on its two stages of mass analysis: the former to pre-select an ion (precursor ion) and the latter to analyze the induced fragments (product ions). Selective precursor-product ion transitions (SRM) are obtained. The setting of the SRM channels for the determination of target analytes is commonly selected considering the signal intensities and structure-specificities of the product ions. $MH^+$ is generally used as the precursor ion. Two SRM transitions are followed with MS/MS instruments (e.g., triple quadrupole), and are enough for reliable identification since 4 IPs result.

## Conclusions

Despite many efforts to develop environmentally friendly alternatives to inhibit biofouling, such as foul-release coatings relying on silicone technology or paints containing natural marine compounds, these novel AF strategies are limited either to fast moving vessels (e.g., large yachts, cruise ships, ferry boats), or to promising AF compounds still in early stages of development. We currently do not have a viable option for the replacement of booster biocides in AF paints, and a long timeline (approximately 10 years) is expected for approval process and widespread use of possible novel AF candidates. Due to the actual large use, and likely for the next years, of AF paints based on organic biocides and potential detrimental effects to the aquatic environment, monitoring data on environmental occurrence of AF biocides is needed and will still be in the future.

This paper overviews the main analytical approaches to the determination of AF biocides in different matrices from the marine environment (coastal waters and sediments) which makes feasible trace level detection of these contaminants in real samples.

Future trends will focus on the improvement in sample preparation, especially in terms of automation and development of online SPE technology, since this reduces sample manipulation and analysis time, and minimises the required amount of sample. In addition, great efforts will be devoted to obtain greener methodologies, involving less consumption of solvent and energy. In this sense, passive samplers are a promising tool since they combine sampling and preconcentration in a single step, but this novel technique has to be still further developed to obtain reliable quantitative results.

As for LC separation, the main advances will concern the application of fast and high separation efficiency approaches using both UHPLC and traditional HPLC systems based on columns packed with sub-2μm and superficially porous particles, respectively.

Future development of generic analytical protocols that will permit the simultaneous determination of AF biocides and other relevant compounds potentially detectable in the coastal marine environment (polar pesticides and emerging contaminants, such as pharmaceuticals and personal care products, alkylphenols) is required. More research devoted to metabolites and transformation products of AF biocides is also needed. In this sense, high resolution MS strategies based on powerful hybrid instruments such as QqTOF and Orbitrap are expected to be applied for the analysis of AF biocides and relevant marine contaminants. These approaches offer the possibility to achieve accurate mass measurements and acquire indispensable qualitative information through full-scan spectra, with the additional advantage of performing a retrospective analysis in order to screen non-target molecules.

Rd

references


1. J. Bones, K. V. Thomas, B. Paull (2006), "Improved method for the determination of zinc pyrithione in environmental water samples incorporating on-line extraction and preconcentration coupled with liquid chromatography atmospheric pressure chemical ionisation mass spectrometry", *Journal of Chromatography A,* 1132, 157-164.
2. K.V. Thomas (1999) "Determination of the antifouling agent zinc pyrithione in water samples by copper chelate formation and high-performance liquid chromatography–atmospheric pressure chemical ionisation mass spectrometry", *Journal of Chromatography A,* 833, 105-109.
3. N. Voulvoulis, M.D. Scrimshaw, J.N. Lester (1999), "Analytical Method Development for the Determination of Four Biocides Used in Antifouling Paints in Estuarine Waters and Sediments by Gas Chromatography-Mass Spectrometry", *Chromatographia* 50, 353-357.
4. S. Lee, J. Chung, H. Won, D. Lee, Y.W. Lee (2011), "Analysis of antifouling agents after regulation of tributyltin compounds in Korea", *Journal of Hazardous Materials,* 185, 1318-1325.
5. S. Eguchi, H. Harino, Y. Yamamoto (2010), "Assessment of Antifouling Biocides Contaminations in Maizuru Bay, Japan", *Arch Environ Contam Toxicol,* 58, 684–693.
6. A. Sànchez Rodríguez, Z. S. Ferrera, J. J. S. Rodrıguez (2011), "A preliminary assessment of levels of antifouling booster biocides in harbours and marinas of the island of Gran Canaria, using SPE-HPLC", *Environ Chem Lett,* 9, 203-208.
7. R.A. Gimeno, C. Aguilar, R.M. Marcé, F. Borrull (2001), "Monitoring of antifouling agents in water samples by on-line solid-phase extraction–liquid chromatography–atmospheric pressure chemical ionization mass spectrometry", *Journal of Chromatography A,* 915, 139-147.
8. H. Harino, Y. Mori, Y. Yamaguchi, K. Shibata, T. Senda (2005), "Monitoring of Antifouling Booster Biocides in Water and Sediment from the Port of Osaka, Japan", *Arch. Environ. Contam. Toxicol.,* 48, 303-310.
9. K.V. Thomas (1998), "Determination of selected antifouling booster biocides by high performance liquid chromatography–atmospheric pressure chemical ionisation mass spectrometry", *Journal of Chromatography A,* 825, 29–35.
10. G. Di Landa, G. Ansanelli, R. Ciccoli, C. Cremisini (2006), "Occurrence of antifouling paint booster biocides in selected harbors and marinas inside the Gulf of Napoli: A preliminary survey", *Marine Pollution Bulletin,* 52 (11), 1541-1546.
11. G. Gatidou, A. Kotrikla, N. S. Thomaidis, T. D. Lekkas (2005), "Determination of the antifouling booster biocides Irgarol 1051 and Diuron and their metabolites in seawater by high performance liquid chromatography-diode array detector", *Analytica Chimica Acta,* 528 (1), 89-99.
12. K. Martinez, I. Ferrer, D. Barcelo (2000), "Part-per-trillion level determination of antifouling pesticides and their byproducts in seawater samples by off-line solid-phase extraction followed by high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry", *Journal of Chromatography A,* 879 (1), 27-37.
13. I.K. Konstantinou, D.G. Hela, D.A. Lambropoulou, V.A. Sakkas, and T.A. Albanis (2002), "Comparison of the performance of analytical methods based on solid-phase extraction and on solid-phase microextraction for the determination of antifouling booster biocides in natural waters", *Chromatographia*, 56, 745-751.
14. D. A. Lambropoulou, T. A. Albanis (2004), "Application of solvent microextraction in a single drop for the determination of new antifouling agents in waters", *Journal of Chromatography A,* 1049, 17–23.
15. I. Giráldez, E. Chaguaceda, M. Bujalance, E. Morales (2013), "Determination of five booster biocides in seawater by stir bar sorptive extraction-thermal desorption-gas chromatography-mass spectrometry", *J Chromatogr A,* 1271, 17-26.
16. D.A. Lambropoulou, V.A. Sakkas, T.A. Albanis (2002), "Headspace solid phase microextraction for the analysis of the new antifouling agents Irgarol 1051 and Sea Nine 211 in natural waters", *Analytica Chimica Acta,* 468, 171–180.
17. P.B. Carrasco, S. Díez, J. Jiménez, M.-P. Marco, J. M. Bayona (2003), "Determination of Irgarol 1051 in Western Mediterranean sediments. Development and application of supercritical fluid extraction–immunoaffinity chromatography procedure", *Water Research,* 37, 3658–3665.
18. D. Munaron, N. Tapie, H. Budzinski, B. Andral, J. L. Gonzalez (2012), "Pharmaceuticals, alkylphenols and pesticides in Mediterranean coastal waters: Results from a pilot survey using passive samplers", *Estuarine Coastal and Shelf Science,* 114, 82-92.
19. D. A. Lambropoulou, V. A. Sakkas, T. A. Albanis (2003), "Determination of antifouling compounds in marine sediments by solid-phase microextraction coupled to gas chromatography–mass spectrometry", *Journal of Chromatography A,* 1010, 1–8.
20. S. Biselli, K. Bester, H. Huhnerfuss, K. Fent (2000), "Concentrations of the Antifouling Compound Irgarol 1051 and of Organotins in Water and Sediments of German North and Baltic Sea Marinas", Marine Pollution Bulletin, 40, 233-243.
21. Y. Sapozhnikova, E. Wirth, K. Schiff, M. Fulton (2013), "Antifouling biocides in water and sediments from California marinas", Marine Pollution Bulletin, 69, 189–194.
22. Á. Sánchez-Rodríguez, Z. Sosa-Ferrera, J. J. Santana-Rodríguez (2011), "Applicability of microwave-assisted extraction combined with LC–MS/MS in the evaluation of booster biocide levels in harbour sediments", *Chemosphere,* 82, 96–102.
23. G. Gatidou, J.L. Zhou, N.S., Thomaidis (2004), "Microwave-assisted extraction of Irgarol 1051 and its main degradation product from marine sediments using water as the extractant followed by gas chromatography–mass spectrometry determination", *Journal of Chromatography A,* 1046, 41–48.
24. E. Pocurull, L. Brossa, F. Borrull, R. M. Marce (2000), "Trace determination of antifouling compounds by on-line solid-phase extraction-gas chromatography-mass spectrometry", *Journal of Chromatography A,* 885 (1-2), 361-368.
25. R. Steen, P. E. G. Leonards, U. A. T. Brinkman, W. P. Cofino (1997), "Ultra-trace-level determination of the antifouling agent Irgarol 1051 by gas chromatography with tandem mass spectrometric detection", *Journal of Chromatography A,* 766 (1-2), 153-158.
26. J.L. Zhou (2008), "Occurrence and persistence of antifouling biocide Irgarol 1051 and its main metabolite in the coastal waters of Southern England", *Science of the Total Environment,* 406 (1-2), 239-246.
27. T. Cresswell, J. P. Richards, G. A. Glegg, J. W. Readman (2006), "The impact of legislation on the usage and environmental concentrations of Irgarol 1051 in UK coastal waters", *Marine Pollution Bulletin,* 52 (10), 1169-1175.
28. P.R. Gardinali, M. D. Plasencia, C. Maxey (2004), "Occurrence and transport of Irgarol 1051 and its major metabolite in coastal waters from South Florida", *Marine Pollution Bulletin,* 49 (11-12), 1072-1083.
29. A. Aguera, L. Piedra, M. D. Hernando, A. R. Fernandez-Alba (2000), "Multiresidue method for the analysis of five antifouling agents in marine and coastal waters by gas chromatography-mass spectrometry with large-volume injection", *Journal of Chromatography A,* 889 (1-2), 261-269.
30. I. Ferrer, D. Barcelo (2001), "Identification of a new degradation product of the antifouling agent Irgarol 1051 in natural samples", *Journal of Chromatography A,* 926 (1), 221-228.
31. A. Sanchez-Rodriguez, Z. Sosa-Ferrera, A. S. D. Pino, J. J. Santana-Rodriguez (2011), "Probabilistic risk assessment of common booster biocides in surface waters of the harbours of Gran Canaria (Spain)", *Marine Pollution Bulletin,* 62 (5), 985-991.
32. G. Di Landa, L. Parrella, S. Avagliano, G. Ansanelli, E. Maiello, C. Cremisini (2009), "Assessment of the Potential Ecological Risks Posed by Antifouling Booster Biocides to the Marine Ecosystem of the Gulf of Napoli (Italy)", *Water Air and Soil Pollution,* 200 (1-4), 305-321.
33. M. H. Lamoree, C. P. Swart, A. van der Horst, B. van Hattum (2002), "Determination of Diuron and the antifouling paint biocide Irgarol 1051 in Dutch marinas and coastal waters", *Journal of Chromatography A,* 970 (1-2), 183-190.
34. Y. Sapozhnikova, E. Wirth, N. Singhasemanon, J. Bacey, M. Fulton (2008), "Distribution of antifouling biocides in California marinas", *Journal of Environmental Monitoring,* 10 (9), 1069-1075.
35. V.W.H. Tsang, N. Y. Lei, M. H. W. Lam (2009), "Determination of Irgarol-1051 and its related s-triazine species in coastal sediments and mussel tissues by HPLC-ESI-MS/MS", *Marine Pollution Bulletin,* 58 (10), 1462-1471.
36. N.C. Maragou, N. S. Thomaidis, M. A. Koupparis (2011), "Optimization and Comparison of ESI and APCI LC-MS/MS Methods: A Case Study of Irgarol 1051, Diuron, and their Degradation Products in Environmental Samples", *Journal of the American Society for Mass Spectrometry,* 22 (10), 1826-1838.
37. P. Massanisso, C. Marcoaldi, E. Nardi (2014), "Passive sampling of antifouling compounds in compliance with the Water Framework Directive", *Energia, Ambiente e Innovazione,* this issue, next article.

ENVIRONMENTAL OCCURRENCE AND CONCERNS OF ANTIFOULING BIOCIDES

# Passive sampling of antifouling compounds in compliance with the Water Framework Directive

The utilization of passive sampling allows the quantification of extremely low pollution levels and gives information concerning time-weighted average concentrations of the pollutants. These characteristics are fundamental for the employment of these systems as complementary methods in the design of monitoring programmes, in compliance with WFD

*DOI: 10.12910/EAI2014-47*

*Paolo Massanisso, Caterina Marcoaldi, Elisa Nardi*

## Introduction

Passive sampling, widely used to monitor air pollutants, has been gaining acceptance for monitoring organic contaminants in water [1-7]. More than 50% of the total number of publications over the last decade describes the use of passive samplers to monitor water environmental quality condition [8]. Contrary to grab sampling, passive sampling is less sensitive to extreme fast variations of the organic pollutant concentration in natural waters, and is suited to determining time-weighted average concentrations of pollutants.

A potential risk for the marine environment comes from the gradual release of biocides by antifouling paints, used to protect the boat hulls from the undesirable accumulation of micro-organisms, plants, and animals (marine biological fouling) and, consequently, to reduce the negative effects of fouling (slower speed, increased fuel consumption and maintenance costs, etc…) [9]. European legislation has established the Environmental Quality Standards (EQS) for a list of substances, including antifouling biocides: tributyltin (TBT) and diuron (Water Framework Directive WFD; Directive 2000/60/EC, annex X list of priority substances). These are subject to bioaccumulation and biomagnification processes and therefore create a potential risk to human health and ecosystems. Limited data and information are available on the environmental occurrence, fate, toxicity, and persistence of these biocides; hence, any system able to improve the information concerning the environmental presence of these compounds is of great interest [5,10]. In the present work the utilization of passive sampling for the evaluation of antifouling agents in the seawater and the possible utilization of this new system of sampling in compliance with the WFD will be examined.

## Passive sampling: principle of operation

Passive sampling is based on free flow (according to the Fick's first law of diffusion) of analyte molecules from the sampled medium to a collecting medium. The diffusion driving forces and separation mechanisms

**Paolo Massanisso, Caterina Marcoaldi, Elisa Nardi**
*ENEA, Technical Unit for Environmental Characterization, Prevention, and Remediation - Laboratory of environmental biogeochemistry*

depend on the different chemical potentials of trapped and non-trapped (remaining in the sample) analytes. Therefore, passive samplers are able to measure only the freely dissolved (bio-available) amount of these compounds.

The sort of analytical data obtained as a consequence of the utilization of passive sampling system depends, to a great extent, on the accumulation regimes in which passive samplers operate during field exposure: two main accumulation regimes (linear and equilibrium, Figure 1) can be distinguished in the operation of a sampler during field deployment, and the exchange kinetics between a passive device and the water phase can be described by a first-order, one-compartment mathematical model [11]:

$$Cs(t) = Cw \frac{k_1}{k_2} (1 - e^{-k_2 t}) \text{ (Eq. 1)},$$

where Cs(t) is the concentration of the analyte in the sampler at exposure time t, Cw is the analyte concentration in the water phase, and $k_1$ and $k_2$ are the uptake and offload rate constants, respectively.

The passive samplers can operate using these two different regimes [8]:

1) In the linear uptake, passive samplers and/or non-equilibrium passive samplers, the rate of mass transfer to the receiving phase is linearly proportional to the difference in the chemical potential of the contaminant in the receiving phase for the compounds to be analysed (kinetic and time-integrative uptake phase). Based on the application of this type of passive sampler, average contaminant concentrations present in the monitored part of the environment over the entire sampling period can be obtained.
2) In equilibrium passive sampling, the regime is described by a partition coefficient between the receiving phase and the sample matrix. When equilibrium passive sampling is used for sample collection the sampler should be deployed long enough to ensure that the thermodynamic equilibrium is reached between the environmental media and the receiving phase.

The most useful utilization of the passive sampler in monitoring is the linear uptake design [12]. Indeed, the main advantage of using an integrative sampler is that

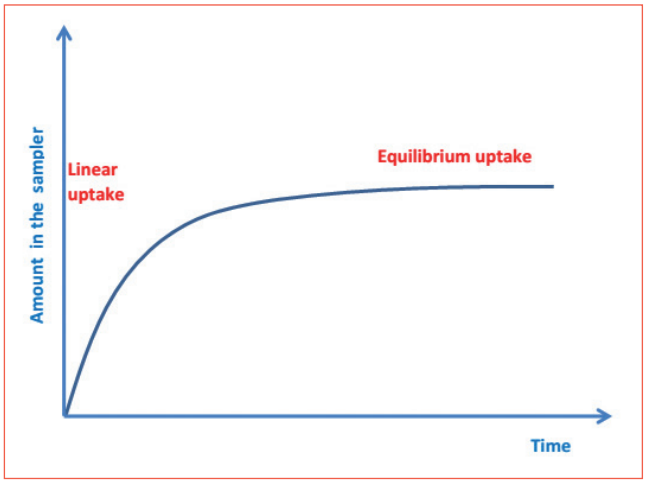



**FIGURE 1** Mass uptake in passive sampler: two main accumulation regimes can be considered

episodic events (surface runoff, spills, and other unpredictable sources of contamination) can be sampled without the cost of trained staff and challenges of trying to catch the events; however, because of the sampling nature of the devices, it is impossible to determine when the event occurred during the deployment period, nor to know the maximum concentration of a chemical related to the event. Integrative samplers provide data of Cw as a time-weighted average concentration of a chemical within the whole exposure period.

Equation 1 can be rearranged to an equivalent relationship [11] $Cw = \frac{M_s}{Rs * t}$ (Eq. 2), where $M_s$ is the mass of analyte accumulated in the receiving phase after an exposure time t, and Rs is the proportionality constant (sampling rate), which is the product of the first-order rate constant for uptake of pollutant (k1) and the volume of water that gives the same chemical activity as the volume of the receiving phase. The sampling rate (Rs) can be stated as the number of litres of water per day that are sampled 'through' the sampler during the exposure time. The higher Cw, the higher the amount of the substance obtained from that volume of water that goes through the sampler. When Rs is known, Cw (the time-weighted average (TWA) concentration of a pollutant in the water phase) may be calculated since the exposure time is also known, and the amount of the analyte trapped by the

receiving phase can be measured after extraction from the receiving phase.
The measurement of Rs is defined as the calibration of the passive sampling device and it is performed in laboratory or in field with the utilization of performance reference compounds [13]. To predict accurately TWA water concentrations of contaminants from the levels accumulated in passive samplers, extensive calibration studies, aimed at characterizing the uptake of chemicals under various exposure conditions, are necessary. Uptake kinetics of chemicals depends not only on the physicochemical properties of the compound to be measured, but also on the sampler design and environmental variables, such as temperature, water turbulence and biofouling presence on samplers. The Rs typically falls in the range of 0.5 to 5 l/day, with the most hydrophobic compounds having the higher value [7].
The devices used for passive sampling are usually based on diffusion through a well-defined diffusion barrier or permeation through a membrane. Several designs of passive samplers have been proposed, where the main characteristic is the collecting medium utilized in the system. The most commonly used sampler structures can be separated into two categories [7]:

- "solvent"-filled (semipermeable membrane devices (SPMDs));
- "sorbent"-filled (POCIS and Chemcatcher)

In the SPMD, a tubular low-density polyethylene (LDPE) lay-flat membrane is filled with a high-molecular-weight lipid-usually high purity synthetic triolein 1,2,3-tri-[ cis-9-octacenoyl ] glycerol (>95%) and usually they are used to monitor lipophilic compounds with octanol/water partition coefficients $\log K_{OW}$ >3 (hydrophobic pollutants, PAH, PCBs, etc.) [14].
The POCIS comprises a solid receiving phase material (non-polar sorbent), sandwiched between two microporous polyethersulphone diffusion-limiting membranes. They are used to sample hydrophilic compounds with octanol/water partition coefficients $\log K_{OW}$ <3 (polar organic pollutants, drug residues, pesticides, etc.). In the chemcatcher passive sampler the receiving phase is typically a C18 Empore disk and it is suitable for monitoring organic compounds with $\log K_{OW}$ between 2 and 4 [15, 16].
Up to now, few data have been published on antifouling compounds occurrence in the seawater sampled with passive devices, and the results are reported in Table 1. The significant point to be highlighted is the extremely low concentration (sub ng/L), that is possible to quantify with all the passive sampling devices employed in the selected studies.

## Passive sampling with respect to WFD

For priority pollutants, annual average and maximum acceptable concentration environmental quality standards (AA-EQS and MAC-EQS, respectively) are to be used in compliance with the WFD (Directive on Environmental Quality Standards - Directive 2008/105/EC, EQSD).
In some cases the EQS are extremely low, under ng/L

| Analyte | Range of concentrations | Notes |
|---|---|---|
| TBT | 32 - 220 ng Sn/mL SPMD | SPMD, Oslofjord Harbour (Norway) [17] |
| TBT | 0.4 - 10 ng/L | SPMD, Seawater Oslofjord (Norway). Reference in [18] |
| TBT | <1 ng Sn/mL SPMD | SPMD, Pacific Ocean. [19] |
| TBT | 8.3 ng/L | Chemcatcher, Alicante Harbour (Spain) [16] |
| Diuron | 0.06 - 2.5 ng/L | SPMD, Great Barrier Reef (Australia). Reference in [18] |
| Diuron | 50 - 1400 ng/L | Chemcatcher, Portsmouth Harbour (Portsmouth, UK) [20] |
| Diuron | 0.4 - 2.5 ng/L | POCIS, Mediterranean Sea (Spain) [21] |
| Irgarol | 0.02 – 0.7 ng/L | POCIS, Mediterranean Sea (Spain) [21] |
| Irgarol | 10 - 230 ng/L | Chemcatcher, Portsmouth Harbour (Portsmouth, UK) [20] |
| Chlorothalonil | 2.7 - 48 ng/L | SPMD, Estuarine ecosystems (FL,USA). Reference in [18] |

**TABLE 1** Concentration of antifouling biocides worldwide using passive sampling devices

(AA-EQS for TBT 0.08 ng/L as tin) and the utilization of the conventional method of spot sampling do not permit to reach the limit of quantification (LOQ) of the most advanced methods of analysis. Passive samplers have been validated and provide high sampling rates (litre/day) for various contaminants, thus allowing to quantify extremely low pollution levels in water using the same methods of analysis [22]. In addition, since one of the primary objectives of WFD is the assessment of the average concentrations of pollutants in water bodies, the determination of time-integrated concentrations using passive samplers seems to be a promising approach [23].

The Commission Directive 2009/90/EC on technical specifications for chemical analysis and monitoring of water status (pursuant to Directive 2000/60/EC of the European Parliament and of the Council) sets out the technical specifications for chemical analysis and monitoring of water. The objective of this Directive is to establish common quality rules for chemical analysis and monitoring of water, sediment and biota carried out by Member States.

In this technical specifications, minimum performance criteria have been defined for the LOQ and the measurement uncertainty "U" (expanded uncertainty of measurement). They are, wherever possible, linked to the EQS.

If no suitable analytical method is available that meets these minimum performance criteria for a particular priority substance, e.g., TBT, monitoring has to be carried out using the best available techniques not entailing excessive costs. Passive sampling may be the best available technique for evidencing very low concentrations not detectable in water samples collected in the traditional way (using spot sample). Furthermore, passive sampling can also be used in parallel with spot sampling in order to confirm or refute the results for water samples taken in the traditional way, particularly in situations in which contaminant concentrations fluctuate considerably over time [24, 25].

Recently, a monitoring campaign on TBT has been carried out by ENEA in the Gulf of La Spezia with the utilization of grab sample and SPMD devices. The results (Table 2) show that the data obtained are comparable in the Port and confirm that only SPMD allows to measure TBT levels in the protected area (< 1 ng/L), reaching quantification limits similar to the requested EQS for this contaminant [26].

| | SPMD | Grab samples |
|---|---|---|
| Port of La Spezia | 1.6 ± 0.1 | 1.3 ± 0.4 |
| Cinque Terre Marine Protected Area | 0.2 ± 0.05 | n.d |

**TABLE 2** TBT (as Sn, ng/L) concentration in the Gulf of La Spezia using SPMD devices and the classical sampling method (n.d, non-detected) [26]

## Conclusion

The main benefit of the passive approach over grab sampling and/or extraction is that only one device is necessary at a given sampling location for the duration of sampling. In grab sampling, where the sample represents the conditions at the sampling site at a given moment in time, the number of samples collected over the duration of the sampling survey can be larger if the same time-averaged information is obtained. Passive sampling requires only a few analyses over the monitoring period, hence analytical costs can be substantially reduced. Passive sampling devices might be useful for identifying pollution sources, in particular, if extremely low levels have to be detected or when the pollution source is not constant. Moreover, the use of passing sampling for measuring the time-weighted average concentration is in compliance with the EQS (annual average in particular) defined by the WFD.

The debate on the issue of passive sampling for the WFD has been developed in the guidance document on surface water chemical monitoring [24], where passive sampling is indicated as one of the complementary methods that can be used for both monitoring network design and surveillance monitoring. An ongoing issue is that the compliance checking of water quality under WFD, with respect to organic compounds, is based on total water concentrations, and that passive sampling only measures the concentration of freely dissolved (bio-available) fractions. However, total concentrations in water can be calculated using averaged measured

DOC concentrations, concentrations of suspended matter and total organic matter levels in the suspended matter with equilibrium partitioning, on the basis of the freely dissolved concentration determined with passive sampling. Finally, another interesting possible development in the field of passive sampling is the use of these devices (the extracts), in combination with biological tests to measure toxicity and genotoxicity for a better definition of the EQS in compliance with the WFD [23].



references


1. Alvarez D., (2013), Development of semipermeable membrane devices (SPMDs) and polar organic chemical integrative samplers (pocis) for environmental monitoring, *Environmental Toxicology and Chemistry* (32), 2179–2181.
2. Namiensik J., Zabiegala B., Wasik A., Partyka M., (2005), Passive sampling and/or extraction techniques in environmental analysis: a review, *Analytical Bioanalytical Chemistry* (381), 279-301.
3. Mills G., Greenwood R., Vrana B., Allan I., (2011), Measurement of environmental pollutants using passive sampling devices - a commentary on the current state of the art, *Journal of Environmental Monitoring* (13), 2979.
4. Turrillas F., Yusa V., Pastor A., Guardia M., (2008), New perspectives in the use of semipermeable membrane devices as passive sampler, *Talanta* (74), 443-457.
5. Greenwood R., Mills G., Vrana B., (2009), Potential applications of passive sampling for monitoring non polar industrial pollutants in the aqueous environment in support of REACH, *Journal of Chromatography* (1216), 631-639.
6. Petty J., Orazio C., Huckins J., Gale R., (2000), Considerations involved with the use of semipermeable membrane devices for monitoring environmental contaminants, *Journal of Chromatography* (879), 83-95.
7. Laurisden F., (2005), Review of passive accumulation devices for monitoring organic micropollutants in the aquatic environment, *Environmental Pollution* (136), 503-524.
8. Zabiegala B., Wasik A., Urbanowicx M., Namiesnik J., (2010), Passive sampling as a tool for obtaining reliable analytical information in environmental quality monitoring, *Analytical Bioanalitycal Chemistry* (396), 273-296.
9. Ladislao B., (2008), Environmental levels, toxicity and human exposure to tributyltin (TBT)- contaminated marine environment, *Environment International* (34), 292-308.
10. Allan I., Mills G. Vrana B., Knutsson J., (2006), Strategic monitoring for the European Water Framework Directive, Trends in *Analytical Chemistry* (25), 7.
11. Vrana B., Mills G., Allan I., Dominiak E., (2005), Passive sampling techniques for monitoring pollutants in water, Trends in *Analytical Chemistry* (24), 10.
12. Madrid Y., Zayas Z., (2007), Water sampling: Traditional methods and new approaches in water sampling strategy, Trends in *Analytical Chemistry* (26), 4.
13. Seethapathy S., Gorecky T., Li X., (2008), Passive sampling in environmental analysis, *Journal of Chromatography* (1184), 234-253.
14. Turrillas F., Pastor A., Yusa V., Guardia M., (2007), Using semipermeable membrane devices as passive samplers, Trends in *Analytical Chemistry* (26), 7.
15. Soderstrom H., Lindberg R., Fick J., (2009), Strategies for monitoring polar organic contaminants in water with emphasis on integrative passive sampling, *Journal of Chromatography* (1216), 623-630
16. Aguilar Martinez R., Palacios MA., Greenwood R., Vrana B., (2008), Calibration and use of the Chemcatcher passive sampler for monitoring organotin compounds in water, *Analytical Chemistry Acta* (2), 67-157.
17. Folsvik N., Brevik E., Berge J., (2000), Monitoring of organotin compounds in seawater using semipermeable membrane devices (SPMDs) tentative results, *Journal of Environmental monitoring* (2), 281-284.
18. Francesc A., Yusà V., Pastor A., (2008), New perspectives in the use of semipermeable membrane devices as passive samplers, *Journal of Environmental monitoring* (74), 443-457.
19. Gokosyr A., Tollefsen KE., Grung M., Lie E., (2009), Balsa raft crossing the Pacific finds low contaminant levels. *Environmental Science Technology* (43), 4783-4790.
20. Kingston J., Greenwood R., Mills G., Morrison G, (2000), Development of a novel passive sampling system for the time- average measurement of a range of organic pollutants in aquatic environments, *Journal of Environmental Monitoring* (2), 487-495.
21. Martínez Bueno M., Hernando M., Aguera A., Fernandez-Alba A., (2009), Application of passive sampling devices for screening of micro-pollutants in marine aquaculture using LC–MS/MS, Journal of Environmental monitoring (77), 1518-1527.
22. Miège C., S. Schiavone, A. Dabrin, M. Coquery, N. Mazzella, C. Berho, J.-P. Ghestem, A. Togola, C. Gonzalez, J.-L. Gonzalez, B. Lalere, S. Lardy-Fontan, B. Lepot, D. Munaron, C. Tixier, (2012), An in situ intercomparison exercise on passive samplers for monitoring metals, polycyclic aromatic hydrocarbons and pesticides in surface waters, Trends in *Analytical Chemistry* (36), 128-143.
23. Hering D., Borja A., Elliott M., Feld C., (2010), The European Water Framework Directive at the age of 10: a critical review of the achievements with recommendations for the future, *Science of the total Environment* (408), 4007-4019.
24. Common implementation strategy for the water framework directive, (2009), Guidance on surface water chemical monitoring under the water framework directive, Official Publications of the European Communities (19), Luxembourg.
25. Foppe Smedes, Dick Bakker, Jasperien de Weert, 2010, The use of passive sampling in WFD monitoring, DELTARES, Dutch research institute for water, subsurface and infrastructure. http://kennisonline.deltares.nl/product/30272 (February 2014).
26. Marcoaldi Caterina, (2014), Determinazione dei composti organostannici tramite campionamento passivo nel Golfo Di La Spezia, Master's Degree Thesis, University of Rome "La Sapienza".

ENVIRONMENTAL OCCURRENCE AND CONCERNS OF ANTIFOULING BIOCIDES

# Undesirable effects of the antifouling biocides Irgarol and Diuron upon some non-target marine organisms

In this report, results obtained with organisms belonging to different phyla, at different levels of biological complexity and of the trophic chain, have been summarized. *Algae Dunaliella tertiolecta, Tetraselmis suecica, Isoscrysis galbana*, bacteria *Vibrio fischeri,* crustacean *Artemia salina*, echinoids *Paracentrotus lividus*, and fishes *Sparus auratus*, *Dicentrarchus labrax* were tested for their sensitivity to the antifouling biocides Irgarol and Diuron

*DOI: 10.12910/EAI2014-48*

*Sonia Manzo*

## Introduction

The extensive use of antifouling (AF) biocides on boat shells and other submerged surfaces was often responsible for the contamination of water and sediments by many toxic substances, especially booster biocides such as Irgarol and Diuron [1], used in combination with other compounds such as copper salts [2].

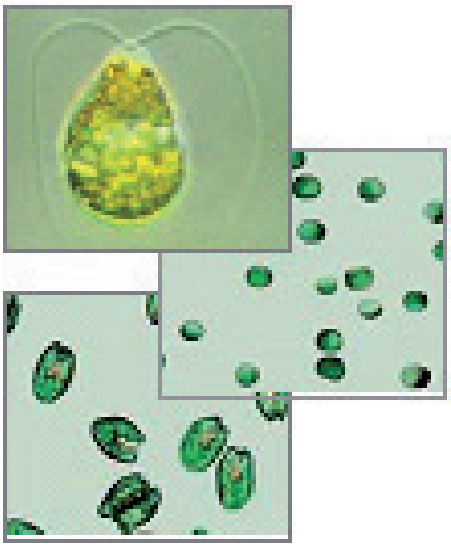
However, some of these chemicals have also been used as pesticides for agricultural use (e.g., Diuron), leading to possible confusion in identifying contamination sources [3]. There is evidence that these compounds were highly toxic for freshwater and marine autotrophs [4], influencing key species in both environments. The previous TBT experience (shell malformation in oyster, mortality of mussel larvae and imposex in gasteropods at ng/L concentration) generated the necessity to investigate any possible adverse effect on the marine ecosystem of these herbicides.

Ecotoxicological assessment of the adverse effects of these compounds in estuarine and coastal systems is, therefore, a matter of concern for many stakeholders involved in the conservation and exploitation of these areas (e.g., oyster or mussel farmers, fishermen).

Ecotoxicology is the science studying the contaminants effects on the biosphere constituents. Albeit a relatively new field, ecotoxicological research is rapidly developing due to concern induced by the industrial development. Ecotoxicology has therefore become an important part in environmental and ecological risk assessment and in the definition of environmental policies. As a matter of fact, unlike analytical chemistry approaches, ecotoxicological tests integrate all toxic signals, thus adding toxicity-based criteria to the currently adopted policies for a more comprehensive evaluation of the environmental hazard. Additionally, the laboratory results obtained with pure chemicals allow to evaluate the effects observed in the environmental samples and, then, to estimate the possible contribution of each biocide to the overall toxicity.

**Sonia Manzo**
*ENEA, Technical Unit for Technologies Development - Portici Research Centre*

The ecotoxicological approach is generally based on a battery of bioassays with organisms belonging to several species, since the use of a combination of assays and/or organisms increases the ecological reliability and easiness of interpretation of results, which in turn offers a powerful tool for assessing the potential bias of individual organisms and also the mode of action of contaminants.
This paper will explore the ecotoxicological effects of Irgarol and Diuron, registered for the marine environment, on non-target species at the ENEA's Portici Research Centre, during the last few years. Toxicity test with organisms (bacteria, microalgae, crustaceans, echinoids and fishes) belonging to different trophic levels were performed. The toxicity data was utilised to create concentration-response curves and to calculate EC50, LOEC and NOEC parameters. In addition, the toxicity results were fitted by interpolation models, in order to obtain continuous dose-response curves.



Algae

## *Algae* test

Marine algae are highly diffused in coastal ecosystems [5], therefore they are particularly susceptible to contaminants associated with anthropogenic pollution.

The evaluation of the effects of AF biocides upon marine phytoplankton is a necessary step to predict their potential impact on coastal marine food webs and on the whole ecosystems they support.

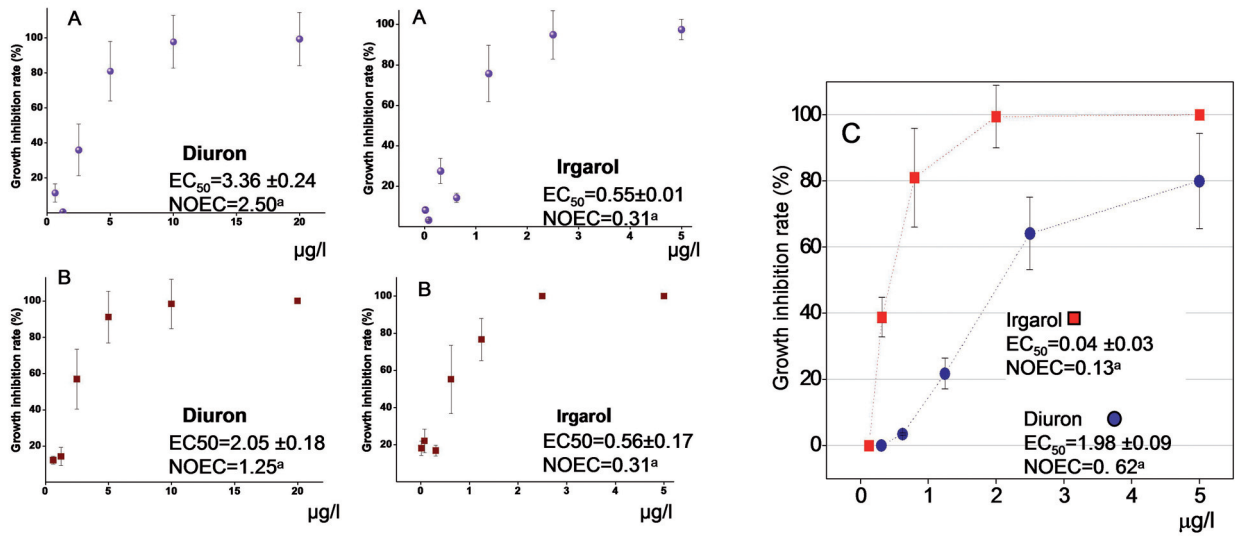



**FIGURE 1** Dose-response curves (mean percentage of growth inhibition rate with respect to control) for Irgarol and Diuron with different marine algal species *I. galbana* (A), *T. suecica* (B), *D.tertiolecta* (C) after 96h incubation.a ($p<0.05$)

The chronic test was carried out according to UNI EN ISO 10253. Briefly, an algal suspension at concentration of $1x10^6$ was added to each replicate [3] to reach the final concentration of $1x10^4$. Artificial seawater [6] was used for sample dilution, and culture medium was utilized as negative control (6 replicates). The test flasks were placed in a thermostatic chamber at 20 °C with a light source in the 7000-8000 lux range for 72h. The cell density of each sample is measured after 72h by the Burker chamber. Growth inhibition percentage and EC50 were calculated for each sample with respect to the control.

### *Results*

Being a herbicide, Irgarol is toxic to algae and its prevalent effect is the inhibition of photosynthesis, acting on Photosystem-II (PSII) in particular [7].
The tested microalgae showed a high sensitivity to Irgarol. However, the EC50 values, LOEC and NOEC resulted quite similar for the tested species.
Figure 1 showed the dose response curves of these phytoplanctonic species. It is possible to note effects starting from the lowest tested concentrations. The curves had an increasing trend, and for *D. tertiolecta* (C) and *I. galbana* (A) an 80% toxic effect at concentration of about 1 g/L Irgarol was observable.
Among the tested species, *D. tertiolecta* was the most sensitive one to both biocides.

## *Artemia Salina* test

Crustaceans are frequently used as bioindicators and biomonitors in various aquatic systems. The brine shrimp *Artemia salina* is a zooplankton organism found in hypersaline habitats such as inland salt lakes, coastal salt pans and man-managed saltworks worldwide. Their life cycle begins by hatching dormant cysts where these cysts are inactive but, once in salt water, they become rehydrated and resume their development. Brine shrimp larvae are commonly used for toxicity assays.
*A. salina* cysts were hatched by using the procedure described in APAT-IRSA, 2003 [8]. The encysted organisms were first hydrated in a volume of artificial seawater [6] for 1h at 25 °C at 3000-4000 lux. Then they were incubated for 24h in the dark at the same temperature. Acute toxicity test (48h) was conducted according to APAT-IRSA. Ten nauplii were transferred in beaker with 40 ml of sample. Each sample was tested in triplicate. The negative control consisted of 6 artificial seawater replicates. The treatments were

*Artemia Salina*

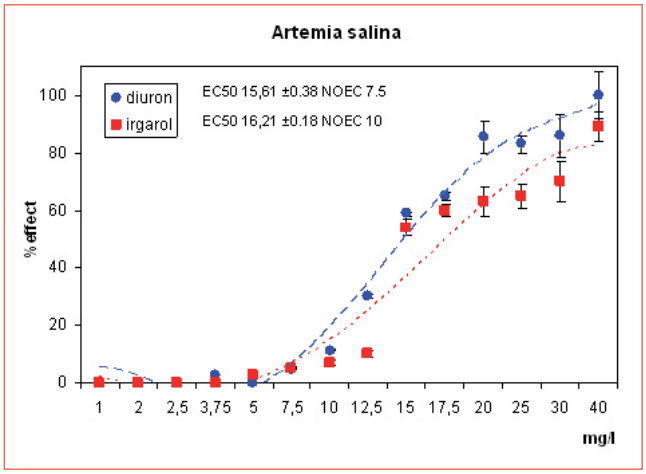



**FIGURE 2** Dose response curves (mean percentage of effect (mortality) with respect to control) obtained for *A. Salina* exposed to Irgarol and Diuron solutions for 48 h

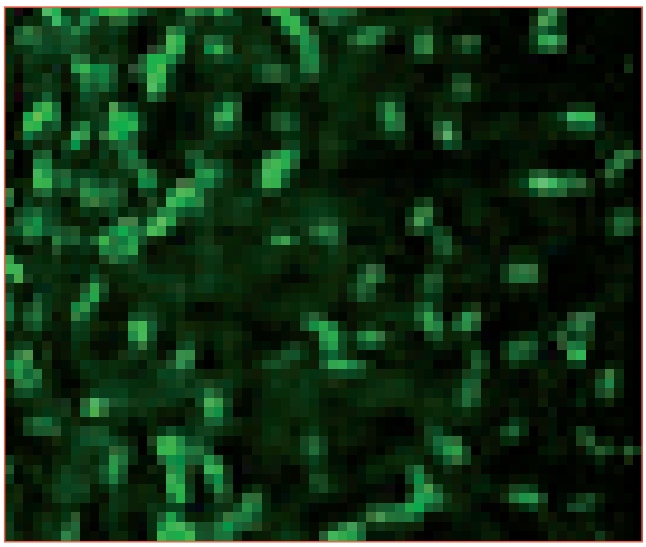
incubated at 25 °C, with a light regime of 14:10h light: dark. No food was provided during the course of the exposure. Every 24h the number of the live individuals was recorded. The effect percentage for each sample was calculated with respect to the control.

### *Results*

In Figure 2, the dose response curves obtained for *A. salina* exposed to Irgarol and Diuron solutions for 48h are reported. As can be observed, this organism showed scarce sensitivity to these biocides also at the lowest concentrations, in fact the NOEC values are around 10 mg /L.

## *Vibrio Fischeri* test

Microtox® is a standardised toxicity test system which is rapid, sensitive, reproducible, ecologically relevant and cost effective. It is recognised and used throughout the world as a standard test for aquatic toxicity testing. The Procedure employs the bioluminescent marine bacterium *(Vibrio fischeri)* as test organism. The bacteria are exposed to a range of concentrations of the material being tested. The reduction in intensity of light emitted from the bacteria is measured along with standard solutions and control samples. The change in light output and concentration of the toxicant produces a dose/response relationship. The results are normalised and the EC50 (concentration producing a 50% reduction in light) is calculated.



*Vibrio Fischeri*

*V. fischeri* bacteria were exposed to serial dilutions (1: 2) and to a negative control Microtox diluent (NaCl 2%). The luminescence decrease was evaluated after 5, 15 and 30 minutes of exposure. The luminescence was measured using a Microbics Model 500 Toxicity Analyzer and following the manufacturer's instructions (Microbics Corporation). The results were expressed as luminescence inhibition percentage with respect to the control.

### *Results*

In Figure 3 are reported the dose response curves

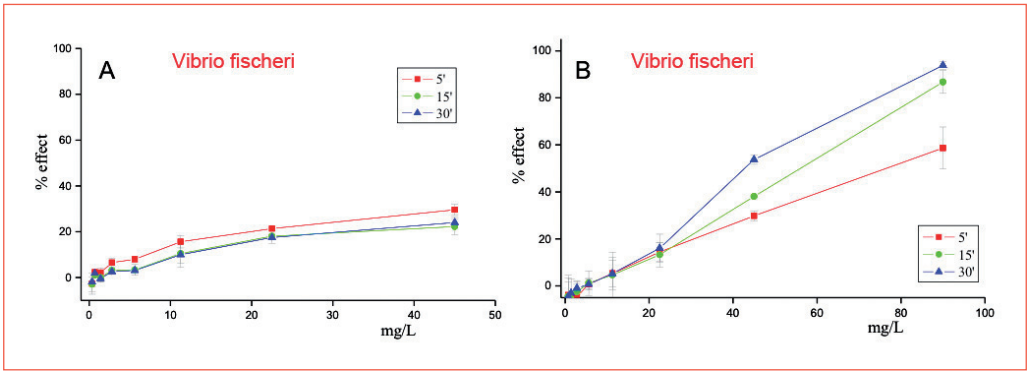



**FIGURE 3** Dose response curves (mean percentage of effect (bioluminescence inhibition) with respect to control for *V. fischeri* at three different exposure times to A) Irgarol, B) Diuron

for the bacteria at three different exposure times. For Irgarol the trends did not show significant differences and NOEC and LOEC values were comparable (Figure 3 A). This biocide resulted slightly toxic for *V. fischeri* with the highest toxic effect of 25% with respect to the control. Actually Diuron exerted a lower toxicity with an EC50 of 73 mg /L, and NOEC and LOEC values over 10 mg/L (11.25 mg/L and 22.50 mg/L, respectively) (Figure 3B). In this case, starting from concentration of 30 mg/L a dependence with the exposure times was also observable.

## *Paracentrotus Lividus* test

Sea urchin embryos and gametes are often utilized to assess the toxicity of chemicals in the marine ecosystem due to their sensitivity and availability. In addition, spermiotoxicity and embryotoxicity tests offer the possibility of comparing the effects of the same substance upon two different biological systems. Fertilization was carried out by adding pooled-sperm to the egg suspension and incubating it at 18 °C for 20 minutes. A volume of the egg suspension corresponding to 250–300 fertilized eggs was treated with 10 mL of test solution. Three replicates for each treatment were prepared. The eggs were then incubated at 18 °C, for 48–50 h. After this time, 100 µL of 40% buffered formalin was added in each vessel and developmental

*Paracentrotus lividus*

abnormalities were determined in each replicate by direct observation of 100 individuals, randomly chosen. For each treatment schedule, 100 plutei were scored for the frequencies of: normal larvae, according to their symmetry, shape, and size, malformed larvae affected in skeletal and/or gut differentiation and/or pigmentation, embryos unable to go to larval differentiation, such as abnormal blastula or gastrulae.

Spermiotoxicity test was performed, according to

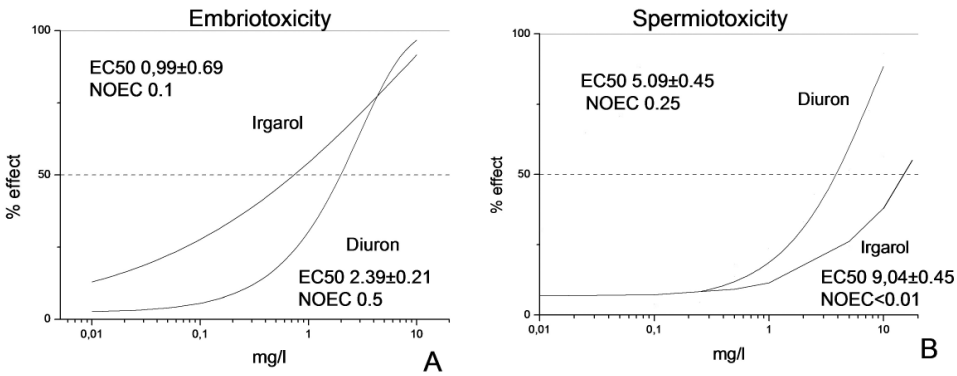



**FIGURE 4** Dose response curves obtained for *P. lividus* embryos (A) and sperms (B) treated with Irgarol and Diuron

Manzo et al. 2006 [9].
Sperm was collected "dry" from each male and stored on ice. 10 µL of concentrated sperm was diluted in 10 mL of sample. The solution were incubated for 30 min at room temperature, then 50 µL of treated sperm was added to 10 mL of FSW containing untreated eggs. Experimental wells were incubated at 18 °C for 20 min. Three replicates were carried out for each sample. The fertilization rate was determined on a sample of 100 eggs. The effect percentage for each sample was calculated with respect to the control.

*Results*

**Embriotoxicity**

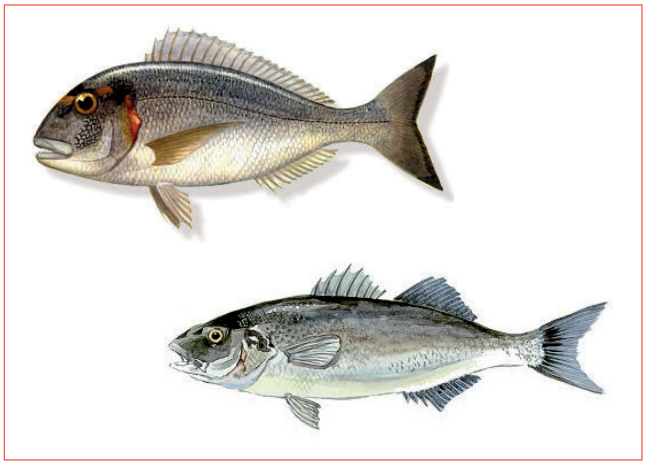
The embriotoxic effects of Irgarol and Diuron are reported in Figure 4.A.
The Irgarol toxicity values quickly increase up to EC50 0.99 (± 0.69) mg/L, and seem to stabilize from 1 to 5 mg/L dose (laying around 60% toxic effects) and at higher concentrations a corresponding increase is observed, with the maximum at 10 mg/L.
Diuron toxicity shows values with an increasing trend up to the maximum effect (SD = 0) at 7.5 mg/L. EC50 is 2.39 (± 0.21) mg/L and NOEL 0.25 mg/L.

**Spermiotoxicity**

Spermiotoxicity values are reported in Figure 4B.
The toxicity pattern of Irgarol on sperm fertilization ability can be evidenced already at 0.01 mg/L concentration (NOEL < 0.01 mg/L), but then the effects remain under 25% up to 5 mg/L. The EC50 is 9.04 (± 0.45) mg/L. Significant effects on the fertilization rate (FR) were observed for Diuron. FR shows a significant progressive decrease due to a reduction in the fertilization ability of exposed sperms. The Diuron EC50 is 5.09 (±0.45) mg/L, and NOEL 0.5 mg/L.

## Fish test *(Sparus aurata, Dicentrarchus labrax)*

The purpose of the toxicity test with fish species is to help assess possible risk to similar species in natural environments, as an aid in determination of possible water quality criteria for regulatory purposes, and for use in correlation with acute testing of other species for comparative purposes.



*Sparus aurata, Dicentrarchus labrax*

*Fish early life stage toxicity test [10]*

Larvae were exposed to increasing concentrations of Irgarol and Diuron in static conditions and did not feed during the test. Filtered seawater was used to dilute the solutions and as control. In addition, a DMSO control has been also used. The experiments were conducted in triplicate using a testing volume of 200 ml and 20 individuals in each replicate. The experiment exposure was 24 and 48 h at T 18 °C with regular photoperiod.

*Fish Acute Toxicity test [11]*

Juveniles of *Sparus aurata* were exposed to increasing concentrations of Irgarol and Diuron. Filtered seawater was used to dilute the solutions and as control. In addition, a DMSO control has been also used. The experiments were conducted in triplicate using a testing volume of 8 L and 5 individuals for each replicate and exposure condition for 96 h at T 18 °C with regular photoperiod and continuous aeration.

*Results*

Fish larvae were also very sensitive to Irgarol (*D. labrax,* NOEC= 2.5 mg/L) with respect to Diuron (S. aurata larvae NOEC < 0.01mg/L), while the juveniles *(S. aurata)* showed a high resistance (NOEC= 2 mg/L): therefore, during a brief exposure (48h), larvae

| | | Irgarol | | | Diuron | | |
|---|---|---|---|---|---|---|---|
| **Test organism** | | **EC50** | **LOEC** | **NOEC** | **EC50** | **LOEC** | **NOEC** |
| *Vibrio fischeri* | *5 min* | >45 | 2.81 | 1.41 | 73.12 | 22.50 | 11.25 |
| *Dunaliella tertiolecta* | | [a]0.40±0.03 | [a]0.32 | [a]0.13 | [a]1.98±0.09 | [a] 1.25 | [a] 0.62 |
| *Tetraselmis suecica* | | [a]0.56±0.17 | [a]0.40 | [a]0.31 | [a] 2.05±0.18 | [a] 2.50 | [a] 1.25 |
| *Isochrysis galbana* | | [a]0.45±0.02 | [a]0.31 | [a]0.08 | [a]3.36±0.24 | [a] 5.00 | [a] 2.50 |
| *Artemia salina* | | >15.00 | 15.00 | 10.00 | 15.61±0.38 | 10.00 | 7.50 |
| *Paracentrotus lividus* | *spermiotox* | 0.99±0.69 | 0.01 | <0.01 | 2.39±0.21 | 0.5 | 0.25 |
| | *embriotox* | 9.04±0.45 | 0.50 | 0.10 | 5.09±0.45 | 1 | 0.5 |
| *Sparus auratus (juveniles)* | | - | - | [b]2 | >1 | - | [a] < 10 |
| *Dicentrarchus labrax (larvae)* | | [a]134.59±23.96 | [a]12.50 | [a]2.50 | - | - | - |

a: µg/L; b: max concentration tested

**TABLE 1** Toxic effects of Irgarol and Diuron for different marine organisms

resulted to be more sensitive compared to juveniles, probably due to an easier absorption. However, some observations about the insurgence of sublethal effects were evidenced also in the juveniles, such as scarce reactivity, and altered orientation.

## Conclusions

The sensitivity range of the different organisms to Irgarol and Diuron was quite similar. The two biocides showed a high toxicity for algae species, whereas they resulted moderately toxic toward the other species. The highest sensitivity of algae is linked to the mechanism of action of these compounds; both compounds are photosynthesis inhibitors acting upon transport electron chain in the photosystem II.

The NOECs were between 0.08-0.31 mg/L and 0.62- 2.5 mg/L for Irgarol and Diuron, respectively. Among the tested species, *T. suecica* was the less sensitive to Irgarol while I. galbana to Diuron. Similarly, looking at the EC50s, Irgarol always resulted the most toxic compound.

Sea urchin embryos seem to be very sensitive to Irgarol. The spermiotoxicity test shows an EC50 value in the same range as those reported for crustaceans [12] and a NOEC of 0.10 mg/L. To our knowledge, the mode of action of triazine upon aquatic invertebrates is not well known. In our spermiotoxicity and embriotoxicity tests, we observed a predominance of malformed larva, mainly affected by skeletal alterations.

Exposed sperms show a dose-related decrease in fertilization ability but with less sensitivity than for embryos, probably because they are differentiated cells. On the contrary, the maximum defect in offspring is obtained at the lowest test concentration (0.01 mg/L). The herbicide affected only the sperm fertilization ability, producing an acute spermiotoxicity. Although belonging to a different chemical class of pesticide (phenylureas) than Irgarol, Diuron is a photosynthesis

inhibitor, too, but the mode of action at the biochemical level has not been precisely determined so far. Diuron ecotoxicological data in recent literature are quite scarce, particularly with reference to marine species. Hernando et al. [13] reported toxicity effects on *D. magna* (EC50 8.6 mg/L ± 1.3) and *V. fisheri* (EC50 100 mg/L ± 7.8).

Although the sensitivity of tested organisms indicated that concentrations necessary to cause severe toxicity are higher than the reported environmental levels reviewed by Konstantinou and Albanis 2004 [12], this may not indicate any absence of risks, since interactions and synergic effects with other contaminants can take place [14].

It is also important to consider that, from 2008 onwards, tributyltin-based paints have been totally banned and the environmental levels of the replacing organotin-free biocide can considerably increase. Moreover, these active compounds can accumulate in marine sediments, especially if introduced as paint particles [15]. Compared with leached biocides, those bound to particles are considerably more persistent and, therefore, likely to pose a longer term threat to marine organisms.

references


1. L.W. Hall, P. Gardinali (2004), "Ecological risk assessment for Irgarol 1051 and its major metabolite in United States surface waters", *Human and Ecological Risk Assessment,* 10, 525-542.
2. I. Omae (2003), "Organotin antifouling paints and their alternatives", *Applied Organometallic Chemistry,* 17, 81-105.
3. G. Di Landa, L.Parrella, S. Avagliano, G. Ansanelli, E. Maiello, C. Cremisini (2009), "Assessment of the potential ecological risks posed by antifouling booster biocides to the marine ecosystem of the Gulf of Napoli (Italy)", *Water Air and Soil Pollution,* 200, 305-321.
4. H. Okamura, T. Nishida Y. Ono, W.J. Shim (2003), "Phytotoxic effects of antifouling compounds on nontarget plant species", *Bulletin of Environment Contamination and Toxicology,* 71, 881-886.
5. M.J. Behrenfeld, RT, O'Malley , D.A., Siegel C.R,.McClain, J.L. Sarmiento, G.C. Feldman, et al. (2006), "Climate-driven trends in contemporary ocean productivity", *Nature*, 444,752–5.
6. ASTM Standard guide for acute toxicity test with the rotifer Brachionus. *Annual Book of ASTM Standards Philadelphia* (1998),1440-1491.
7. B. Dahl, H. Blanck (1996), "Toxic effects of the antifouling agent Irgarol 1051 on periphyton communities in coastal water microcosms", *Marine Pollution Bullettin,* 32, 342–350.
8. APAT-IRSA (2003). APAT-IRSA – METODI ECOTOSSICOLOGICI. 8060. Metodo di valutazione della tossicità acuta con Artemia sp.
9. S. Manzo, S. Buono, C. Cremisini (2006), "Toxic effects of Irgarol and Diuron on sea urchin Paracentrotus lividus early development,fertilization, and offspring quality". *Archives of Environmental Contamination and Toxicology,* 51, 61-68.
10. USEPA, (1996), "Fish early stage toxicity test Ecological Effects Test guidelines", *OPPTS* 850.1400.
11. USEPA, (1996), "Fish acute toxicity test, freshwater and marine. Ecological Effects Test guidelines". OPPTS 850.1075.
12. I.K. Kostantinou, T.A. Albanis (2004), "Worldwide occurrence and effects of antifouling paint booster biocides in the aquatic environment: a review", *Environment International,* 30, 253–248
13. M.D. Hernando, M. Ejerhoon, A.R. Fernandez-Alba, Y. Chisti (2003), "Combined toxicity effects of MTBE and pesticides measured with Vibrio fisheri and Daphnia magna bioassays", *Water Research,* 37, 4091–4095.
14. S. Manzo, S. Buono and C. Cremisini (2008), "Predictability of Copper, Irgarol and Diuron combined effects on sea urchin Paracentrotus lividus", *Archives of Environmental Contamination and Toxicology,* 54, 57-68.
15. K.V. Thomas, M. McHugh,, M. Waldock (2002), "Antifouling paint booster biocides in UK coastal waters: inputs, occurrence and environmental fate", *Science of Total Environment,* 293, 117–127.

ENVIRONMENTAL OCCURRENCE AND CONCERNS OF ANTIFOULING BIOCIDES

# CARISMA Project: an example of integrated approach in the study of the adverse effects posed by antifouling agents in the Southern Adriatic Sea

In the framework of CARISMA project – which aims to assess the quality of the Southern Adriatic Sea area between Italy (Apulia) and Albania, and the impact due to the use of antifouling paints – a preliminary survey in ports and marinas along coastal areas of both countries was conducted. Chemical analyses were complemented with ecotoxicological assays. In addition, in order to assess potential adverse ecological effects posed by selected antifouling agents on non-target marine organisms, Ecological Risk Assessment was applied

*DOI: 10.12910/EAI2014-49*

*Sonia Manzo, Giuliana Ansanelli, Luisa Parrella, Giuseppe Di Landa, Paolo Massanisso, Simona Schiavo, Carmine Minopoli, Bruno Lanza, Juri Rimauro, Antonio Salluzzo, Pellumb Aleksi, Afrim Tabaku*

**Sonia Manzo, Giuliana Ansanelli, Giuseppe Di Landa, Simona Schiavo, Carmine Minopoli, Bruno Lanza, Juri Rimauro, Antonio Salluzzo**
*ENEA, Technical Unit for Technologies Development - Portici Research Centre*

**Luisa Parrella**
*University of Naples "Federico II"*

**Paolo Massanisso**
*ENEA, Technical Unit for Environmental Characterization, Prevention, and Remediation*

**Pellumb Aleksi**
*Food Safety and Veterinary Institute, Tirana, Albania*

**Afrim Tabaku**
*Aldent University, Tirana, Albania*

## Introduction

Carisma (Characterization and ecological risk analysis of antifouling biocides in the Southern Adriatic Sea), a project funded the by the Italian Ministry of Foreign Affairs, aims to assess the quality of the portion of the Adriatic Sea between Italy (Apulia region) and Albania and, specifically, the impact due to the use of antifouling paints.

Antifouling (AF) paints are routinely used to prevent any living organisms from undesirably adhering to the submerged surfaces of ships, boats and aquatic structures; they act realising effective biocides from the coated surface.

Formulations containing organotin (OT) compounds (e.g., tributyltin, TBT) were the most successful against biofouling but they were banned in 2008, due to their detrimental impact on sea life. Currently, most antifouling paints contain copper or zinc as an active ingredient and a "booster" biocide, such as Irgarol and Diuron, to strengthen the effectiveness of the formulation. The toxicity of AF biocides can also be exerted on non-target species, after their release in water column. Likewise, copper and zinc at high concentrations and in a bioavailable form can be toxic to algae and other water organisms [1]. Therefore, these AF agents need to be monitored in order to assess the possible environmental damage related to their use.

A preliminary survey was carried out on the occurrence of Diuron, Irgarol, OT compounds and some heavy metals, in ports along the Apulia (Italy) and Albania coasts.

The sampling strategy was limited to harbors and marinas as they can represent the worst scenario for the exposure of marine organisms to AF compounds. In fact, such sites are usually characterized by intense boat traffic and by a conformation that does not favour water exchange, so that contaminants tend to accumulate, reaching higher levels than in the open sea.

As far as we know, no monitoring data of organic booster biocides are available for Albanian marine waters whereas previous studies have been carried out in Italy (e. g., Di Landa et al.) [2].

Moreover, to assess the toxicity of biologically available contaminants [3], even those not taken into account or detected by chemical analyses [4], as well as their action as mixtures [5,6], toxicological assays were performed [7,8].

Lastly, a deterministic Ecological Risk Assessment (ERA) has been accomplished for assessing potential adverse ecological effects posed by AF biocides (i.e., TBT, Irgarol, Diuron) to non-target marine organisms in the studied area. Through this ERA approach, high-risk or low-risk situations can be identified by the estimation of the numerical hazard quotient (HQ).

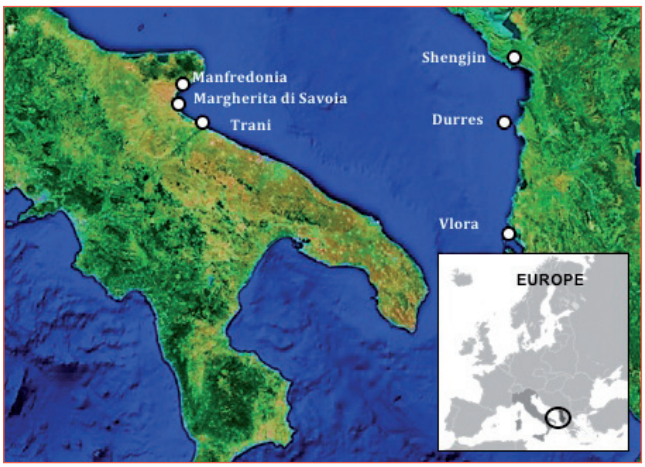



FIGURE 1 Italian and Albanian monitored sites

## Study areas

Three ports from medium to large size – Manfredonia, Trani and Margherita of Savoia – were selected north of Apulia (Italy). This region is located right in front of Albania, from which it is separated by the Adriatic sea, with distances ranging between 72 and 290 km. The ports of Trani and Margherita of Savoia mainly host fishing boats and pleasure crafts. The port of Manfredonia, instead, is frequented by ferries, commercial ships, fishing boats, and pleasure crafts.

Albania has a 472 km coastline, but there are few relevant ports, all destined to freight and passenger traffic as well as to mooring of fishing vessels, while recreational boating is still very poorly developed. So low environmental loading is expected for AF agents.

Sampling was carried out in the three main Albanian ports: Durres, Vlora and Shengjin. Durres has currently 78% of maritime trade at the national level and is also a key location for transit networks and passenger ferries. Shengjin houses mainly fishing vessels and Vlora has two distinct ports, one dedicated to goods and passenger traffic, and the other one to fishing boats. Only the latter was sampled in this preliminary campaign.

Figure 1 shows the Italian and Albanian sites selected for monitoring, while in Table 1 the geographic coordinates and the main characteristics of the sampled points are reported.

Rd

| Sampled harbors | Abbreviation | Position | Site description | Berths |
|---|---|---|---|---|
| **Italy** | | | | |
| Manfredonia | MN1 | 41°37'27.94" N - 15° 55' 13.490"E | harbor; marina | 365 |
| Manfredonia quay | MN2 | 41°37'30.73" N –15°54' 53.600"E | | |
| Margherita di Savoia | MDS1 | 41°23'17.19"N - 16°08'2.770"E | marina; fishery port | 200 |
| Margherita di Savoia quay | MDS2 | 41°23'02.63"N – 16°07'55.190"E | | |
| Trani | TR1 | 41°16'51.399"N - 16°25'17.234"E | marina; fishery port | 550 |
| Trani quay | TR2 | 41°16'44.966"N - 16°25' 10.346"E | | |
| Trani reference [a] | TRref | 41° 17' 30.000"N - 16°26'6.000"E | | |
| **Albania** | | | | |
| Shengjin | SH1 | 41°48'42.900"N - 19°35'17.400"E | fishery port | 28 [b] |
| Shengjin quay | SH2 | 41°48'49.320"N - 19°35'11.400"E | | |
| Durres | DR1 | 41°18'22.800"N - 19°27'19.740"E | harbor | 98 [b] |
| Durres quay | DR2 | 41°18'10.440"N - 19°27'14.100"E | | |
| Vlora | VL1 | 40°29'4.800"N - 19°25'58.200"E | fishery port | 61 [b] |
| Vlora quay | VL2 | 40° 29'3.300"N - 19°25' 51.720"E | | |
| Vlora reference [a] | VLref | 40°28'25.920"N-19°24'38.640"E | | |

[a] Blank seawater samples, collected 1 mile offshore; [b] Registered fishery vessels

**TABLE 1** Description of sampling sites

| Variables | Sampling stations | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MN1 | MN2 | MDS1 | MDS2 | TR1 | TR2 | SH1 | SH2 | DR1s | DR2 | VL1 | VL2 |
| | Seawater | | | | | | | | | | | |
| **Organic compounds** | | | | | | | | | | | | |
| Diuron (ng/L) | 12.9 | 12.4 | 16.5 | 583.5 | 448.7 | 68.9 | 1.9 | 8.4 | 78.8 | 93.9 | 28.8 | 33.3 |
| Irgarol (ng/L) | 10.0 | 8.9 | 0.6 | 14.7 | 5.1 | 16.1 | < 0.2 | 0.5 | 0.8 | 0.7 | 8.5 | 9.3 |
| TBT (ng/L, as cation) | 76.0 | 105.0 | 12.0 | 110.0 | 24.0 | 22.0 | 5.0 | 22.0 | 24.0 | 24.0 | 34.0 | 44.0 |
| **Physical parameters** | | | | | | | | | | | | |
| T (°C) | 28.5 | | 27.3 | | 26.7 | | 26.0 | | 25.4 | | 26.7 | |
| O2 (% saturation) | 81 | | 39 | | 65 | | 84 | | 85 | | 108 | |
| Salinity | 34.9 | | 36.6 | | 36.6 | | 37.7 | | 38.3 | | 38.1 | |
| pH | 7.95 | | 7.81 | | 7.87 | | 7.88 | | 8.04 | | 8.00 | |
| **Ecotoxicological assays** | | | | | | | | | | | | |
| A. salina (% effect) | 17 | 23 | 13 | 6 | 18 | 16 | 11 | 26 | 10 | 15 | 4 | 4 |
| D. tertiolecta (% effect) | 66 | 76 | 79 | 100 | 75 | 62 | 99 | 87 | 85 | 85 | 88 | 88 |
| P. lividus (% effect) | 34 | 47 | 43 | 76 | 34 | 32 | 27 | 37 | 43 | 40 | 47 | 40 |
| V. fischeri (% effect) | - 31.6 | 2.7 | -19.6 | -34.4 | 29.9 | -24.8 | -39.0 | -44.7 | -11.0 | -25.0 | -31.2 | -33.0 |
| **HQ (Risk Analysis)** | | | | | | | | | | | | |
| Diuron | 0.004 | 0.004 | 0.005 | 0.187 | 0.144 | 0.022 | 0.001 | 0.003 | 0.025 | 0.030 | 0.009 | 0.011 |
| Irgarol | 0.053 | 0.047 | 0.003 | 0.078 | 0.027 | 0.085 | 0.001a | 0.003 | 0.004 | 0.003 | 0.045 | 0.049 |
| TBT | 25.333 | 35.000 | 4.000 | 36.667 | 8.000 | 7.333 | 1.667 | 7.333 | 8.000 | 8.000 | 11.333 | 14.667 |

| Variables | Sampling stations | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MN1 | MN2 | MDS1 | MDS2 | TR1 | TR2 | SH1 | SH2 | DR1s | DR2 | VL1 | VL2 |
| **Sediments** | | | | | | | | | | | | |
| **OT compounds** | | | | | | | | | | | | |
| TBT (µg/kg, as cation) | 51 | | 5 | | 7 | | 41 | | 71 | | n.s. | |
| **Metals** | | | | | | | | | | | | |
| $^{52}$Cr (mg/kg) | 99.3 | | 39.4 | | 71.1 | | 293.4 | | 439.1 | | n.s. | |
| $^{55}$Mn (mg/kg) | 705.9 | | 88.6 | | 1044.4 | | 1041.8 | | 537.1 | | n.s. | |
| $^{63}$Cu (mg/kg) | 89.7 | | 26.8 | | 54.7 | | 47.7 | | 52.8 | | n.s. | |
| $^{75}$As (mg/kg) | 15.6 | | 5.6 | | 21.5 | | 13.4 | | 11.1 | | n.s. | |
| $^{114}$Cd (mg/kg) | < 0.1 | | < 0.1 | | < 0.1 | | < 0.1 | | < 0.1 | | n.s. | |
| $^{208}$Pb (mg/kg) | 49.6 | | 17.1 | | 34.1 | | 11.3 | | 17.2 | | n.s. | |
| $^{78}$Se (mg/kg) | < 0.1 | | < 0.1 | | < 0.1 | | < 0.1 | | < 0.1 | | n.s. | |
| $^{118}$Sn (mg/kg) | 5.9 | | 1.4 | | 4.1 | | < 0.1 | | < 0.1 | | n.s. | |
| $^{66}$Zn (mg/kg) | 168.6 | | 49.8 | | 126.6 | | 112.2 | | 117.6 | | n.s. | |
| $^{121}$Sb (mg/kg) | 0.1 | | 0.3 | | 0.6 | | < 0.1 | | < 0.1 | | n.s. | |
| $^{115}$In (mg/kg) | < 0.1 | | < 0.1 | | < 0.1 | | < 0.1 | | < 0.1 | | n.s. | |
| $^{98}$Mo (mg/kg) | 2.1 | | 0.6 | | 2.1 | | 1.1 | | 1.0 | | n.s. | |
| $^{60}$Ni (mg/kg) | 45.8 | | 13.9 | | 38.2 | | 205.5 | | 226.8 | | n.s. | |
| $^{51}$V (mg/kg) | 128.0 | | 58.5 | | 101.6 | | 94.9 | | 95.5 | | n.s. | |
| $^{59}$Co (mg/kg) | 14.9 | | 25.5 | | 16.1 | | 23.0 | | 21.6 | | n.s. | |
| **Ecotoxicological assays** | | | | | | | | | | | | |
| A. salina -Eluatriates (% effect) | 3 | | 4 | | 3 | | 10 | | 13 | | n.s. | |
| A. salina - Pore water (% effect) | 3 | | - | | 3 | | 16 | | 16 | | n.s. | |
| D. tertiolecta - Eluatriates (% effect) | 98 | | 98 | | 88 | | 95 | | 95 | | n.s. | |
| D. tertiolecta - Pore water (% effect) | 88 | | - | | 88 | | 85 | | 96 | | n.s. | |
| P. lividus - Eluatriates (% effect) | 74 | | 75 | | 72 | | 74 | | 75 | | n.s. | |
| V. fischeri - Eluatriates (% effect) | 36.2 | | - | | - | | - | | - | | n.s. | |
| V. fischeri - Pore water (% effect) | 3.3 | | - | | - | | - | | - | | n.s. | |
| V. fischeri - Whole sediment (EC 50% mg /mL) | NM[b] | | 1.4 | | 30.9 | | 3.6 | | NM[c] | | n.s. | |
| **Mussels** | | | | | | | | | | | | |
| **OT compounds** | | | | | | | | | | | | |
| TBT (µg/kg) | 732 | | 854 | | 220 | | 122 | | n.s. | | n.s. | |

n.s.: not sampled. NM: Not Measurable. [a] Value derived from a concentration arbitrarily set equal to one half of the detection limit. [b] Hormesis. [c] Highest toxic effect = 90%(200mg/mL)

**TABLE 2** Physical, chemical and ecotoxicological data for Italian (Apulia) and Albanian ports

## Sampling

Water, sediment and biota samples were collected in Italy and Albania in September 2012, when boating activity is still intense and the contamination from AF paints is expected to be significant.
At each site, water sampling was carried out in the middle of the basin and close to quay, with the aim to evaluate changes in concentration for the investigated chemicals.
Surface sediment samples were collected only at the centre of each harbor, by a stainless steel Van Veen grab sampler.
Where available, sea urchins *(Paracentrotus lividus)* and mussels *(Mytilus galloprovincialis)* were taken too.
For seawater samples, also measurements of temperature (T, °C), conductivity (mS/cm)/salinity, pH and dissolved oxygen (DO, % saturation), were performed in situ, using a portable multi-meter.

## Results and discussion

All results are summarized in Table 2.

### *Occurrence of booster biocides*

The two most persistent booster biocides, Diuron and Irgarol, were monitored in seawater. Analyses were performed according to the method described in Di Landa et al. [9].
Concentrations of Irgarol and Diuron in Italy were almost always higher than in Albania.
Diuron was detected in all the surveyed Italian and Albanian harbors and always exhibited higher concentrations than Irgarol, and the Diuron/Irgarol concentration ratios ranged from 1.3 to 87.7 in Apulia and from 3.6 to 145.1 in Albania.
Since Diuron is largely used in agriculture as herbicide as well as for weed control in non-agricultural applications, we suppose that seawater contamination by Diuron is also due to these uses in addition to antifouling paints, in both countries.
Average concentrations for Diuron in ports of Apulia were comparable to those reported by other authors (<7 and 366 ng/L) [10,11], but lower than those measured elsewhere in the world (up to 2160 ng/L) [1].
Albanian levels were similar to those recorded in Seto Inland Sea, Japan (10-62 ng/L) [12] and in California (<2-68 ng/L) [13].
Irgarol was found in all samples from the ports of Apulia while, as regards Albania, it was below the detection limit (< 0.2 ng/L) at Shengjin.
Irgarol concentrations in samples from Apulia were considerably lower than those detected in harbors and marinas worldwide, where levels up to 1300 ng/L have been achieved [2, 14].
Unlike the ports of Apulia, the Albanian ones are characterized by basins with good water circulation, hence both Irgarol and Diuron concentrations were quite similar in samples collected from quayside and centre of basin. The greatest differences in concentrations were registered at MDS. Moreover, it is interesting to note that in the harbor of Trani, contrary to what is usually observed, Diuron levels in the dock (68.9 ng/L) were much lower than those at the centre of the port (448.7 ng/L), probably because of a contamination source nearby.

### *Occurrence of OT compounds*

Despite the total ban of TBT-based paints, TBT was still a commonly encountered contaminant [15] and we found it in all samples collected from both Albanian and Italian coastal areas.
The highest TBT concentrations in water were observed in samples collected near quayside in both a large commercial harbor (MN) and a little marina (MDS).
Monitored sites of Apulia's coastal area resulted more contaminated with TBT (range 12– 110 ng/L as cation) than Albanian sites (range 5 – 44 ng/L as cation). The results are in agreement with recent studies in marine environment [15], where maximum concentrations in water rarely exceed 100 ng/L.
Environmental Quality Standards (Directive on EQS, 2008/105/EC) identified TBT as a priority hazardous substance and set the maximum allowable concentration (MAC-EQS) at 1.5 ng/L as cation. TBT levels found in this work were not negligible compared to its MAC-EQS.
TBT concentrations in sediment samples ranged between 5 and 71, with almost all results higher than the EQS for sediment (5 µg/kg, Legislative Decree 219/2010).

Mussel analyses confirmed that TBT is ubiquitous, with higher pressure on the coast of Apulia.
TBT concentrations found in both sediments and mussels were in agreement with the results reported in literature for countries [15] where the TBT has been banned.

*Metals*

Concentration levels of As, Cd, Co, Cr, Cu, In, Mn, Mo, Ni, Pb, Sb, Se, Sn, V, and Zn were determined in sediments collected in the ports of Apulia and Albania.
Cd, In and Se were always below the detection limit (0.1 mg/kg).
Very low amounts were found for Sb (≤ 0.6 mg/kg) and Mo (0.6 -2.1 mg/kg).
By comparing the results obtained for Italian ports with the quality standards (QS) reported in the DM 260/2010, a good ecological status was found only for the sediment sample collected at MDS. Conversely, sediments from MN and TR showed higher values than QS for Pb (QS 30 mg/kg dry weight, d.w.), As (QS 12 mg/kg, d.w.), Ni (QS 30 mg/kg, d.w.) and, mostly, for Cr (QS 50 mg/kg d.w.). Very high levels, up to 1044 mg/kg, were measured also for Mn, the main sources of which are from industrial processes, agricultural activities and combustion of coal. The Mn levels detected in this work are in accordance with the results found by other authors in the Adriatic sea [16].
Quality standard limits for Cu and Zn in sediments are not available in the Italian legislation, but the National Oceanic and Atmosphere Administration (NOAA) [17] indicated 34 mg/kg for Cu and 150 mg/kg for Zn as the concentrations below which adverse effects rarely occur. However, the results reported in literature showed that even lower concentrations can be toxic to aquatic organisms [18,19,20].
Levels of Sn, largely used in the past in AF paint based on OT compounds, ranged from 1.4 to 5.9 mg/kg in the Italian harbors, where they may also indicate unknown mineralisation or contamination by industrial activities. As regards sediments from Albanian ports (SH and DR), Sn was always below the detection limit and also Pb amounts were lower than in Apulia. Instead, As, Co, V, Mn, Cu and Zn exhibited concentrations comparable to Italian ports. Finally, Cr and Ni showed significantly higher levels than those detected in the Italian sediment samples. These high amounts may be due to agricultural and industrial activities (metallurgical and chemical plants for Cr, refineries, sewage sludge and phosphate fertilizers for Ni), producing discharges transported into the sea by rivers flowing across the country. In addition, the Albanian territory is characterized by the rich deposits of Cr and Ni, which might contribute to the high levels found for these two elements.

*Ecological risk assessment*

In the present study the ERA procedure, developed by US-EPA and described in detail in the Guideline for Ecological Risk Assessment [21] and elsewhere in this journal [22], was applied.
The numerical hazard quotients (HQ) were obtained as the ratio of the measured exposure concentrations to the 5th percentile of species sensitivity distributions, used as toxicity benchmarks.
The estimated 5th percentile from literature toxicity data was 189 ng/L, 3126 ng/L and 3 ng/L for Irgarol, Diuron and TBT, respectively.
HQ lower than 1 were obtained for Diuron (0.001-0.187) and Irgarol (0.001-0.085), considered as both single contaminants and a mixture.
For TBT, instead, the individual HQ values were always higher than 1 (1.67-36.67): it means that even if TBT has been banned, deleterious effects on aquatic exposed organisms can still be exerted.

*Ecotoxicity*

The organisms used for the tests responded to the samples showing a different sensitivity. The D. tertiolecta algae test on seawater samples, always showed the highest effects with peaks registered at MDS2 (EC50 of 3%) for the Italian samples and at VL2 (55%) for the Albanian ones. This peculiar sensitivity, ascribed to the chronic exposure (72 hours, ISO 10253), was also evidenced in other studies with different matrices [23,24]. For all seawater samples, *V. fischeri* showed biostimulation, with the only exception of TR1 (30%); *A. salina* showed an effect lower than 20%, while the *P. lividus* spermiotoxicity test evidenced high toxicity.
The algal test for sediments (aqueous matrices)

always showed the highest effect in Apulia with values near to 100% for MDS and MN elutriates, while, pore waters exerted low effects in all samples. Despite the general view of a higher contaminant concentration in aqueous matrices deriving from sediment compared to seawaters, this never happened for our Albanian and Italian samples. This can be ascribed to a recent contamination that mainly affected algae populations, while presumably in these sediments, contaminants were of different nature [25, 26] or strongly stuck to particles; for example, Irgarol in sediments can be present in association with paint particles and this fact restricts its bioavailability [27].

*A. salina* always showed an effect lower than 20% for both elutriates and pore waters, while *V. fischeri* evidenced higher toxicity in the elutriates. *P. lividus* test, carried out only on elutriates, always showed effect values over 50%.

Except for MN, for which hormesis was detected, toxic effects (90%) were observable with *V. fisheri* in all whole sediments, while generally they were not found in the corresponding aqueous matrices (elutriates and pore water). This can be linked to the occurrence of some low soluble chemicals, scarcely released during the elutriation treatment. Actually, in sediment toxicity evaluation, since toxicity is evaluated on both dissolved and adsorbed contaminants, the test with whole sediments can be considered more ecologically relevant than the test performed with elutriates or pore waters [28].

## Considerations on chemical, ecotoxicological, and risk analyses results

Water chemical analyses showed a different extent of contamination by Irgarol and Diuron between Albania and Apulia, the latter exhibiting higher levels for both biocides, as expected since marine traffic in Albania is much lower.

However, HQ calculated by ERA indicated that, in both countries, the risk posed by Irgarol and Diuron to aquatic organisms was always low, even when their mixture was considered. Conversely, a very high risk was determined for TBT in water. This biocide was detected in all the sampling sites but, differently from Irgarol and Diuron, its levels in water were comparable to those observed in Albania, except in a few cases. In particular, in the waters of MN and in the inner channel of Margherita di Savoia (MDS2), especially high concentrations of TBT (31-45 ng/L) were recorded.

It is worth noting that MDS2 was a hotspot for all the AF biocides investigated. This was due to the particular lay of the harbor, having an inner channel with a high density of moored boats and a very poor water exchange in contrast to the middle of the port, free of berths and connected to the open sea (MDS1).

In agreement with chemical results, the growth inhibition test with seawater samples performed on the marine algae *D. tertiolecta* showed the highest effect (100%) right at MDS2 as well as the spermiotoxicity test with sea urchin *P. lividus,* which showed an effect about as twice (76%) as that observed in all the other sampling stations (32-47%).

In contrast to the results of chemical analyses, an effect near to 100% was determined in the algal test with elutriates from MDS1, a site exhibiting a low degree of contamination in sediments, as regards TBT and selected heavy metals, and also in water, as regards Irgarol, Diuron and TBT.

These results suggest the presence of contaminants not taken into account by chemical analyses.

Again, differently from chemical results, the algal test highlighted slightly higher toxic effects for Albanian seawaters (average 86.8%) than for Italian ones (average 76.3%). In particular it should be noted that, despite the less contaminated waters from the monitored biocides, SH exhibited a very high toxic effect for algae (99%) and the major response from bacteria in bioluminescence tests (- 44.7%).

Spatial distribution assessment of Irgarol, Diuron and TBT within each port evidenced different concentrations for seawater samples collected from the quay and from the centre of the basin in Italy, except at Manfredonia, while in Albania the spatial variability was rarely observed.

In agreement with chemical findings, ecotoxicological bioassays, carried out on seawater samples, evidenced

higher toxic effects at the quay in the ports of Apulia, whereas in Albania similar values were obtained.
The same distribution was observed for the monitored AF biocides at MDS, with higher levels at the dock. However, the pattern distribution was not always similar for the investigated AF agents, thus suggesting that the concentration changes could be due to the proximity of pollution sources in addition to the dynamics of the currents. For example, at MN both Irgarol and Diuron exhibited comparable levels in the two sampling points, while TBT showed a higher concentration in the dock. At Trani, the opposite was true: Diuron and Irgarol, to a much lesser extent, showed a spatial variability, while TBT did not. In particular, for Diuron, a much higher level was found in the centre of the harbor (TR1), while in all the other ports it was always observed the opposite. Similarly, the ecotoxicological test showed that the water sample from TR1 was more toxic than the one from the dock. Furthermore, TR1 seawater was the only one eliciting 30% toxic effect for *V. fischeri,* which usually exhibited biostimulation.
At Shengjin, water concentrations of Diuron and TBT were higher in the dock while no variation in Irgarol concentrations was observed. Conversely, higher toxic effects have been found at the centre of the port (SH1), once again suggesting the presence of not analysed toxicants.
The high concentrations of Ni and Cr found in all analyzed sediments, together with the high levels frequently determined for TBT, could contribute to the high toxic effect (> 70%) obtained for all elutriates with the *P. lividus* test, and to the even higher toxic effect (> 90%) recorded for all samples (whole sediment) with the *Vibrio fischeri* test.
Sediments should always be taken into account when assessing the quality of an aquatic ecosystem to determine the most polluted areas which demand treatment and remediation. In fact, contamination of sediments in a water body not only results in water quality deterioration but also involves a continuous and long-term risk for ecosystems and human health due to the diffusion and re-suspension in pore water and in the water column of contaminants, and to the transfer of pollutants at different trophic levels through the food chain.

## Conclusions

This work gives an example of how the combined use of diverse and complementary methodologies enables a deep and robust interpretation of data, allowing to capture different aspects of the system.
In the coastal areas of the Southern Adriatic Sea, we evaluated the occurrence of Irgarol, Diuron, TBT and some heavy metals, their effects by ecotoxicological assays, and their associated risk by ERA. The chemical characterization showed that coastal waters in Albania were less polluted than in Apulia with regard to Irgarol and Diuron. In contrast, the algal test highlighted slightly higher toxic effects in Albania than in Italy. This result suggests the presence of contaminants not taken into account by chemical analyses. With reference to sediments, instead, two hot spots were identified by the algal test in Italy, at MDS and MN.
Surprisingly, TBT was detected in all the sampling sites and in all samples (water, sediments and mussels), even at concentrations by far higher than the fixed quality standard limits, although it has been banned for years. Moreover, ERA indicated a high likelihood of adverse effects for TBT, while for Irgarol and Diuron no risk was found.
Therefore there is an urgent need for further investigation on the spread of TBT in the marine environment and the frequency of exceedence of TBT quality standards (MAC-EQS), in order to both evaluate the associated risk and to understand the possible sources of this dangerous biocide.

## Acknowledgements

This study was funded by the Italian Ministry of Foreign Affairs (MAE), Project CARISMA PGR00123 for cooperation with Albanian scientists. Our thanks to the Italian Coastal Guard, (Apulia) and the Albanian port authorities for their support during sample collection.

references


1. K. A. Dafforn, J. A. Lewis and E. L. Johnston (2011), Antifouling strategies: History and regulation, ecological impacts and mitigation, Mar. Pollut. Bull., 62, 453-465.
2. G. Di Landa, L. Parrella, S. Avagliano, G. Ansanelli, E. Maiello and C. Cremisini (2009), Assessment of the Potential Ecological Risks Posed by Antifouling Booster Biocides to the Marine Ecosystem of the Gulf of Napoli (Italy), Water. Air. Soil. Pollut., 200, 305-321.
3. L. Leitgib, J. Kálmán, K. Gruiz (2007), "Comparison of bioassays by testing whole soil and their water extract from contaminated sites", Chemosphere, 66, 428-434.
4. C. Lors, J. F. Ponge, M. Martínez Aldaya, D. Damidot (2010), "Comparison of solid-phase bioassay and eco-scores to evaluate the toxicity of contaminated soils", Environmental Pollution, 158, 2640-2647.
5. S. Manzo, F. De Nicola, F. De Luca-Picione, G. Maisto, A. Alfini (2008), "Assessment of the effects of soil PAH accumulation by a battery of ecotoxicological tests", Chemosphere, 71, 1937-1944.
6. K. O' Halloran (2006), "Toxicological considerations of contaminants in the terrestrial environmental for ecological risk assessment", Human and Ecological Risk Assessment, 12, 74-83.
7. M. T. K. Tsui, L. M. Chu (2003), "Aquatic toxicity of glyphosate based formulations: comparison between different organisms and the effects of environmental factors", Chemosphere, 52, 1189-1197.
8. K. Fent (2003), "Ecotoxicological problems associated with contaminated sites", Toxicology Letters, 140, 353-363.
9. Giuseppe Di Landa, Giuliana Ansanelli, Roberto Ciccoli, Carlo Cremisini (2006), Occurrence of antifouling paint booster biocides in selected harbors and marinas inside the Gulf of Napoli: A preliminary survey, Marine Pollution Bulletin, 52, 1541–1546.
10. R. A. Gimeno, C. Aguilar, R. M. Marce and F. Borrull (2001), Monitoring of antifouling agents in water samples by on-line solid-phase extraction–liquid chromatography–atmospheric pressure chemical ionization mass spectrometry, J. Chromatogr. A., 915, 139-147.
11. G. Gatidou, N. S. Thomaidis and J. L. Zhou (2007), Fate of Irgarol 1051, Diuron and their main metabolites in two UK marine systems after restrictions in antifouling paints, Environ. Int., 33, 70-77.
12. S. Balakrishnan, K. Takeda and H. Sakugawa (2012), Occurrence of Diuron and Irgarol in seawater, sediments and planktons of Seto Inland Sea, Japan, Geochem. J., 46, 169-177.
13. Y. Sapozhnikova, E. Wirth, K. Schiff and M. Fulton (2013), Antifouling biocides in water and sediments from California marinas, Mar. Pollut. Bull., 69, 189-194.
14. K. Carbery, R. Owen, T. Frickers, E. Otero and J. Readman (2006), Contamination of Caribbean coastal waters by the antifouling herbicide Irgarol 1051, Mar. Pollut. Bull., 52, 635-644.
15. B. Antizar-Ladislao (2008), "Environmental levels, toxicity and human exposure to tributyltin (TBT)-contaminated marine environment. A review", Environment International, 34, 292-308.
16. S.P.C. Tankéré, N.B. Price, P.J. Stantham (2000), "Mass balance of trace metals in the Adriatic Sea", Journal of Marine systems, 25, 269-286.
17. E.R. Long, L.G. Morgan, (1990), "The potential for biological effects of sediment sorbed contaminants tested in the National Status and Trends Program". NOAA Tec. Memo. NOS OMA 52. US National Oceanic and Atmospheric Administration, Seattle, Washington. pp. 175.
18. A. Kungolos, S. Hadjispyrou, M. Petala, V. Tsiridis, P. Samaras, G.P. Sakellaropoulos (2004), "Toxic properties of metals and organotin compounds and their interactions on Daphnia mangna and Vibrio fischeri", Water, Air and Soil Pollution, Focus 4, 101-110.
19. S.L.R. Melo, M. Nipper (2007), "Sediment toxicity tests using the burrowing amphipod Tiburinella viscana (Amphipoda: Platyischnopidae)". Ecotoxicology and Environmental Safety, 66, 412-420.
20. A. Sabdono (2009), "Heavy metal Levels and Their Potential Toxic Effect on Coral Galaxea fascicularis from Java Sea, Indonesia", Research Journal of Environmental Science, 3, 96-102.
21. U.S. EPA, Framework for ecological risk assessment, DC: Risk Assessment Forum, Washington, 1992.
22. L. Parrella (2014), "Ecological risk assessment and potential adverse effects posed by antifouling biocides to the salt water environment", EAI Energia, Ambiente e Innovazione, this number.
23. M. L. Miglietta, G. Rametta, G. Di Francia, S. Manzo, A. Rocco, R. Carotenuto, F. De Luca Picione, S. Buono (2011), "Characterization of nanoparticles in seawater for toxicity assessment towards aquatic organisms", Sensors and Microsystem, 91,425-429.
24. E. Prato, I. Parlapiano, F. Biandolino (2012), "Evaluation of a bioassays battery for ecotoxicological screening of marine sediments from Ionian Sea (Mediterranean Sea, Southern Italy)", Environmental Monitoring Assessment, 184, 5225-5238.
25. J. Eggleton, K. V. Thomas (2004), "A review of factors affecting the release and bioavailability of contaminants during sediment disturbance events", Environmental International, 30, 973-980.
26. J. T. F. Ashley, J. E. Baker (2009), "Hydrophobic organic contaminants in superficial sediments of Baltimore harbor: Inventories and sources", Environmental toxicology and chemistry, 18, 838-849.
27. J. C. Bowman, J. W. Readman, J. L. Zhou (2003), "Seasonal variability in the concentrations of Irgarol 1051 in Brighton marina, UK; including the impact of dredging", Marine Pollution Bulletin, 46, 444–451.
28. G. Libralato, C. Losso, A. Arizzi Novelli, M. Citron, S. Della Sala, E. Zanotto, F. Cepak, A. Volpi Ghirardini (2008), "Ecotoxicological evaluation of industrial port of Venice (Italy) sediment samples after a decontamination treatment", Environmental Pollution,156, 644-650.

NATIONAL/INTERNATIONAL LEGISLATION ON ANTIFOULING

# TBT and antifouling strategies: the Italian and European legislation

The detrimental effects on no-target marine organisms, associated with the widespread presence of TBT in the environments, called for international actions. In 2001, IMO adopted the AFS Convention, banning the application of TBT based antifouling paints after 2003 and requiring their absence from ships' hulls since 2008. The EU anticipated the AFS ban, which entered into force only in 2008, by adopting the Regulation (EC) No 782/2003, which made immediately compulsory the restrictions imposed by the AFS Convention. TBT is part of the priority hazardous substances established within the scope of the Water Framework Directive (WFD; Directive 2000/60/EC), for which environmental quality standards (EQS) have been imposed at European level. Since coordination among the existing environmental regulations is a specific requirement of the Marine Strategy Framework Directive (MSFD; Directive 2008/56/EC), the achievement of these EQS in the European seas and the absence of TBT-related effects in the marine biota would be compulsory for attaining the Good Environmental Status

*DOI: 10.12910/EAI2014-50*

*Seta Noventa, Daniela Berto, Federico Rampazzo, Malgorzata Formalewicz, Claudia Gion, Rossella Boscolo Brusà, Federica Cacciatore, Maria Teresa Berducci, Jessica Bianchi, Fulvio Onorati, Chiara Maggi*

## Introduction

Tributyltin (TBT) based antifouling paints began to be massively used worldwide since the 1970s. Following the assessment of TBT role in inducing a number of detrimental effects on aquatic organisms in the early 1980s (i.e., as immunosuppressive agent and an endocrine disruptor), this biocide underwent ever stricter regulation on the production and applications of such paints.

Organotin compounds (OTC), including TBT, were firstly synthesized in 1853, but they were found to have biocidal properties only 100 years later approximately, when they started to be used in the formulation of several commercial biocide products (i.e., fungicides, miticides, molluscicides, nematocides, ovicides, rodent repellents, wood preservatives). The massive employment of TBT in antifouling coatings was recorded between 1970s-1980s, when it almost completely replaced the most traditional biocides for its unique properties in term of efficiency, versatility and duration. Concern about the hazard generated by the growing presence of TBT in aquatic environments has involved both the scientific and civil communities since 1974, when widespread malformations and developmental abnormalities on aquatic organisms became particularly evident in areas featuring a high density of ships [1]. Among the wide range of biological effects recorded in that period, two had a big resonance because of their significant environmental and economical implications: the shell

**Seta Noventa, Daniela Berto, Federico Rampazzo, Malgorzata Formalewicz, Claudia Gion, Rossella Boscolo Brusà, Federica Cacciatore, Maria Teresa Berducci, Jessica Bianchi, Fulvio Onorati, Chiara Maggi**
*ISPRA*

thickening in common oysters (Crassostrea sp.) farmed along the European Atlantic coast, with a consequent decrease in their market value [2; 3]; the incoming of sexual malformation in wild populations of several gastropod species (i.e., superimposition of male sexual organs in females, a phenomenon known as imposex), often leading to sterility and population decline [4]. The regulation of this biocide at the national and international levels became a priority when several research studies reported the existence of a direct relationship between these abnormalities and the presence of TBT in the aquatic compartments. It was ascertained, in fact, that a number of impacts on many marine species were induced by concentrations even lower that 1 ng/L (approximately 2-3 orders of magnitude lower than those usually recorded in hot-spot areas such as ports, dockyards, marina, and 1-2 orders lower than the levels assessed in coastal areas) [5; 6].

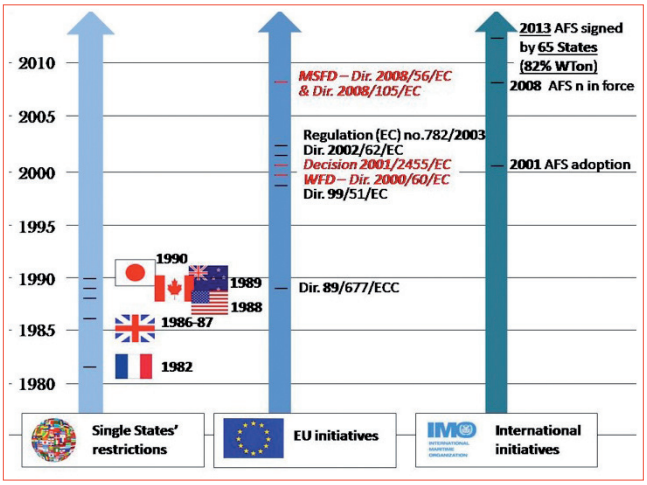



**FIGURE 1** Timeline of the main regulatory initiatives taken at level of single States, European Union and international community (IMO International Maritime Organization) since the early 1980s

## Initial counteractions toward TBT

The first regulatory initiatives were individually taken by single states, as outlined in Figure 1.

France – whose oyster farms located along the Atlantic coast experienced high economic damage due to reduced oyster spatfall, larval development abnormalities and shell deformations between 1975-1982 – was the first to take actions to limit TBT release in the environment by regulating the use, the formulation and the public sale of TBT-containing paints. On 19th January 1982, the French Ministry of the Environment imposed limitations on the application of these coatings on boats less than 25m long. Initially, the prohibition referred to products containing over 3% of biocide and was limited to areas with intensive oyster culture along the English Channel and the Atlantic coast. On 14th September 1982, the ban was extended to the whole French coast. These provisions, which included waivers for paints containing less than 3%, boats longer than 25m and all kinds of submerged structures (light alloys, nets, traps, etc), remained in force almost through the 1980s, when the problem started to be addressed at community level. A few years later, on the other side of the English Channel, the UK Government forbade the application of TBT-coatings on the hulls of small vessels (1985), and established the threshold level at 20 ng $TBT^+$/L (1986), which was reduced to 2 ng $TBT^+$/L on the following year. Similar restrictions (i.e., ban for boats less than 25m long, maximum leaching rate, percentage of TBT content) were also set outside Europe, as in USA (US Antifouling Paint Control Act of 1988), Canada (under the Canadian Pest Control Act, 1989), Australia (1989), Japan (1990), New Zealand (1993) [7; 1; 8].

The first initiative taken by the European Authorities was adopting the Directive 89/677/EEC, which modified the communitarian framework on dangerous substances and preparations (Directive 76/769/EEC) by introducing organostannic compounds within the list of dangerous substances subjected to restrictions (Annex I) (Figure 2).

The Directive 89/677/EEC unified the regulation of TBT-based antifouling paints at European level by banning their use on ships less than 25m long, submerged facilities for fish and shellfish farming and immersed structures; furthermore the sale was reserved to professional users. In the 1999, with Directive 1999/51/EC, this discipline was further restricted by banning all TBT based-free association -paints and by prohibiting the use of TBT in inland waters.

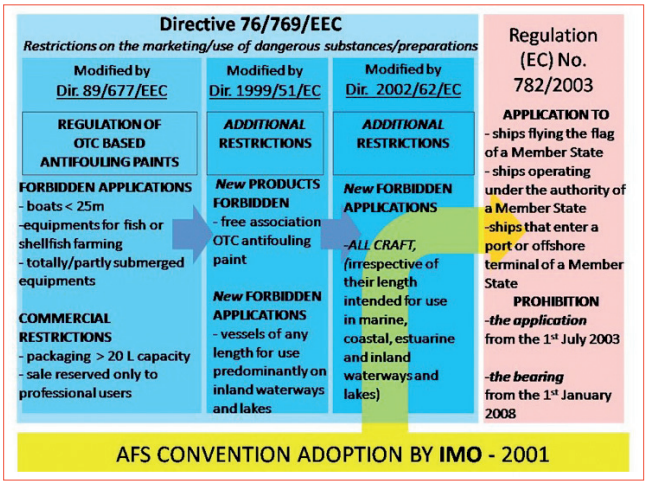



**FIGURE 1** Timeline of the EU regulation of OTC based-antifouling paints

Thanks to the adoption of these Directives by France as well as by the other EU members (in Italy with the D.M. 29/07/1994 and D.M. 13/12/1999), during the 1990s the release of TBT into European marine waters was restrained but not arrested. In fact, the adopted resolutions allowed the application of TBT-based antifouling products, having mean leaching rate lower than 4 μg $cm^{-2}d^{-1}$, on the largest ships (> 25 m long).

## IMO's (International Maritime Organization) Initiative

However it was soon clear that the high toxicological potential of TBT–inducing toxicological effects on the most sensitive aquatic organisms at concentrations less than 1ng/L, [5] made the national individual actions insufficient, to the extent that more severe restrictions, crossing the national boundaries, were indispensable. In 2001, IMO (International Maritime Organization) developed the *Convention on the Control of Harmful Anti-fouling System on Ships*, noted as AFS Convention, banning: 1) new applications of TBT-based antifouling paints from 1st January 2003, and 2) the presence of these coatings on ship surfaces and submerged structures from 1st January 2008. These prescriptions, addressed toward all size-boats flagged or working within the boundaries of signatory countries, couldn't get immediately into force, having to be ratified by at least 25 States covering the 25% of the world gross tonnage.

At first, the EU reacted to the IMO directions with the Directive 2002/62/EC, which introduced as a novelty the prohibition of using antifouling preparations based on organostannic compounds on all kinds of crafts, regardless of their length. One year later, the EU decided to definitively solve the problem in its area of jurisdiction and to adopt an anticipatory action of the AFS prescriptions within the community boundaries: Regulation (EC) No. 782/2003 was adopted, which imposed the immediate respect of the AFS prescription to EU-flagged vessels as well as to all ships approaching the ports and offshore structures of Member States.

Outside the EU boundaries, the global ban of TBT antifouling paints was achieved on 17th September 2008, when the AFS Convention was signed by 25 States, overall representing the 38.11% of the world merchant tonnage. From that date on, the number of countries adopting the AFS prescription has continuously increased and to date 65 States, covering 82.25% of the world tonnage, have banned these products in their territorial waters by signing the international convention[1].

## EU directives on TBT

The progressive adoption of even stricter regulations on TBT antifouling systems has led to the progressive decline of TBT concentrations in aquatic environments since the end of the 1980s. According to the literature, TBT levels have diminished in all marine compartments, especially in water and biota [9, and references therein). Similarly, a progressively ecological recovery worldwide has been recorded at different levels of biological organization (e.g., oyster cultures in France and Southern England, dogwhelks population recovery, decline of imposex, macro-infaunal and epifaunal communities recovery) [10; 11; 7; 8].

Despite the achievement of an almost complete ban on TBT used as biocide, there is scientific agreement about the need to keep monitoring OTC levels in aquatic

environments. This is because OTC are persistent environmental pollutants tending to biomagnificate along the food chain [6;12], and concentrations able to induce harm for ecosystem and human health are still found. In particular, high OTC concentrations are still present in sediments, especially in hot spots areas such as ship channels, ports, harbours and marinas [13;14], and it is ascertained that they are acting as secondary source of pollution for the surrounding area [15].

In the EU, TBT is one of the aquatic pollutant considered within the European Water Framework Directive (WFD; Directive 2000/60/EC;). This Directive is aimed at achieving, by 2015, the *good environmental status of waters* by the attainment of both ecological and chemical quality objectives. Pursuant art. 16, the good chemical status is met when concentrations of specific substances, considered priority because presenting significant risk to or via the aquatic environment, do not exceed the EQS established in Annex IX and under Article 16 [7]. TBT is part of a subset of this group, priority hazardous substances, the discharging, emissions and losses of which have to cease or phase-out; hence, stricter objectives have been established. The complete list of *priority* and *priority hazardous* substances is provided for within the Decision n. 2455/2001/EC, whereas Directive 2008/105/EC (EQS Directive) sets Environmental Quality Standards (EQS) in the water matrix. Annex I fixes the limit of 0.0002 μg $TBT^+$/L as annual average concentration, whereas 0.0015 μg $TBT^+$/L as maximum permitted concentration. Whilst the WFD is focused on water concentrations for tracing the chemical status and the quality improvements – given thanks to the undertaken measures – the European Authorities allow Member States to establish EQS for sediment and/or biota at the national level and to apply those EQS instead of the EQS for water (art. 16 [7] of the WFD; art. 3 of the Directive 2008/105/EC). In Italy, the Ministerial Decree 260/2010 defines national quality standards in sediments of marine and transitional water for several priority substances including TBT establishing the EQS value of 5μg $TBT^+$/kg.

More recently, the protection of European marine ecosystems from the detrimental effects of the most harmful chemical contaminants, including TBT, has been added within the scopes of the Marine Strategy Framework Directive (MSFD; Directive 2008/56/EC). MSFD establishes a framework for community action in the field of marine environmental policy, having the final aim of promoting sustainable use of EU seas and conserving marine ecosystems. The overall goal is the achievement or maintenance of the *Good Environmental Status* (GES) of the Community's marine environments by 2020, by applying an ecosystem-based approach to the management of human activities, marine goods and services. With the MSFD, the EU asked to each Member State to concretely develop a marine strategy of its own for its marine waters and undertake a program of measures to achieve GES considering both the specificities to its own waters as well as the overall perspective of the marine region/subregion it belongs to. To ensure consistency and allow for comparison within/between marine regions/subregions, the European Commission stated a set of eleven Descriptors of the extent to which GES is being achieved. The issue of marine pollution is specifically faced in Descriptor 8, stating that *"Concentrations of contaminants must be at levels not giving rise to pollution effects".* As for all other Descriptors, within Decision 2010/477/EU distinctive technical features (*criteria*) are tagged together with a list of related *indicators*, which make the criteria operational and allow subsequent progress. Basically, Member States have to trace the progress status towards contamination levels not compromising the achievement GES, by focusing on: Criteria 8.1) concentration of contaminants, ensuring the comparability with the assessments under Directive 2000/60/EC (Indicator 8.1.1), and Criteria 8.2) effects of contaminants for which the cause/effect relationship has been established and needs to be monitored (Indicator 8.2.1), and physical impact of acute pollution events on biota (Indicator 8.2.2).

TBT fate in marine environments, already considered within the WFD and daughter Directives, is among the objectives which have to be considered for the achievement of Descriptor 8-GES of MSFD. In particular, as regards the monitoring of pollutants effects (indicator 8.2.1), the measurement of imposex development in wild gastropod populations is an effective candidate as bio-tool to be included within the monitoring programs. In fact, even if it is likely that

other toxicants might be able to induce imposex in marine snails by disrupting the hormonal system [16], it was largely demonstrated that TBT is the primary pollutant responsible for this effect, and is therefore a mature and valuable technique for assessing the environmental significance of TBT contamination. The analysis of imposex in marine gastropods species is already part of the monitoring protocols in use within some regional convention areas. In particular, since 2003 the evaluation of imposex in common whelks (Nucella, Littorina, Buccinum, Neptunea) is a mandatory commitment of OSPAR Contracting Parties (Convention for the Protection of the marine Environment of the North-East Atlantic) under the CEMP (Co-ordinated Environmental Monitoring Programme). In fact, OSPAR defined imposex in whelks as an Ecological Quality Element and, in collaboration with ICES, established the associated Ecological Quality Objective (EcoQO) as a reference for assessing the achievement of the desired level of marine quality (JAMP Guidelines for contaminant specific biological effects monitoring - OSPAR Agreement 2008-9; Provisional JAMP Assessment Criteria for TBT - Specific Biological Effects - *Reference Number 2004-15*). Also within the Baltic area (HELCOM - Helsinki Commission), during the recent CORESET expert workshop (CORESET HS 5/2013; <http://meeting.helcom.fi/web/guest/home>) the monitoring relevance of imposex was stated as a core indicator of TBT within the Baltic Sea Action Plan, at least for the next decade. By considering that the MSFD wishes for coordination among the existing Sea Conventions, whenever practical and appropriate, it is likely that this already developed bio-tool will be taken into account.



notes

1. <http://www.imo.org/About/Conventions/StatusOfConventions/Pages/Default.aspx>; updated 31st July 2013.

references


1. Hoch M. 2001. Organotin compounds in the environment: an overview. Applied Geochemistry 16, 719–43
2. Alzieu C, Héral M, Thibaud Y, Dardignac M J, Feuillet M. 1981-82. Influence des peintures antisallisures à base d'organostanniques sur la calcification de la coquille de l'huître Crassostrea gigas. Revue de travaux de l'Institut des peches Maritimes 45(2), 101-116
3. Alzieu C. 1991. Environmental problems caused by TBT in France: assessment, regulations, prospects. Marine Environmental Research 32, 7-17
4. Bryan GW, Gibbs PE, Burt GR, Hummerstone LG. 1986. The decline of the gastropod Nucella lapillus around South-West England: evidence for the effects of tributyltin from anti-fouling paints. Journal of Marine Biological Association UK 66, 611-640
5. Alzieu C. 1989. L'étain et les organoétains en milieu marine: Biogéochimie et Ecotoxicologie. Rapports scientific and technique de l'INFREMER 17, 1-93
6. EPA, Ambient Aquatic Life Water Quality Criteria for Tributyltin (TBT) – Draft. EPA-822-B-02-001, December 2002, 155 p.
7. Champ MA. 2000. A review of organotin strategies, pending actions, related coast and benefits. The Science of Total Environment 258, 21-71
8. Alzieu C. 1998. Tributyltin: case study of a chronic contaminant in the coastal environment. Ocean & Coastal Management 40, 23-36
9. Evans SM, Leksono T, And Mckinnell PD. 1995. Tributyltin Pollution: a diminishing problem following legislation limiting the use of TBT-based anti-fouling paints. Marine Pollution Bulletin 30(1), 14-21
10. Smith R, Bolam SG, Rees HL, Mason C. 2008. Macrofaunal recovery following TBT ban. Environmental Monitoring Assessment 136, 245-256
11. Rees HL, Waldock R, Matthiessen P, Pendle MA. 2001. Improvents in the epifauna of the Crouch Estuary (UK) following the decline in TBT concentrations. Marine Pollution Bulletin 42(2), 137-144
12. Fortibuoni T, Noventa S, Rampazzo F, Gion C, Formalewicz M, Berto D, Raicevich S. 2013. Evidence of butyltin biomagnification along the northern Adriatic food-web (Mediterranean Sea) elucidated by stable isotope ratios. Environmental Science and Technology 47, 3370–3377
13. Berto D, Giani M, Boscolo R, Covelli S, Giovanardi O, Massironi M, Grassia L. 2007. Organotins (TBT and DBT) in water, sediments and gastropods of the southern Venice lagoon (Italy). Marine Pollution Bullettin 55(10-12), 425-35
14. Sousa A, Matsudaira C, Takahashi S, Tanabe S, Barroso C. 2007. Integrative assessment of organotin contamination in a southern European estuarine system (Ria de Aveiro, NW Portugal): tracking temporal trends in order to evaluate the effectiveness of the EU ban. Marine Pollution Bullettin 54 (2007), 1645-1653
15. Maggi C, Ausili A, Boscolo R, Cacciatore F, Bonometto A, Cornello M, Berto D. 2012. Sediment and biota in trend monitoring of contaminants in transitional waters. Trends in Analytical Chemistry, 36: 82-91.
16. Maran C, Centanni E, Pellizzato F, Pavoni B. 2006. Organochlorine compounds (polychlorinated biphenyls and pesticides) and polycyclic aromatic hydrocarbons in populations of Hexaplex trunculus affected by imposex in the lagoon of Venice, Italy. Environmental Toxicology and Chemistry 25( 2), 486–495

Rd

legislative references


- European Council Directive (76/769/EEC) of 27 July 1976 on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances and preparations. Official Journal L 262, 201. 27/9/1976.
- European Council Directive (89/677/EEC) of 21 December 1989 amending for the eighth time Directive 76/769/EEC on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances and preparations. Official Journal L 398. 30/12/1989.
- European Commission Directive (1999/51/EC) of 26 May 1999 adapting to technical progress for the fifth time Annex I to Council Directive 76/769/EEC on the approximations of the laws, regulations, and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances and preparations (tin, PCP and cadmium). Official Journal L 142. 05/06/1999.
- European Commission Directive (2002/62/EC) of 9 July 2002 adapting to technical progress for the ninth time Annex I to Council Directive 76/769/EEC on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances and preparations (organostannic compounds). Official Journal L 183/58. 12/7/2002.
- European Parliament and Council Regulation (No. 782/2003) of 4 November 2003 on the prohibition of the use of organotin compounds on ships. Official Journal L 115/1. 9/5/2003.
- IMO (International Maritime Organisation), 2001. International convention on the control of harmful antifouling systems on ships. International Maritime Organisation, London UK.
- European Parliament and Council Directive (2000/60/EC) of 23 October 2000 establishing a framework for Community action in the field of water policy. Official Journal L 327/1. 22/12/2000.
- European Parliament and of the Council Decision (No. 2455/2001/EC) of the of 20 November 2001 establishing the list of priority substances in the field of water policy and amending Directive 2000/60/EC. Official Journal L 3. 15/12/2001.
- European Parliament and of the Council Directive (2008/105/EC) of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council. Official Journal L 348/84. 24/12/2008.
- European Parliament and of the Council Directive (2008/56/EC) of 17 June 2008 establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive). Official Journal L 164/19. 25/6/2008.
- European Commission Decision (2010/477/EU) of 1 September 2010 on criteria and methodological standards on good environmental status of marine waters. Official Journal L 232/14. 2/9/2010.
- Decreto Ministeriale 29 luglio 1994. Attuazione delle direttive CEE numeri 89/677, 91/173, 91/338 e 91/339 recanti, rispettivamente, l'ottava, la nona, la decima e l'undicesima modifica della direttiva CEE n. 76/769 per il ravvicinamento delle disposizioni legislative, regolamentari ed amministrative degli Stati membri relative alle restrizioni in materia di immissione sul mercato e di uso di talune sostanze e preparati pericolosi, ai sensi dell'art. 27 della legge 22 febbraio 1994, n. 146 (Gazzetta ufficiale 13 settembre 1994, n. 214 e 10 dicembre 1994, n. 288, suppl. ord.).
- Decreto Ministeriale 13 dicembre 1999 (D.M. 13-12-1999/1999). Recepimento delle direttive 99/43/CE recanti modifiche alla direttiva 76/769/CEE del Consiglio del 27 luglio 1976 relativa alle restrizioni in materia di immissione sul mercato e di uso di talune sostanze e preparati pericolosi (Gazzetta ufficiale n. 67 del 21/03/2000)
- Decreto del Ministero dell'Ambiente e della Tutela del Territorio e del Mare (260/2010) del 8 novembre 2010. Regolamento recante i criteri tecnici per la classificazione dello stato dei corpi idrici superficiali, per la modifica delle norme tecniche del decreto legislativo 3 aprile 2006, n. 152, recante norme in materia ambientale, predisposto ai sensi dell'articolo 75, comma 3, del medesimo decreto legislativo (Gazzetta ufficiale n. 30 del 7/2/2011).


NATIONAL/INTERNATIONAL LEGISLATION ON ANTIFOULING

# Managing of antifouling paints following the new Biocidal Product Regulation (BPR): a new running for products affecting the marine environment

Biocidal products in antifouling paints, used for protecting boat hulls from the unwanted accumulation of micro-organisms, plants, and animals on artificial surfaces (marine biological fouling), constitute a potential risk for the marine environment because of the presence, among other potentially toxic components, of organic compounds in their formulation, acting as biocide.
Due to their intrinsic properties and uses, biocidal products may pose health risks and be harmful to the environment. It is therefore crucial to ensure that only safe biocidal products are placed on the market. To this aim in the latest years several European directives and regulations have come into force

*DOI: 10.12910/EAI2014-51*

*Francesca Carfì, Roberto Carletti, Stefano Castelli, Federica Ceccarelli*

## Antifouling paints, utilities and uses

Boats spend a large proportion of their working life partly submerged in water. As with all objects subject to long periods of time in water, boat hulls are subject to colonization by the many micro-organisms which inhabit the aquatic environment. This colonization is known as *"fouling"*. Boat hulls are susceptible to all types of fouling, which can cause increased drag on the hull when it is not attended to, leading to increased fuel consumption, and eventually significant damage to the boat structure. It is, therefore, necessary to apply some coatings to protect the hull against infestation. These coatings are generally known as antifouling paints and are applied to the hull at regular intervals.
Antifouling paints usually contain a biocide, or toxin, held within the structure of the paint [1]. The coating is designed to leach biocide slowly into the marine environment, preventing any organism from adhering to the paint by poisoning the settling organisms.
The nature of a biocide is such that it can potentially have harmful effects, not only on the fouling organism it

**Francesca Carfì, Stefano Castelli**
*ENEA, Technical Unit for Environmental Technologies*

**Roberto Carletti**
*ENEA, Technical Unit for the Development of Application of Radiation*

**Federica Ceccarelli**
*Technical expert on biocides*

| Active substances | International Chemical Identification | N. CAS | CLP Classification |
|---|---|---|---|
| Chlorothalonil | tetrachloroisophthalonitrile | 1897-45-6 | Skin Sens. 1; Eye Dam. 1; Acute Tox. 2; STOT SE 3; Carc.2; Aquatic Acute 1; Aquatic Chronic 1(*) |
| Dichlofluanid | N-dichlorofluoromethylthio-N',N'-dimethyl-N-phenylsulfamide | 1085-98-9 | Skin Sens. 1; Eye Irrit. 2 Acute Tox. 4; Aquatic Acute 1 (*) |
| Diuron | 3-(3,4-dichlorophenyl)-1,1-dimethylurea | 330-54-1 | Acute Tox. 4; Carc. 2 STOT RE 2; Aquatic Acute 1; Aquatic Chronic 1 (*) |
| Irgarol 1051 | N'-tert-butyl-N-cyclopropyl-6-(methylthio)-1,3,5-triazine-2,4-diamine | 28159-98-0 | Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1 |
| Maneb | manganese ethylenebis(dithiocarbamate) (polymeric) | 12427-38-2 | Skin Sens. 1Eye Irrit. 2 Acute Tox. 4 Repr. 2 Aquatic Acute 1 Aquatic Chronic 1 (*) |
| Sea-Nine211 | 4,5-dichloro-2-octyl-2H-isothiazol-3-one | 64359-81-5 | Acute Tox. 4 Skin Corr. 1B Skin Sens. 1 Acute Tox. 3 Eye Dam. 1 Aquatic Acute 1 |
| TCMS piridina | methyl-2,3,5,6-tetrachloro-4-pyridylsulphone | 13108-52-6 | Acute Tox. 4 Skin Sens. 1 Eye Irrit. 2 (*) |
| Thiram | tetramethylthiuram disulphide | 137-26-8 | Acute Tox. 4 Skin Irrit. 2 Skin Sens. 1 Eye Irrit. 2 Acute Tox. 4 STOT RE 2 Aquatic Acute 1, Aquatic Chronic 1 (*) |
| pyrithione zinc | pyrithione zinc | 13463-41-7 | Acute Tox. 3 Eye Dam. 1 Acute Tox. 3 Aquatic Acute 1 |
| fenoprop | 2(2,4,5trichlorophenoxy)propionic acid | 93-72-1 | Acute Tox. 4 Skin Irrit. 2 Aquatic Acute 1 Aquatic Chronic 1 (*) |
| Zineb | Zinc,ethylenebis(dithiocarbamate) (polymeric) | 12122-67-7 | Skin Sens. 1 STOT SE 3 (*) |
| Ziram | zinc bis dimethyldithiocarbamate | 137-30-4 | Acute Tox. 4 Skin Sens. 1 Eye Dam. 1 Acute Tox. 2 STOT SE 3 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1 (*) |

(*) Harmonised classification, Annex VI of Regulation (EC) No 1272/2008 (CLP Regulation)

**TABLE 1** Active substances most commonly used in antifouling paints

is designed to deter, but also on other marine life forms unconnected with fouling activity.

Organotin compounds (TBT or tributyltin) replaced the use of cuprous oxide ($Cu_2O$), giving better performance antifouling paints and increased service life. However, it became evident in the 1980s that their continued use was causing severe damage to shellfish communities and, in particular, dog whelk populations [2]. In fact, TBT

causes reproductive anomalies and population effects in certain species of marine snails at concentrations in the parts-per-trillion range, and has been implicated in endocrine effects on other organisms [3,4]. TBT is associated with immune suppression and other adverse effects in marine species, it is slow to degrade, and is very persistent in sediments, where many affected species live and feed [5].

This resulted in the implementation, in 1987, of a Europe-wide ban on the use of TBT in antifouling paints on boats. TBT-free antifouling paints have been developed since 1990. The ban on TBT resulted in a shift back to paints containing copper as the main biocide. Copper is included in antifouling paints most commonly as cuprous oxide, but also as cuprous thiocyanate and metallic copper powder. It is widely felt that although the performance of copper biocides cannot approach that of TBT, they remain the most effective alternative [6]. Currently there is a great deal of research into alternative forms of biocides, particularly those of organic origin. These, however, tend to be less universally effective than TBT and, in particular, may deter only specific types of fouling organisms. As a result of these 'species-specific' characteristics, antifouling paints on the market today contain a mixture of biocides in order to be effective against most of marine micro-organisms.

The most widely used biocides in paints today are shown in Table 1, with their classification according to Regulation (EC) No. 1272/2008/EU (CLP) [7].

## Overview of legislation on antifouling paints

After an initial phase of national legislative measures to regulate the use of biocidal products in antifouling paints, in 2001 an action phase at European level began with the "Convention on the Control of Harmful Antifouling System on Ships" (AFS Convention) that prohibited the use of harmful organotins in antifouling paints used on ships, and established a mechanism to prevent against the potential future use of other harmful substances in antifouling systems.

Later Regulation (EC) 782/2003 [8] on the prohibition of organotin compounds on ships, imposed Member States the same deadlines and conditions of the AFS Convention; in this way also the Member States that had not ratified the Convention were forced to comply with the European legislation.

At the same time, the environmental legislation enacted in the same years had an impact on the use of organotin compounds in antifouling paints, particularly TBT. Directive 2000/60/EC (EU Framework Water Directive) [9] provided for the establishment of a priority list of substances as a basis for shared actions aimed at reducing or eliminating discharges and releases of hazardous pollutants in the aquatic environment (Decision 2455/2001/EC [10]) and the establishment of environmental quality standards (EQS) for the substances in surface waters (Directive 2008/105/EC [11]).

TBT was included among the priority hazardous substances of Decision 2455/2001/EC and its environmental quality standards were included in Annex I of Directive 2008/105/EC. The European environmental legislation in the first instance applied to surface water was then extended to the marine environment with Marine Strategy Framework Directive (Directive 2008/56/EC [12]), which aims to achieve good environmental status of the European seas by 2020.

Until 1 September, 2013, Biocidal Product Directive (BPD) 98/8/EC [13], concerning the placing of biocidal products on the market, was applied to antifouling paints. Among its objectives, this Directive had the establishment of a list of active substances which may be used in biocidal products, authorizing the placing on the market of biocidal products in the Member States and the mutual recognition of authorizations within the European Community. Starting from 1 September, 2013, the BPD has been repealed by the new Reg. (UE) n. 528/2012.

The Biocidal Products Regulation (BPR, Regulation (EU) 528/2012) [14] concerns the placing on the market and use of biocidal products, which are used to protect humans, animals, materials or articles against harmful organisms like pests or bacteria, by the action of the active substances contained in the biocidal product.

This regulation is aimed at improving the functioning of the biocidal products market in the EU, while ensuring a high level of protection for humans and the environment. The new Regulation will also remedy a number of weaknesses that were identified during the

11 years of implementation of Directive 98/8/EC.
In fact, the new text simplifies and streamlines the requirements for approving active substances and authorizing products. The new provisions will also reduce animal testing by making data sharing compulsory and encouraging a more flexible and intelligent approach to testing. A dedicated IT platform (the Register for Biocidal Products) will be used for submitting applications as well as recording decisions and disseminating information to the public [15].
The text is also a major breakthrough for the internal market with the creation of a Union authorisation of biocidal products, which will allow industry to directly place their products on the entire Union market.
The text of the BPR was adopted on 22nd May, 2012, and it entered into operation on 1st September, 2013, with a transitional period for certain provisions, repealing the Biocidal Products Directive (Directive 98/8/EC).

## Definitions

The BPR (art. 3.1.a) defines active substances and biocidal products as follows:
**"Active substance"** is *"a substance or a micro-organism that has an action on or against harmful organisms."*
**"Biocidal product"** is:
- *any substance or mixture, in the form in which it is supplied to the user, consisting of, containing or generating one or more active substances, with the intention of destroying, deterring, rendering harmless, preventing the action of, or otherwise exerting a controlling effect on, any harmful organism by any means other than mere physical or mechanical action;*
- *any substance or mixture, generated from substances or mixtures which do not themselves fall under the first indent, to be used with the intention of destroying, deterring, rendering harmless, preventing the action of, or otherwise exerting a controlling effect on, any harmful organism by any means other than mere physical or mechanical action.*

The new Regulation on biocidal products contains provisions, which apply not only to biocidal products but also to all articles which have been treated or incorporate a biocidal product. According to article 3.1.l, a treated article is defined as *"any substance, mixture or article which has been treated with, or intentionally incorporates, one or more biocidal products".*
According to the regulation, articles can only be treated with biocidal products containing active substances approved in the EU. This is a change from the BPD (repealed by the BPR from 1st September, 2013), where articles imported from third countries could be treated with substances not approved in the EU – such as, wood treated with arsenic, and sofas and shoes containing DMF.

## Differences between old and new legislation

The aim of the new Regulation is to improve the functioning of the internal market in biocidal products whilst ensuring a high level of environmental and human health protection.
Furthermore, the new Regulation aims to simplify the approval of active substances and authorisation of biocidal products and introduces timelines for Member State evaluations, opinion-forming and decision-making. It also promotes the reduction of animal testing by introducing mandatory data sharing obligations and encouraging the use of alternative testing methods.
As in the previous directive, the approval of active substances takes place at Union level and the subsequent authorisation of the biocidal products at Member State level.
This authorisation can be extended to other Member States by mutual recognition. However, the new regulation also provides applicants with the possibility of a new type of authorisation at Union level (Union authorisation – art. 3.1.n) for biocidal products which have similar conditions of use, with the exception of biocidal products that contain active substances that fall under Article 5 (exclusion criteria) and those of some product- types – e.g., rodenticides, avicides, piscicides, control of other vertebrates and antifouling products (art. 42.1).
Before they are put on the market, all biocidal products must be authorized and all the active substances present in the biocidal products must be previously approved.
Compared to the previous regulatory framework, the main differences concern greater safety of products on the market, the simplification of the authorization procedure and greater speed in the marketing.

In terms of safety, controls are strengthened to prevent biocides from being harmful to humans and the environment; most hazardous substances, such as carcinogens, mutagens or toxic to reproduction will be prohibited in principle; specific rules for security checks are provided on products marketed in nanoform, for which there is also a labeling requirement.
In terms of simplification, the existing authorisation procedures are simplified, except for biocidal products containing nanomaterials; the sale will be made more quickly by setting new deadlines for submission of authorization applications; mutual recognition between Member States becomes easier.

## The Revision Programme

According to the BPD, active substances in biocidal products, placed on the EU market prior to 14th May, 2000 (all notified active substances), had to be reviewed in a Community program to be carried out within 14 years. If, after the review, they were accepted for use in biocidal products in specific product types, they would be included in ANNEX I, IA or IB to the BPD.
The first phase of the review program was established by Commission Regulation (EC) 1896/2000 [16], which provided for the identification or notification by producers and formulators to the European Chemicals Bureau of all existing active substances before 28th March, 2002. The second phase of the review program was established by Commission Regulation (EC) 2032/2003 [17]. This Regulation has been amended by Commission Regulation (EC) 1048/2005 [18] and by Commission Regulation (EC) 1849/2006 [19].
On 4th December, 2007, the Commission adopted Regulation (EC) No 1451/2007 [20], which repeals Regulation (EC) No 2032/2003 and entered into force on 31st December, 2007. The Regulation (EC) 1451/2007 regards the second phase of the 10-year work program established by article 16.2 of BPD.

## Approval stage of active substances

Most of the active substances used in antifouling paints are still included in the review program of the BPD. At the moment only one substance (dichlofluanid - Dir. 2007/20/CE) has been approved. Three substances were banned for this use: Chlorothalonil, Diuron and Ziram. Some other substances are under evaluation for this use.
The next antifouling active substances (biocidal Product Types 21 – PT21) [21] for which a decision is expected to be taken are Zineb, DCOIT and copper pyrithione.
For this reason the European Commission's DG Environment is studying a work programme finalized to decide which active substances could be used in antifouling paints. To this aim the following actions are proposed:

- To approve all active substances in antifouling products (PT21) on the basis on the same generic conditions. Additional specific conditions could be added on a case-by-case basis (for instance, if the substance is a skin sensitizer, the standard paragraph related to treated articles should be added).
- To establish the same expiry date of approval for all existing active substances (ASs) placed on the market for PT21, in order to evaluate the renewal of their approval at the same time.
- To flag specific concerns related to each individual active substance in the assessment report.
- Furthermore it is proposed to have a common date of expiry of the approval: this date could be set on 31/12/2025.

In order to respect this date and then to have a clear situation on which active substances can be used safely in antifouling paints, the following time schedule has been proposed (Figure 1).
In view of this work programme, authorisations for antifouling paints will be subject to the following conditions:

(1) To manage the risks for industrial/professional users when they apply the biocidal product (BP), safe operational procedures and appropriate organizational measures shall be established. Where exposure cannot be reduced to an acceptable level by other means, products shall be used with appropriate personal protective equipment.
(2) Considering that antifouling products are very specific products, and considering good practices of use of biocidal products, the Commission's services could consider acceptable to impose that

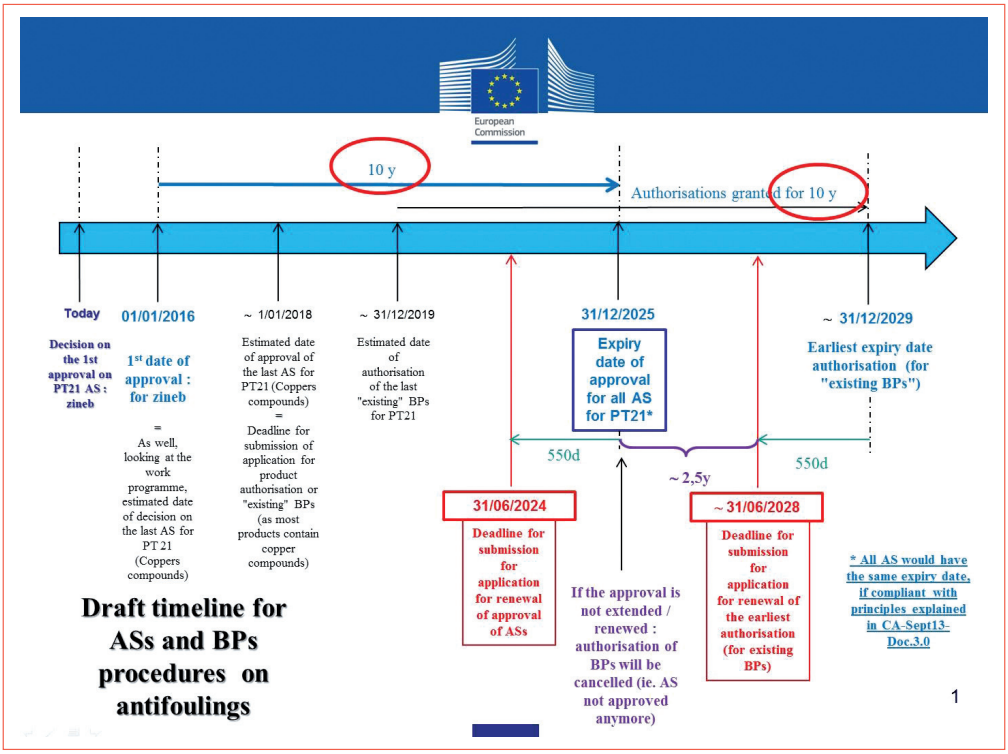



**FIGURE 1** Time schedule for active substances and biocidal products on antifoulings

all products for use by non-professionals are sold with the appropriate protective gloves, and give indications on whether other PPE shall be used. Therefore, Products authorised for non-professionals user shall be sold with the appropriate protective gloves. Labels and, where provided, instructions for use shall indicate whether other personal protective equipments shall be used.

(3) To manage the risks for the environment (soil organisms, groundwater, and run-off to surface water, etc…) during the application, maintenance and repair activities when they apply the biocidal product labels and, where provided, safety data sheets of products authorised shall indicate that application, maintenance and repair activities shall be conducted within a contained area and on impermeable hard standing with binding to prevent against direct leaching and minimize emissions to the environment, and that any leaching or waste containing [the substance] shall be collected for reuse or disposal.

(4) To manage the potential uses where there might be the need to settle or review existing MRLs (fishnets coatings, small professional boats used in mussels/oyster production, paints used to cover artificial ponds for growing fish/seafood products, etc…), for products that may lead to residues in food or feed, the need to set new or to amend existing maximum residue levels (MRLs) in accordance with Reg. (EC) No 470/2009 [22] or Reg. (EC) No 396/2005 [23] shall be verified, and any appropriate risk mitigation measures shall be taken to ensure that the applicable MRLs are not exceeded.

As far as possible, decisions of authorisation of antifouling products should be harmonised. Nevertheless, Member States could derogate from the mutual recognition and decide to refuse to grant, or restrict the use of antifouling products at the regional/

local level, in accordance with Article 37 of the BPR, for instance to ban the use in sensitive areas, specific marinas, specific coastal zones etc.
It has to be noted that boats are "treated articles", as they have been treated with a biocidal product (i.e., antifouling paint). Boats that are *placed on the market* (i.e., the first made available on the EU market according to Article 3(1)(j)) have to comply with provisions of the BPR. So have fishnets treated with an antifouling, or other aquaculture equipments.

## Conclusions

To date it is not possible to avoid the use of antifouling paints. The deadlines foreseen by the European Commission still imply a long use of these products, with consequences on the environment. The new EU regulation on biocides will have the result of banning some products, introducing some measures for increasing human health protection and some geographical restrictions, but antifouling paints containing biocidal products will continue to be sold for decades. This environmental and safety issue cannot be solved only by regulating the substances, but also by meaningful R&D outcomes.
At present, mitigating measures could be represented by silicone-based antifouling paints, which work by preventing or greatly reducing the adhesion of marine "fouling" to boat hulls. They are used from time to time on immersed parts of some military ships and on submarines where metal-free paints are needed. Recently these silicon based paints have been used on immersed parts of great freight ships.
Other developments could arise from the use of paints containing biomolecules with antifouling properties and from antifouling action developed by physical means, as reported in a recent communication of an Italian company, describing the antifouling action of $CO_2$ bubbles developed on immersed parts by enzyme-based paints [24].



references


[1] Nuovi biocidi per le vernici antivegetative – I. Mazziotti, P. Massanisso, C. Cremisini, S. Chiavarini, M. Fantini, R. Morabito - Energia, Ambiente e Innovazione, 5/2005.
[2] Effects of TBT on Dogwhelks at Marine Inlets and Marinas – C.J. Loretto, S.V. Proud - Marine Conservation Society, 1993
[3] Concise International Chemical Assessment Documents, No.13, Triphenyltin compounds, IPCS (1999), World Health Organization, Geneva.
[4] Concise International Chemical Assessment Documents, No. 14, Tributyltin oxide, IPCS (1999), World Health Organization, Geneva.
[5] Persistence and fate of tributyl tin in aquatic ecosystems – P.F. Seligman, R.J. Maguire, R.F. Lee, K.R. Hinga, A.O. Valkirs, P.M. Stang – 1996 - Organotin - Environmental fate and effects , 1996. Eds Champ, M.A. and Seligman, P.F. Chapman and Hall Publishers London.
[6] Organotin antifouling paints and their alternatives – Iwao Omae – Applied Organometallic Chemistry, 2003; 17: 81-105.
[7] Regulation (EC) No 1272/2008 of European Parliament and of the Council – Official Journal of the European Union 353, 2008, 1–1355.
[8] Regulation (EC) No 782/2003 of European Parliament and of the Council – Official Journal of the European Union 115, 2003, 1-11.
[9] Directive 2000/60/EC of European Parliament and of the Council – Official Journal of the European Union 327, 2000, 1-72.
[10] Decision No 2455/2001/EC of European Parliament and of the Council – Official Journal of the European Union 331, 2001, 1-5 .
[11] Directive 2008/105/EC of European Parliament and of the Council – Official Journal of the European Union 348, 2008, 84-97.
[12] Directive 2008/56/EC of European Parliament and of the Council – Official Journal of the European Union 164, 2008, 19-40.
[13] Directive 98/8/EC of European Parliament and of the Council – Official Journal of the European Union 123, 1998, 1-63.
[14] Regulation (EU) No 528/2012 of European Parliament and of the Council – Official Journal of the European Union 167, 2012, 1-123.
[15] Register for Biocidal Products, http://echa.europa.eu/it/support/dossier-submission-tools/r4bp.
[16] Commission Regulation (EC) No 1896/2000 - Official Journal of the European Union 228, 2000, 6-17.
[17] Commission Regulation (EC) No 2032/2003 - Official Journal of the European Union 307, 2003, 1-96.
[18] Commission Regulation (EC) No 1048/2005 - Official Journal of the European Union 178, 2005, 1-98.
[19] Commission Regulation (EC) No 1849/2006 - Official Journal of the European Union 355, 2006, 63-71.
[20] Commission Regulation (EC) No 1451/2007 - Official Journal of the European Union 325, 2007, 3-65.
[21] CA – Sept 13-Doc.8.4. Possible way forward for the management of antifouling actives substances and the authorisation of biocidal products (PT21).
[22] Regulation (EC) No 470/2009 of European Parliament and of the Council – Official Journal of the European Union 152, 2009, 11-22.
[23] Regulation (EC) No 396/2005 of European Parliament and of the Council – Official Journal of the European Union 70, 2005, 1-16.
[24] Italiainnovatori.gov.it/innovazione/progetto-bubbleboat

NATIONAL/INTERNATIONAL LEGISLATION ON ANTIFOULING

# The control of Anti-Fouling Systems (AFS) on ships: duty of Italian coast guard

The fact-finding and decision-making processes of the international community via the International Maritime Organization have led to a ban on the use of antifouling paints containing organotin compounds: the Italian Coast Guard Corps guarantee compliance with regulations through the Port State Control carried out in ports and onboard vessels

*DOI: 10.12910/EAI2014-52*

*Aurelio Caligiore*

## The International Convention on the control of harmful Anti-Fouling Systems on ships, 2001

In 1992 Agenda 21, adopted by the United Nations Conference on Environment and Development, invited partner States to take measures to reduce pollution caused by organotin compounds used in anti-fouling systems.

The harmful environmental effects of organotin compounds were recognized by IMO (International Maritime Organization) in 1989. Later on IMO Resolution A.895(21), adopted by the Assembly on 25 November, 1999, urged the Organization's Marine Environment Protection Committee (MEPC) to work for the expeditious development of a global legally binding instrument to address the harmful effects of anti-fouling systems as a matter of urgency.

In October 2001, IMO adopted the International Convention on the control of harmful Anti-Fouling System on ship (AFS 2001) [1] which, on 1st January, 2003, introduced the ban on the use of antifouling paints containing TBT and other tin components, fixing 1st January, 2008, as the deadline for the complete retirement of paints containing tin from the hulls of vessels. The convention entered into force on 17th September, 2008.

Under AFS Convention, ships with Gross Tonnage (GT) greater than 400, engaged in international voyages (excluding fixed or floating platforms, FSUs and FPSOs), are required to undergo a preliminary check, carried out by the Flag State, before entering service or before the "International Anti-Fouling System" Certificate (IAFS Certificate) is issued, and inspected in the case of replacement or overhaul of the anti-fouling system on the ship.

The Italian Administration issues IAFS certificates for ships through recognized organizations (Registro Italiano Navale, Bureau Veritas, American Bureau of Shipping and Germanischer Lloyd), which perform survey and control functions relating to the certificate issue, as well as the actual issue of the certificate on behalf of the State Administration.

**Aurelio Caligiore**
*Ministry of the Environment and Protection of Land and Sea, (Head of) Environmental Marine Department of the State Port Control*

Vessels longer than 24 m, but with GT less than 400, engaged in international voyages (excluding fixed or floating platforms, FSUs and FPSOs), however, in place of the certificate must possess a declaration regarding the anti-fouling system in use (Declaration on Anti-Fouling Systems), signed by the ship owner or authorized agent, to which documentation describing the type of anti-fouling product actually used must be attached.

## Italian legislation on the ratification of the Convention AFS

Although Italy is already implementing the European legislation (Regulation (CE) n. 782/2003 of the European Parliament and Council on 14/4/2003 [2] and Regulation (CE) n. 536/2008 of the European Parliament and Council on 13/06/2008 [3]), concerning the ban on organotin compounds on ships, Italy has ratified the AFS Convention, thus introducing additional special rules on penalties.

In fact, by Law no. 163 of 31st August, 2012, (Accession of the Italian Republic to the International Convention on the control of harmful anti-fouling systems applied on ships, with attachments, made in London October 5, 2001, and its execution) [4], Italy has identified the authorities responsible for ensuring proper implementation of the Convention and emphasized the importance of tackling criminal violations of AFS.

The authorities responsible for carrying out the tasks of survey, inspection and control provided for in Articles 10 and 11 of the Convention, are the Ministry of the Environment and Protection of Land and Sea and the Ministry of Infrastructure and Transport, acting through a "recognised classification society" operating on behalf of the Italian government and the Coast Guard Corps for inspection and control activities.

As far as duties of surveillance and maritime policing are concerned, inspection activities are carried out by qualified personnel of the Coast Guards corps, who, during the control effected on board, are entitled to verify the existence of relevant certificates, including also the IAFS Certificate, or valid declaration as well as to provide a check for the presence of organotin compounds used in paints.

With regard to foreign ships, however, inspections and checks are carried out in accordance with "Port State Control" procedures.

Port State Control (PSC) is the power of a State, deriving from international agreements, to carry out checks on foreign ships docking in its ports, with the aim of verifying their compliance with international regulations relating to shipping safety, anti-pollution and on-board living conditions, for the purposes of eventual application of relevant corrective measures.

In this context, as regards Italy the Paris Memorandum of Understanding on State Port Control (Paris MoU on PSC) is applied.

In Italy PSC inspections are implemented by qualified Coast Guard officers, in accordance with Legislative Decree 24 March 2011 n. 53 [5], transposition of Directive 2009/16/CE [6].

## Port State Control - Guidelines for Port State Control officers on control of Anti-Fouling Systems (AFS) on ships

As mentioned before, the rules related to inspections of ships and detection of violations are provided in art. 11 of AFS 2001.

This Convention provides that a ship, in any port, shipyard, or offshore terminal of a Party may be inspected by officers authorized by that Party, for the purpose of determining whether the ship is in compliance with the Convention itself.

Unless there are clear grounds for believing that a ship is in violation of this Convention, any such inspection shall be limited to verifying that there is onboard a valid International Anti-Fouling System Certificate or a Declaration on Anti-Fouling System and, eventually, brief sampling of the ship's anti-fouling system, taking into account the guidelines developed by IMO.

If there are clear grounds to believe that the ship is in violation of the Convention, a more detailed inspection may be carried out taking into account the guidelines developed by IMO.

If the ship is detected to be in violation of this Convention, the Party carrying out the inspection may take steps to warn, detain, dismiss, or exclude the ship from its ports. A Party taking such an action against a

ship that is not compliant with this Convention shall immediately inform the Administration of the ship concerned.

The guidelines for conducting these inspections are described in accordance with the relevant PSC instructions, based on IMO Resolution MEPC.104(49) [7] and MEPC.208(62) [8]. Such guidelines relate to the exercise of the right of the Port State to conduct inspections of anti-fouling systems under Article 11 of the AFS Convention (AFS 2001).

For ships required to carry an IAFS Certificate or Declaration on Anti-Fouling System, the Port State control officer (PSCO) should examine the IAFS Certificate or Declaration on Anti-Fouling System, and the attached Record of Anti-Fouling Systems, if appropriate.

The IAFS Certificate carries information on the ship's details and a series of tick boxes to indicate whether an anti-fouling system controlled under Annex I of AFS 2001 has or has not been applied, removed or been covered with a sealer coat, and if an anti-fouling system controlled under Annex I of AFS 2001 was applied on the ship prior to or after the date specified in AFS 2001.

As a preliminary check, the validity of the IAFS Certificate should be confirmed by verifying that the IAFS Certificate is properly completed and signed/endorsed by the Administration, or by a recognized organization (RO), and stating that the required survey has been performed. In reviewing the IAFS Certificate, particular attention should be paid to verifying that the initial survey matches the dry dock period listed in the ship's log(s), and that only one box should be marked. The Record of Anti-Fouling Systems should be inspected to ensure that the records are attached to the IAFS Certificate and up-to-date. The most recent Record must correlate with the correct checkbox on the front of the IAFS Certificate.

Ships of non-Parties to the AFS Convention are not entitled to be issued with an IAFS Certificate. Therefore the PSCO should ask for documentation that contains the same information as in an IAFS Certificate and take this into account in determining compliance with the requirements.

If the existing anti-fouling system is declared not to be controlled under Annex 1 of the Convention, without being documented by an International Anti-Fouling System Certificate, verification should be carried out to confirm that the anti-fouling system complies with the requirements of the Convention. This verification may be based on sampling and/or testing and/or reliable documentation, as deemed necessary, based on the experience gained and the existing circumstances. Documentation for verification could be, e.g., MSDSs (Material Safety Data Sheets), or similar, a declaration of compliance from the anti-fouling system manufacturer, invoices from the shipyard and/or the anti-fouling system manufacturer.

The records described in Resolution MEPC.195(61) [9], can be used as examples of this types of documentation. Ships of non-Parties may have Statements of Compliance issued in order to comply with regional requirements, for example, Regulation (EC) 782/2003 as amended by Regulation (EC) 536/2008, which could be considered as providing sufficient evidence of compliance.

In all other aspects, the PSCO should be guided by the procedures for ships required to carry an IAFS Certificate, in order to ensure that no more favorable treatment is applied to ships of non-Parties to the AFS Convention.

A more detailed inspection may be carried out whenever clear grounds do exist to believe that the ship does not substantially meet the requirements of the AFS Convention.

Clear grounds for a more detailed inspection may be in case:

a. the ship is from a flag of a non-Party to the Convention and there is no AFS documentation;
b. the ship is from a flag of a Party to the Convention but there is no valid IAFS Certificate;
c. the painting date shown on the IAFS Certificate does not match the dry-dock period of the ship;
d. the ship's hull shows excessive patches of different paints.

If the IAFS Certificate is not properly completed, the following questions may be pertinent:

1. "When was the ship's anti-fouling system last applied?";
2. "If the anti-fouling system is controlled under Annex 1 to the AFS Convention and was removed,

what was the name of the facility and date of the work performed?";
3. "If the anti-fouling system is controlled under Annex 1 of the AFS Convention and has been covered by a sealer coat, what was the name of the facility and date applied?";
4. "What is the name of the anti-fouling/sealer products and the manufacturer or distributor for the existing anti-fouling system?";
5. "If the current anti-fouling system was changed from the previous system, what was the type of anti-fouling system and name of the previous manufacturer or distributor?".

*Action taken under the AFS Convention*

Following the more detailed inspection, a violation may lead to measures of warning, detention, dismissing and exclusion.

The Port State Control Officer could decide to detain the ship following detection of deficiencies during an inspection on board.

Detention could be appropriate if certification is invalid or missing, the ship admits it does not comply (thereby removing the need to prove by sampling) or sampling proves it is non-compliant within the port jurisdiction.

Further action would depend on whether the problem is with the certification or the anti-fouling system itself.

The Port State Control Officer could dismiss the ship, meaning that the Port State Control Officer requests the ship to leave the port, for example if the ship chooses not to bring the AFS into compliance but the Port State is concerned that the ship is leaching tributyltin (TBTs) into its waters.

Dismissal could be appropriate if the ship admits it does not comply or sampling proves it is non-compliant while the ship is still in the port. Since this would also be a detainable deficiency, the PSCO can detain the ship first and require rectification before releasing it.

Dismissal could be appropriate if certification is invalid or missing, the ship admits it does not comply (thereby removing the need to prove by sampling) or sampling proves it is non-compliant within the port jurisdiction.

In these cases the ship would probably already have been detained. However, detention does not force the ship to bring the AFS into compliance (only if she wants to leave the port).

In such a situation the Port State Control Officer may be concerned that the ship is leaching TBTs while it remains in its waters.

The Port State Control Officer could decide to exclude the ship to prevent her from entering its waters.

Exclusion could be appropriate if sampling proves that the ship is non-compliant but the results have been obtained after she has sailed or after she has been dismissed.

*Sampling methodologies*

AFS 2001 specifies that sampling of the ship's anti-fouling system that does not affect the integrity, structure, or operation of the anti-fouling system taking into account the guidelines contained in IMO resolution MEPC.104(49) and MEPC.208(62). However, the time required to process the results of such sampling shall not be used as a basis for preventing against the movement and departure of the ship.

It is to the discretion of the Port State to choose the sampling methodology. The Guidelines for brief sampling of anti-fouling systems on ships adopted by IMO allow that any scientifically recognized method of sampling and analysis of AFS controlled by the Convention other than those described in the appendix to the Guidelines may be used (subject to the satisfaction of the Administration or the Port State).

The sampling methodology will depend, *inter alia,* on the surface hardness of the paint, which may vary considerably.

The amount of paint mass removed may vary correspondingly.

Sampling procedures, based on the removal of paint material from the hull, require the determination of the paint mass. It is important that: the procedures used are validated, produce unambiguous results, and contain an adequate control.

The competent Port State authority can decide to contract specialist companies to carry out sampling. In this case, the PSCO should attend the ship during the sampling procedure to ensure the liaison and arrangements mentioned above are in place.

## Conducting analyses - Use of portable X-ray fluorescence analyzer

The Guidelines for brief sampling of anti-fouling systems on ships envisage a two-stage analysis of samples for both methods presented in the Guidelines. The first stage is a basic test, which can be carried out on site as in the case of Method 2. The second stage is carried out when the first stage results are positive. It is noted that in the IMO Guidelines, these stages are referred to as Steps 1 and 2, as in the case of Method 1. It is to the discretion of the Port State competent authorities to choose which analysis methods are used. The following points are presented for Port State consideration:

- approval procedure for the recognition of laboratories meeting ISO 17025 standards or other appropriate facilities should be set up by the Port State competent authorities. These procedures should define the recognition criteria. Exchange of information between Port States on these procedures, criteria and laboratories/facilities would be beneficial, i.e. for the purposes of exchange of best practices and possible cross-border recognition and provision of services;
- the company that undertakes the analysis and/or samples should comply with national regulations and be independent from paint manufacturers;
- the PSCO carrying out the AFS inspection of a ship should verify the validity of the ISO 17025 certificate and/or the recognition of the laboratory;
- if more time is needed for analysis than available considering the ship's scheduled time of departure, the PSCO shall inform the ship and report the situation to the Port State competent authority. However, the time needed for analysis does not warrant undue delay of the ship; and
- PSCOs should ensure completion of the record sheets for the sampling procedure as proof of analysis. In cases when the laboratory procedures prescribe presentation of the analyses results in a different format, this technical report could be added to the record sheets.

The first-stage analysis serves to detect the total amount of tin in the AFS applied.

It is to the discretion of the Port State competent authority to choose the first-stage analysis methodology. However, the use of a portable X-ray fluorescence analyzer [10] or any other scientifically justified method allowing the conduction of first-stage analyses on site could be considered as best practice.

The Port State competent authority has to decide whether the first-stage analysis should be carried out by PSCOs or by contracted companies.

The Port State competent authority could provide PSCOs with this equipment (e.g., portable X-ray fluorescence analyzer) and provide them with the appropriate training.

## Alternative methods. A new test for the identification and analysis of anti-fouling paints containing TBT

Gueuné and collaborators [11] have proposed a new method, based on recombinant bioluminescent bacteria, with the aim of directly identifying the presence of TBT in paints applied to hulls, by means of a simple device which does not involve invasive sampling.

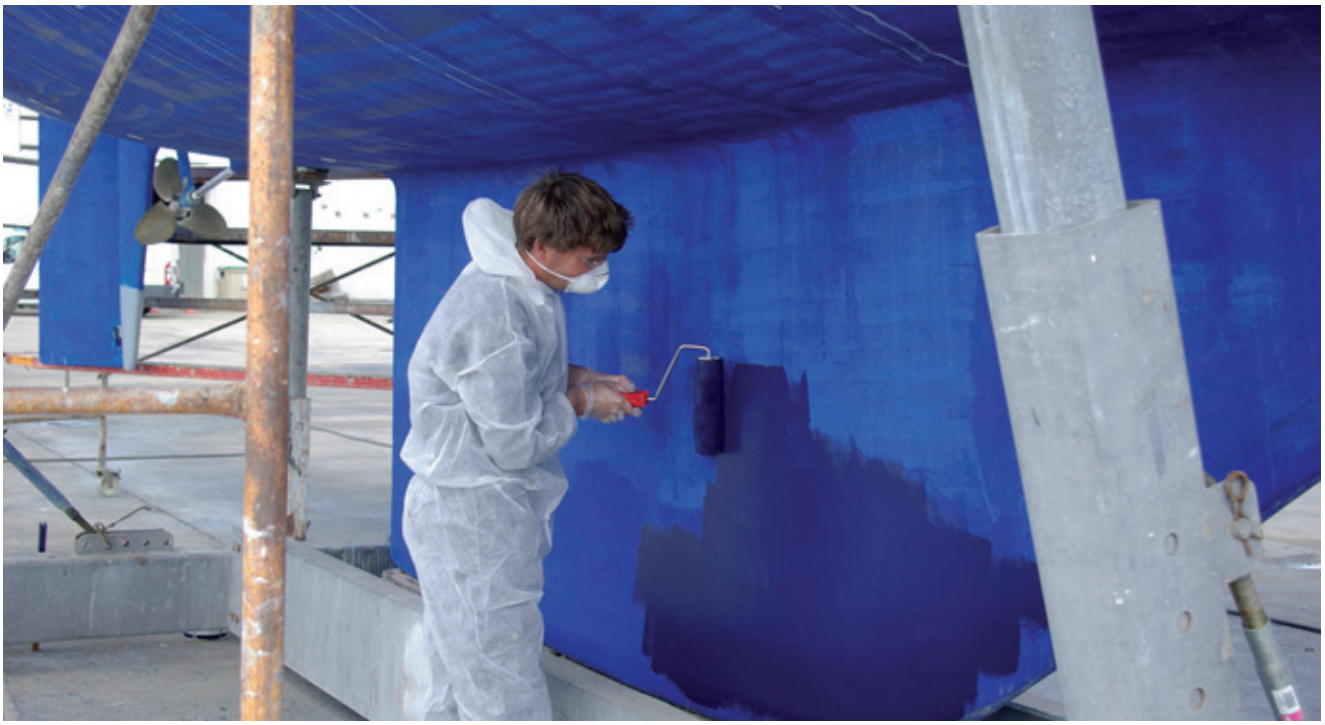
Tests of microbial toxicity, based on the use of recombinant bacteria, are widely used to identify the presence of pollutants. In most cases luxAB genes are inserted downstream of a gene promoter involved in resistance to a metal, or in the biodegradation of organic compounds. In this study the Escherichia coli TBT3 clone was used to identify the presence of TBT in the anti-fouling paints, in view of its optimal characteristics of specificity and sensitivity towards TBT and DBT.

Onsite tests were performed by means of a simple device, consisting of a square polyethylene chamber fixed to the hull, inside which artificial marine water is forced. Once the water has come into contact with the paint on the ship's bottom, it is used as a sample for analysis with the bioluminescent bacteria.

The presence of organotins can be detected directly in less than three hours, without the need to extract or prepare the sample, and without causing delays to the ship's commercial operations It is possible, indeed, to have a result before the ship leaves the port.

Kabiersch [12] report the development and optimization of a bioluminescent yeast assay for the detection of

organotin compounds based on the interaction with a hybrid RXR and subsequent expression of a reporter luciferase gene.
This assay is highly specific toward organotin compounds and natural ligands of the RXR. It detects tributyltin and triphenyltin in nanomolar concentrations (detection limits were found to be 30 nM and 110 nM, respectively).
Also this method is relatively rapid (1 day of work), to allow the subsequent control procedures to be activated in the event of evidence of irregularities.



references


1. International Convention on the Control of Harmful Anti-Fouling System on Ships (AFS 2001). International Maritime Organization, London, 2001.
2. Regolamento (CE) N. 782/2003 del parlamento europeo e del consiglio del 14 aprile 2003 sul divieto dei composti organostannici sulle navi (GU L 115 del 9.5.2003).
3. Regolamento (CE) N. 536/2008 della commissione del 13 giugno 2008 recante attuazione dell'articolo 6, paragrafo 3, e dell'articolo 7 del regolamento (CE) n. 782/2003 del Parlamento europeo e del Consiglio sul divieto dei composti organostannici sulle navi e recante modifica di detto regolamento.
4. Legge 31 agosto 2012, n. 163 Adesione della Repubblica italiana alla Convenzione internazionale per il controllo dei sistemi antivegetativi nocivi applicati sulle navi, con allegati, fatta a Londra il 5 ottobre 2001, e sua esecuzione. (GU n.227 del 28-9-2012 - Suppl. Ordinario n. 187).
5. Decreto Legislativo 24 marzo 2011, n. 53 Attuazione della direttiva 2009/16/CE recante le norme internazionali per la sicurezza delle navi, la prevenzione dell'inquinamento e le condizioni di vita e di lavoro a bordo per le navi che approdano nei porti comunitari e che navigano nelle acque sotto la giurisdizione degli Stati membri.
6. Directive 2009/16/EC of the European Parliament and of the Council of 23 April 2009 on Port State control.
7. IMO Resolution MEPC.104(49) adopted on 18 July 2003. Guidelines for brief sampling of anti-fouling system on ship.
8. IMO Resolution MEPC.208(62) adopted on 15 July 2011. Guidelines for inspection of anti-fouling systems on ships.
9. IMO RESOLUTION MEPC.195(61) adopted on 1 October 2010. Guidelines for survey and certification of anti-fouling systems on ships.
10. European Patent 2 096 431 A1, Kantonen.
11. H. Gueuné, G. Thouand, M.J. Durand (2009), "A new bioassay for the inspection and identification of TBT-containing antifouling paint", Marine Pollution Bulletin, 58 (2009), pp. 1734–1738, Elsevier Ltd.
12. G. Kabiersch, J. Rajasärkkä, M. Tuomela, A. Hatakka, M. Virta, and K. Steffen (2013), "Bioluminescent Yeast Assay for Detection of Organotin Compounds", Anal. Chem. 2013, 85, 5740–5745, American Chemical Society, Washington.

# Energia, Ambiente e Innovazione
## *in rete*

La rivista **Energia, Ambiente e Innovazione** è disponibile **gratuitamente** sul web (per computer ed e-reader) e sui dispositivi mobili Apple:

- sul web alla pagina http://www.enea.it/it/produzione-scientifica/EAI tutti gli articoli pubblicati dal 2003 al 2014 in formato pdf e, a partire dal 2014, anche in formato epub, utilizzato per visualizzare i libri digitali

- sui dispositivi mobili Apple (iPhone, iPad e iPod touch) grazie ad una App liberamente reperibile in App Store