Making barnacles walk away

Effective and environmentally friendly marine antifouling agents based on existing pharmaceuticals.

To prevent marine fouling it is only necessary to prevent the settlement of marine organisms, not to kill them. A number of existing pharmacological products were tested for antifouling activity against barnacles. A paint system with a controlled release rate of one of these products has been shown to be effective, enhancing the motility of barnacles and thus disabling them from attaching to ship hulls. These compounds are unlikely to accumulate in the marine environment to harmful levels.

Lena Mårtensson Lindblad*, Björn Dahlbäck.

* Corresponding Author. Contact: Lena Lindblad, I-Tech AB, Haraldsgatan 5, SE-41314 Göteborg, Sweden, Tel. +46 31 703-1949, Fax +46 31 703-1860, lena.lindblad@vnv.se

Marine fouling is of major importance in shipping - impacting on ship performance, economy and the environment (see Figure 1). There are many antifouling principles, but all suffer either from poor efficacy or significant environmental problems. A new generation of antifouling coatings is needed that are:

- Highly effective;
- Highly versatile;
- Non-hazardous in respect of both health and the environment;
- Based on a thorough understanding of fouling and antifouling mechanisms. The need for new antifouling substances has long been an issue, and the need to understand the basic mechanisms behind fouling was expressed more than fifty years ago by the world’s largest ship owner, the US Navy [1]: “Fouling is, however, a biological phenomenon. If it is to be dealt with effectively from an engineering point of view, it is important that the biological principles which determine its development be understood”.

Present technologies to inhibit attachment and growth of marine fouling organisms rely on bioactive chemicals without a specific mode of action. For the most part, they act by being toxic and lethal. However, killing is not necessary when preventing settlement. With knowledge of basic biology, it is possible to find solutions based on fouling organism biology, such as preventing the secretion of adhesives or changing behavioural patterns.

Biocide use is governed by several regulations

In a wider societal context, antifouling technologies are encompassed by a number of international conventions and EU policies and directives which address issues of marine pollution. Directive 3760/92/EEC requires monitoring of coastal waters.

Directive 76/464 (the Water Framework Directive) and 96/23 (Monitoring of Substances) focus on water quality, which may be adversely affected by antifouling treatments, particularly in the coastal zone where there is a large increase in recreational activities such as the use of leisure boats. Of particular importance for approval of biocidal antifoulants is the Biocidal Products Directive (BPD, Directive 98/8/EC) [2].

Available antifoulings today are very limited

It is not possible to define or plan a new biocide technology without complying with the regulatory process in countries and geographical areas where the new technology is to be introduced.

The BPD was introduced in 1998 in an effort to harmonise European safety requirements for biocide products. A biocide regulatory dossier is composed of two toxicology data sets, human health and safety and environmental fate. Based on the data sets, risk assessments are performed and presented within the dossier. The risk assessment is then judged by a Competent Authority (which each EU country has selected) for review. In Sweden, this is the Swedish Chemicals Agency (KEMI) that evaluates and presents the regulatory dossier to the DG Environment board for approval and inclusion in Annex 1, which allows for usage and sale within the European market.

After the BPD’s introduction in 1998, different biocide product categories became subject to the directive, including antifouling substances (product type 21). The notification period ended in April 2006 and a full dossier had to be presented to a Competent Authority if it was to be allowed to stay on the market. Only a few notified substances are now under evaluation in this process. Out of 46 substances notified in 2002, only ten have now entered the BPD process. These are listed in Table 1 [3, 4]. Three are copper products, and among the other seven, several have the same or nearly the same product profile, which effectively limits the biocides available for antifouling purposes even more.

It should also be noted that use of copper is scrutinised both in Europe, especially regarding the Baltic Sea [5], and in the USA, for example, in the state of California [6, 7] and Chesapeake Bay [8]. The new regulatory context has created a great need for new substances and products, especially for long term, sustainable use in the marine environment and to prevent the development of tolerance.

Existing pharmaceuticals in new roles

In 1999, a patent was filed on medetomidine (“Catemine”) as an antifouling agent effective against barnacles. The patent was based on an idea presented by the US Navy (see above) 50 years earlier, taking advantage of biological knowledge in preventing marine biofouling. A company (I-Tech AB) was later formed to take the catemine concept further, as well as becoming a partner to the academic researchers engaged in research and development of catemines.

The research strategy is compatible with pharmaceutical industry strategies in providing small molecules, well defined with a known mode of action. This concept offered several advantages and was not within the mainstream of academic antifouling research.

By using available substances with a specific biological profile, substances that were characterised regarding their mode of action, some existing toxicological data and the possibility to industrially synthesise materials in larger quantities made it possible to speed up the invention process and consequently, the time to market.

To reach the market with a new antifoulant substance a multidisciplinary research approach is needed, including regulatory and commercialisation competence. Out of the different substances tested, one in particular showed unique properties, namely medetomidine. This substance was a thousandfold more potent than any other molecule tested in the early investigations on inhibition of settlement of barnacle larvae, not by killing them, but by preventing attachment [9].

Following the initial barnacle larvae settlement studies, the research has focused on barnacle physiology and mode of action, paint formulation and ecotoxicology risk assessment.
Catemines stop fouling by preventing barnacle attachment
All cells are connected by communication systems, either neuronal or circulating hormones. The signal molecules, whether neurotransmitters or hormones, interact by binding to a specific protein on the cell surface, the receptor. The receptor then transmits the signal from the outside to the inside of the cell inducing a biological response. The biological response is therefore dependent on the signalling molecule and the signalling transfer by the specific receptors. One class of receptors on the cell surface is termed G-protein coupled receptors (GPCRs). Within the pharmaceutical industry, many products act by either blocking or stimulating these types of receptors. Barnacles, in this perspective, are no different from humans [10, 11]. From among the vast number of GPCR ligands, three different molecules were identified: medetomidine (Catemine 1), clonidine (Catemine 2), and S18616 (Catemine 3). They are all far more potent in inhibiting barnacle settlement than any other imidazole substances with receptor activity so far tested. They are characterised by being effective against barnacles in low nanomolar concentrations and induce increased motility. Other compounds with the same pharmacological profile, or with similar chemical imidazoline structures, lack these features [12]. Among these three catemines, S18616 is the most potent; medetomidine has surface retention, whereas clonidine is the least effective, lacking surface retention. Thus, both S18616 and medetomidine can function as barnacle deterrents in antifouling paint [13, 14].

The first hypothesis regarding the mode of action was that the catemines inhibited cement secretion. The reasoning was that if no cement (adhesive proteins) were secreted, it would be impossible for the barnacle cyprid larva to settle. This hypothesis was proved wrong and instead, an increase in motility was seen when adding the different catemines. Two different assays were developed that were able to quantify the increased mobility, either as leg motility per minute or swimming velocity. A barnacle cyprid larva can swim as fast as 1 mm/s [15] (or twice its own length each second, a better proportional speed than human swimming records). At such a swimming speed the larval exploratory surface behaviour, necessary for settlement, is effectively blocked. Instead, deterrent behaviour is promoted and attachment and permanent settlement is therefore inhibited. Within this context, it has been shown that receptor activation is a possible target for new biocide inventions in the area of marine biofouling, and most importantly, they act without having to kill the organism, only inhibit it.

Controlled release from paints
A marine paint formulation contains many different types of chemicals of which the most important are solvent, binder, filler, pigment and stabiliser. The selection of each and their respective fractions of the dry paint are based on economy and performance. Most often, paints are developed to optimise economy and mechanical properties but not to the same extent with regard to biocides or to a specific slow release system.

Self-eroding paints in which the surface layer is hydrolysed by contact with water, thus creating an erosive zone, are considered state-of-the-art. Biocidal loss is restricted to the erosive zone and this per se becomes a slow release system since the biocides are only able to enter the marine environment from within the erosive layer. Medetomidine has a deterrent effect even without a specialised slow release system. However, it has been found possible to refine the release control by adding a small amount of metal oxide nanoparticles. Imidazoline groups (as in medetomidine) adsorb to transition metal oxides very strongly in an apolar solvent, such as xylene. By adding a small amount of, for example, ZnO nanoparticles [16, 17] it is possible to retain medetomidine in the dry film. When the marine water hydrolyses the outer layer and the solvent changes from xylene to water in the erosive layer, the medetomidine desorbs from the metal oxide nanoparticles, diffuses and is free to act as a biocide in the boundary layer.

The metal oxide slow release system has been tested in two systems, a static raft test measuring its efficacy (see Figure 2) and in a flow chamber (Figure 3). Both tests validate the hypothesis, showing both better efficacy and leakage rates when metal oxide nanoparticles were added to the paint. The effective loss rate was estimated to be as low as 1.8 ng/cm²/day [18].

Catemines have low ecotoxicology and hazard rating
In predicting environmental hazards, research takes advantage of modelling programs that estimate the predicted environmental concentration (PEC) from different marine activity scenarios such as shipping lanes, large commercial harbours or non-tidal, poorly flushed marinas. One such model is the MAMPEC program [19]. With MAMPEC, a PEC value is estimated and then related to the predicted no-effect concentration (PNEC) of the substance. The PNEC value is an estimate from ecotoxicological studies and a risk factor, known as the assessment factor. The value of the assessment factor is regulated by the authorities and is based on the studies performed, taking into account number, species and length of time. In the regulatory context, this is called the PEC/PNEC ratio and should be less than 1 to be regarded as safe for the environment.

The ecotoxicology and hazard assessment of medetomidine is based on tests of a large number of marine non-target organisms to create a fine ecotoxicological mesh (see Figure 4). The studies have identified several important responses e.g. metabolic down-regulation, interference with detoxification processes, and pigmentation disturbances, but in concentrations significantly higher than needed to deter barnacles.

The most sensitive bioassay, the Turbot EROD-activity (a detoxification enzyme that could be induced by low levels of medetomidine) was first initiated by concentrations greater than 50 times higher than the predicted environmental concentration in the worst-case harbour scenarios. The emission of catemine used in the evaluation is for the best paint formulation so far, and is based on the nanoparticle concept developed to control release from marine coatings. In another aspect of efforts to reduce risk, an initial PBT assessment of medetomidine was carried out. PBT stands for "Persistent, Bioaccumulative and Toxic." PBT deals with the concern that hazardous substances might accumulate in parts of the marine environment which could lead to long-term effects that are difficult to predict. So far, the results suggest that medetomidine does not accumulate and therefore should not be regarded as a potential PBT chemical.

To conclude, the ecotoxicological research performed by the Marine Paint research organization at Göteborg University suggests that medetomidine has the potential for becoming a strong candidate for effectively controlling fouling by barnacles without imposing unacceptable risks to the environment [20].

Further restrictions on antifoulings are likely
The antifouling market is now in a turnaround phase starting
with the national and international banning of TBT and ending when the approved substances are listed in the
Annex 1 of the European Biocide Product Directive (BPD). After the 2nd review phase according to the BPD
regulations, only ten antifouling substances have entered the review process by submission of a registration dossier to the chosen Competent Authorities (see Table 1).

The present trend of increasing the amount of copper in the paints will probably change, both due to growing environmental concerns regarding copper in the marine environment as well as increasing copper prices.

At present there is uncertainty regarding the environmental risks of antifouling compounds, indicated by the fact that countries seem to make different judgements even in Europe. Sweden approves a minor number of active substances such as Irgarol, while Denmark prohibits Irgarol, giving preference to Diuron. Both Sweden and the Netherlands regulate the use of copper, while the UK and other EU states so far allow several other booster biocides to be used.

The overall changes in attitude and striving toward a sustainable marine environment as well as new legislative positions create an opportunity and an advantage for the introduction of a new antifouling substance such as medetomidine.

ACKNOWLEDGEMENTS

This paper is based on research results from and on the close collaboration within the multidisciplinary Marine Paint research programme at Goteborg University and Chalmers University of Technology in Sweden. The contributions of all members of Marine Paint are gratefully acknowledged. Marine Paint is funded by the Foundation for Strategic Environmental Research, Mistra. The assistance of Mr. Per Jansson for input toward the fruitful collaboration between the Marine Paint research programme and I-Tech AB is also gratefully acknowledged.

REFERENCES

[1] U.S. Naval Institute, Marine fouling and its prevention. Copyright 1952, Annapolis, Maryland, USA
compounds prevent the settlement of cyprid larvae of Balanus improvisus, Biofouling 16 (2-4), 191-203
[18] M. Nydén, Unpublished data

Results at a glance

There is increasing concern over the toxic effects of marine antifoulings, and the introduction of the Biocidal Products Directive will further restrict the choice of biocides available for use in European waters.
- However, to prevent fouling it is only necessary to prevent the settlement of marine organisms on the coating, not to kill them.
- Three products already in pharmaceutical use and known collectively as catemines were tested for antifouling activity against barnacles and two were considered effective. They force the barnacle larvae to keep moving, so they cannot settle and bond to the surface.
- A slow release paint system has been developed, using metal oxide nanoparticles to slow down the release rate of one of the catemines (medetomidine).
- Ecotoxicity and hazard tests indicate that catemines are unlikely to accumulate in the marine environment to harmful levels.

The authors:

-> Associate Professor Lena Mårtensson Lindblad is one of the initiators of the catemine concept and the Marine Paint research programme at Göteborg University and Chalmers University of Technology in Sweden. She has a background in pharmacology and zoophysiology and is currently the RD & Manager at I-Tech AB, responsible for regulatory affairs and the progress of barnacle biology research.
-> Dr. Björn Dahlbäck is Programme Director of the Marine Paint research programme funded by the Foundation for Strategic Environmental Research, Mistra. His research background is in marine microbiology.

This paper was presented at the European Coatings Conference "Novel Biocide Technology III", Berlin, 15/16 February 2007
Figure 2: One year static test outside the Swedish west coast proving the efficacy of catemine in preventing barnacle settlement.
Figure 3: A flow chamber used to measure loss rates, confirming that the slow release concept with catemine bound to ZnO nanoparticles is functional even at different flow velocities (photo: Ann Larsson)
Figure 4: A vast number of ecotoxicological studies on non-target organisms have been performed to provide assurance that catemine can be safely used in the marine environment (photo: Åke Granmo)
### Table 1: Antifouling biocides now being evaluated under the Biocidal Products Directive

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<th>Name</th>
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<td>Tolyfluanid</td>
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<td>Lanxess Deutschland GmbH</td>
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<td>Copper thiocyanate</td>
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<td>Dicopper oxide</td>
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<td>TNO Nutrition and Food Research</td>
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