Bioassays and field immersion tests: a comparison of the antifouling activity of copper-free poly(methacrylic)-based coatings containing tertiary amines and ammonium salt groups

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This paper focuses on the activity spectrum of three dimethylalkyl tertiary amines as potential active molecules and the corresponding ammonium salt-based antifouling (AF) paints. Bioassays (using marine bacteria, microalgae and barnacles) and field tests were combined to assess the AF activity of coatings. Bioassay results demonstrated that the ammonium salt-based paints did not inhibit the growth of microorganisms (except the dimethyldodecylammonium-based coatings) and that the tertiary amines were potent towards bacteria, diatoms, and barnacle larvae at non-toxic concentrations (therapeutic ratio, LC50/EC50, < 1). The results from field tests indicated that the ammonium salt-based coatings inhibited the settlement of macrofouling and the dimethylhexadecylammonium-based coatings provided protection against slime in comparison with PVC blank panels. Thus, results from laboratory assays did not fully concur with the AF activity of the paints in the field trial.

Keywords: antifouling; bioassays; marine coating; screening; raft immersion

Introduction

Any unprotected substratum immersed in seawater will rapidly be colonized by marine organisms (bacteria, fungi, microalgae, macroalgae and invertebrates) resulting in severe problems on both dynamic and static structures. Biofouling of ships’ hulls reduces speed and can increase fuel consumption (Anderson et al. 2003). Moreover, on piers and static platforms, fouling both accelerates corrosion and increases the risk of damage from heavy waves, impact, or vibration (Almeida et al. 2007). Antifouling (AF) coatings are used on man-made surfaces likely to come into contact with fouling organisms. Such coating compositions generally comprise polymer matrixes (called binders) and biocides that impede or inhibit the settlement of marine organisms. The most successful AF paints for many years have been triorganotin-based self-polishing AF paints (Omae 2003; Yebra et al. 2004). The combination of widespread use, persistence, and toxicity led the International Maritime Organisation (IMO) to introduce the AFS Convention, banning the application of tributyltin (TBT)-containing AF on all vessels (Chambers et al. 2006; Maréchal and Hellio 2009). As a consequence, self-polishing copolymers or controlled deplention paints are continuously under investigation to mimic the well-known TBT-based self-polishing copolymers (Yebra et al. 2004, 2005; Finnie and Williams 2010). These commercial paints contain biologically active compounds (see Thomas and Brooks 2010) which are listed in the Biocidal Products Directive (98/8/EC). Major research efforts have been initiated to develop environmentally-friendly solutions based on non-toxic technologies such as ‘non-stick’ or fouling-release (FR) coatings. While these coatings are known to release hard and soft foulers under suitable hydrodynamic conditions, a high degree of settlement occurs under static and low-flow conditions (Majumdar et al. 2008). An ideal AF formulation would have the following properties: be able to control biofouling for at least 5 years, be durable resistant to damage, repairable, low maintenance, easy to apply, hydraulically smooth, compatible with existing anticorrosion coatings, cost effective, non-toxic to non-target species, and effective in port and at sea (Ralston and Swain 2009). The initial step in biofouling is the acquisition of a conditioning layer (Jain and Bhosle 2009), typically followed by the adhesion of microorganisms (mainly bacteria and diatoms) (Molino and Wetherbee 2008; Molino et al. 2009a,b). The efficiency of new AF formulations is usually first evaluated through laboratory bioassays towards key biofoulers including...
bacteria, microalgae, fungi, macroalgae and invertebrates (Briand 2009; Dahms and Hellio 2009). These bioassays have the advantage of being fast, reproducible and can be performed on a small scale. To be selected as a promising new AF substance/formulation, compounds need to have an effective concentration (EC_{50}, the concentration of a compound that inhibits 50% of the growth or adhesion of the target organisms when administered as a single exposure) lower than the lethal concentration (LC_{50}, the concentration of a compound that kills 50% of the target organisms when administered as a single exposure) (Dahms and Hellio 2009). Field experiments are the next step in assessing the AF performance, but they require more of the test compound(s) and are conducted over a longer time scale. So far, few studies had focused on the potential correlation between data obtained from AF performance in laboratory and field tests (eg raft trials) (eg Stafslien et al. 2007; Rittschof et al. 2008) and because of the lack of field data, the reliability and the validity of *in vitro* bioassays cannot be critically discussed.

This article focuses on the assessment of the efficiency of chemically-active AF paints through laboratory and field immersion tests. Poly(meth)acrylic resins bearing ionic complexes were selected as tin-free substitutes (Bressy et al. 2009a,b). This patented tin-free paint technology is based on seawater-erodible polymers having pendant acid functional groups in the form of ammonium salts (Finnie and Lenney 1996; Cambon 1996). The amino compound could be a primary, secondary, or tertiary amine biocide (Rahn and Van Eseltine 1947; Finlay and Callow 1996). Secondary amines were reported to be more effective against macrofouling organisms such as barnacles, whereas primary amines were reportedly more effective against algae (Hunter et al. 1995). Long-chain aliphatic tertiary amines are reported to be active against the microalgae, *Amphora coffeaeformis* and *Dunaliella parva* and toxic to nauplii larvae of the crustacean, *Artemia salina* (Finlay and Callow 1996). In addition, their toxicity was shown to depend on the pH of the solution, resulting in the ionized or unionized forms of the amine having different efficacy against the microorganisms tested. Tertiary amines are reported to be readily biodegraded, the biodegradation pathway of a long-chain aliphatic amine such as dimethyldodecylamine by a *Pseudomonas* sp. proceeding by the N-dealkylation of the dodecyl hydrocarbon chain from the amine (Alexander 1999). The resulting dodecyl chain was transformed by β-oxidation into CO_{2}, H_{2}O, and subsequently biomass (Kroon et al. 1994). All of these previous investigations showed the potential of amines as active molecules for AF coatings.

Thus, ‘ion-exchange’ binders were prepared from a commercial poly(meth)acrylic resin through a chemical modification of the carboxylic acid function with dimethylalkyl tertiary amines (Hugues et al. 2003; Bressy et al. 2008). Laboratory assays on marine bacteria (*Shewanella baltica*, *Polaribacter igeri*, *Halomonas marina*, *Pseudoalteromonas elyakovii*, *Pseudoalteromonas citrea*), microalgae (*Hymenomonas coronata*, *Rhodosorus marinus*, *Pleurochrysis roscofensis*, *Pleurochrysis carterae*, *Sacrinchrysis sp.*) and larvae of barnacles (*Amphibalanus amphitrite*, previously named *Balanus amphitrite* (Clare and Hoeg 2008)) were performed to enlarge the activity spectrum of tertiary amines. A multi-well plate screening method was used to assess the activity of the tertiary amines and the corresponding ammonium salt-based coatings towards microorganisms (Hellio et al. 2004a, 2005). Field tests (Toulon, Mediterranean Sea) were performed to assess the *in situ* AF performance. Correlations with results obtained from laboratory and field immersion trials are discussed.

**Materials and methods**

**Chemical compounds**

An acrylic resin Elvacite® 2669 (RE, Lucite International) containing 49 mol% of methyl methacrylate, 29 mol% of ethyl acrylate, and 22 mol% of methacrylic acid monomer units was used as the raw material. Dimethylolctylamine (C8N), dimethyldodecylamine (C12N), and dimethylhexadecylamine (C16N) were purchased from Aldrich and used without further purification. 1-methoxy-2-propanol (Aldrich) was used as solvent. A tributyltin based copolymer containing 68% of methyl methacrylate and 32% of tributyltin methacrylate monomer units (Cutinox 1120®, 50 wt% in toluene, from ACIMA) was used as a reference. Xylene (from Aldrich) was used to dissolve the TBT-reference (REF). Rutile titanium dioxide (TiO_{2}) (purity ≥ 98%, average particle size of 0.5 μm) supplied by Kronos was used as a hydrophobic pigment.

**Formulation of paints**

Ammonium salt-based acrylic binders were prepared by mixing the poly(methacrylic) resin RE with C8N, C12N, and C16N in 1-methoxy-2-propanol (Bressy et al. 2008). Because the reaction between the acrylic resin and the tertiary amines was previously demonstrated not to be quantitative, the resulting coatings were composed of polymer chains bearing both carboxylic acid and ammonium salt groups and also non-bonded tertiary amine (Figure 1). Simple AF paints were prepared by adding TiO_{2} to the ammonium salt-based solution using a laboratory dissolver DISPERMAT®. A pigment volume concentration (PVC) of 9% and a solid content of 37% were defined.
The fineness of grind was estimated at between 0 and 10 μm using an Elcometer 2020/2 gauge (10 on NorthScale range). These simple paints are named PECKXY, where CXN corresponds to the tertiary amine with X the number of the longer alkyl chain, and Y to the stoichiometric molar ratio between the carboxylic acid and the amino groups.

**Antifouling efficiency of the film coating and solutions of amines**

*Laboratory bioassays*

The simplest method for preparing film samples is to deposit solutions of the polymer or paint formulations with a pipette into an array of wells. Polystyrene plates (96-well) were selected for coating deposition and biological analysis. The well bottoms were modified with poly(vinyl chloride) PVC coverslips, which were compatible with the solvent-based paint formulations. Coverslips prevent the polystyrene plates from dissolution in organic solvents, and result in uniform films, free of contamination. Coatings were dried at room temperature for 2 weeks before they were used for assays. Paint formulations were classified as active or inactive towards the organisms tested.

Stock solutions of amines (C8N, C12N and C16N) were prepared at 1000 μg ml⁻¹ directly in 0.2 μl filtered seawater (FSW) (0.45 μm) and placed in an ultrasonic bath for 15 min in order to obtain homogeneous suspensions. From these stock suspensions, serial dilutions were made to obtain test concentrations in the range of 0.001–100 μg ml⁻¹.

Bioactivity was tested towards marine bacteria, microalgae and barnacle larvae. Toxicity tests were performed using nauplii of Amphibalanus amphitrite. All the bioassays were performed using six replicates and using two separate batches of organisms (Maréchal et al. 2004).

**Antibacterial assays**

Five strains of marine bacteria involved in marine surface biofilms were used, viz *Pseudoalteromonas elyakovii* (ATCC 700519), *Polaribacter ingensii* (ATCC 700398), *Shewanella baltica* (ATCC BAA-1091), *Halomonas marina* (ATCC 25374), and *Pseudoalteromonas citrea* (ATCC 29719). Bacterial strains were maintained on agar plates (LB medium [Luria-Betani Broth, Sigma, Andover, UK] enriched with NaCl [35 g l⁻¹] and containing 15% agar). Experiments were run as previously described by Maréchal et al. (2004). The wells, containing the coatings, were incubated with the bacteria (2 x 10⁸ cells ml⁻¹) in 100 μl of LB medium (supplemented with NaCl [35 g l⁻¹]), at 28°C for 48 h. A formulation was considered to be active in inhibiting bacterial growth if no growth was recorded in five or six out of the six wells. For the amines tested, EC₅₀ values were calculated using SigmaPlot (Tsoukatou et al. 2007).

**Antialgal assays**

The coatings and solutions of amines were assayed for their inhibition of growth of the benthic phase of five coastal marine microalgae obtained from Algobank (University of Caen-Basse Normandie, France), viz. *Hyphomenonas coronata* (AC115), *Rhodosorus marinus* (AC117), *Pleurochrysis roscoffensis* (AC32), *Pleurochrysis carterae* (AC1), and *Exanthechrysis gayraliae* (AC15). All microalgal cultures and assay plates were maintained under controlled conditions in a constant temperature chamber at 18 ± 2°C. The photoperiod was 15:9 light:dark (54 μmol photons m⁻² s⁻¹ cool-white fluorescent lamp). Stock cultures were kept on agar plates (F/2, agar 12.5%) (Guillard and Ryther 1962). Experiments were carried out as previously described in Tsoukatou et al. (2002). One hundred μl of a culture at 1mg ml⁻¹ of chlorophyll a were introduced in 96-well plates containing the coating. After 48 h, the optical density of the algal suspension was measured at 600 nm and compared with the controls. EC₅₀ values were calculated as explained above.

**Anti-settlement tests with cyprids of A. amphitrite**

The effect of coatings on the settlement of cyprids of *A. amphitrite* was carried out as previously described (Hellio et al. 2004b). Cyprids were reared by conventional methodology (Rittschof et al. 1992) and were aged for 3 days at 6°C (d3 cyprids) before use in settlement assays. Bioassays were conducted by adding 10 cyprids to the individual wells of a 96-well plate, each of which contained FSW and a paint formulation. The control consisted of FSW. Test plates were incubated at 28°C in darkness and examined after 24 h. Each larva was inspected under a dissecting microscope and its condition recorded (Lau and Qian 2000). Results were expressed as a percentage inhibition of settlement, and of EC₅₀ values (Tsoukatou et al. 2007).
Toxicity tests using naupliar larvae of A. amphitrite

Toxicity tests were conducted on nauplii of *A. amphitrite* according to the method of Wu et al. (1997). Ten stage-II nauplii were added to the FSW solution in the wells of a 96-well plate containing the coating or a solution of amine. The number of swimming and dead nauplii was recorded after exposure for 24 h at 28°C. For the purposes of the analysis, non-swimming larvae were regarded as dead (Rittschof et al. 1992). Toxicity results are presented as 24 h LC50 values with 95% confidence intervals for different concentrations of amine (Hellio et al. 2005).

Field immersion test

Paint formulations were applied with a bar-coater onto sand-blasted poly(vinyl chloride) panels (5 cm × 10 cm) with a dry film thickness of about 100 μm. The ammonium-salt based coatings were immersed in natural seawater (Mediterranean Sea, Toulon, + 43° 06′ 19.42″N, + 5° 53’ 07.84″E) for 9 months. Duplicate panels were immersed at a depth of 1 m. A TBT-based binder was used as a performance reference. Uncoated PVC panels were used as controls. The values of pH recorded during the immersion tests varied between 8.1 and 8.3, the lowest temperature was 13°C and the highest temperature was 28°C. The conductivity ranged from 55.8 to 57.7 mS cm⁻¹.

Results and discussion

The ammonium salt-based binders are characterized by the polymer backbone bearing acid functional pendant groups blocked by biocidal tertiary amines containing an organic group of at least 8 carbon atoms and up to 16 carbon atoms. Previous quantitative investigations on the reaction between a poly (methacrylic) resin and dimethylalkylamines demonstrated that 36–42 mol% of the acid functional groups are blocked by the tertiary amines (ie 36–42 mol% of the acid groups are in ionized form). This result suggests that free amines are still incorporated within the film coating (Hugues et al. 2003; Bressy et al. 2008). Table 1 summarizes the weight content of each component derived from simple paint formulations.

Defining the extent of ionization in the polymers is required because ionized (cationic) and unionized forms (neutral) of amine groups may play different roles in their antibacterial and antialgal action (Finlay and Callow 1997; Palermo and Kuroda 2009). Bioassays and *in situ* immersion tests were performed at a pH close to 8.2. The pKa values for the three tertiary amines were previously estimated as below 7.8 by potentiometric titration (Bressy et al. 2008). Therefore, the ammonium salt groups near the surface are converted into neutral amine groups with seawater through ion-exchange reactions (Figure 2).

Laboratory experiments

Inhibition of growth of marine bacteria and diatoms

The AF activity of ammonium salt-based coatings was tested against five marine bacteria and five marine benthic diatoms and was expressed as the number of strains for which the coating was active (Figure 3). It appears that the growth of neither bacteria nor diatoms was affected by the coatings containing C8N and C16N as tertiary amines. However, C12N significantly inhibited the growth of all the marine benthic diatoms, whereas only one strain of marine bacteria (*P. citrea*) was affected after 48h of incubation. From these results, the tertiary amine C12N can be considered to be an efficient active product that controls the development of benthic diatoms.

Solutions of free amines were tested for their potential bioactivity towards microorganisms. EC50 values > 100 μg ml⁻¹ (demonstrating the absence of bioactivity) were obtained for C8N and C16N for all the strains tested, as well as for C12N for *P. elyakovii*, *P. irgensii*, *S. baltica*, and *H. marina* (data not shown). The results obtained for the effect of C12N on the five

| Reference | Mol % of blocked acid groups | Wt % of resin | Wt % of complexed amines | Wt % of free amines |
|-----------|-----------------------------|---------------|-------------------------|-------------------|
| PE        | 0                           | 100           | 0                       | 0                 |
| PEC8N1    | 42                          | 74            | 11                      | 15                |
| PEC12N0.35| 41                          | 86            | 6                       | 8                 |
| PEC12N1   | 41                          | 68            | 13                      | 19                |
| PEC12N2   | 41                          | 51            | 10                      | 39                |
| PEC16N0.35| 36                          | 83            | 6                       | 11                |
| PEC16N1   | 36                          | 63            | 13                      | 24                |
| PEC16N2   | 36                          | 46            | 10                      | 44                |

Figure 2. General chemical structure and main reaction with seawater of ammonium salt-based poly(meth)acrylic copolymers. R = -CH₃ or -H, R₁, R₂, R₃ = -C₆H₂n+1.
marine diatoms and the bacterial strain *P. citrea* are presented in Table 2 and show a direct correlation with the results observed from the screening of the bioactivity of the coatings. Thus, the same strains of microorganisms were inhibited both by the coating containing C12N or C12N in solution. The EC50 values allow a ranking regarding the efficacy of C12N to the bacterial strains, viz. *E. gayraliae > H. coronata > R. marinus > P. roscoffensis > P. carterae > P. citrea*. The activity of the dimethylalkyl tertiary amines towards diatoms followed a similar pattern to that described for other organisms (Finlay and Callow 1996), increasing with carbon chain length up to a region of maximum value, after which further increases in chain length resulted in a decrease in activity. The decrease in the activity for C16N can be explained by adsorption of the compounds to inert surfaces and/or a decrease of solubility which may prevent biocide/membrane interactions. Therefore, C12N appears to be an interesting candidate for controlling a spectrum of fouling organisms. Finlay and Callow (1996) previously reported that C12N demonstrated the highest and most consistent activity with the algae *A. coffeaeformis* and *Dunaliella parva*.

**Settlement assays with cyprids of *A. amphitrite***

Settlement assays showed that the ammonium salt-based coatings were efficient in inhibiting the settlement of cyprids of *A. amphitrite* with a level of activity similar to the TBT-based coating. No swimming or settled larvae were recorded after exposure to the coatings for 48 h. Figure 4 shows the bioactivity of the three amines (C8N, C12N and C16N) in solution towards the inhibition of cyprid settlement. Settlement was not reduced with C8N up to a concentration of 1 μg ml⁻¹, whereas at 10 μg ml⁻¹ no settlement was observed, although almost all larvae were still alive. The result demonstrates that this compound has a repulsive effect rather than a biocidal effect. Regarding the bioactivity of C12N, settlement was not affected when C12N was used at concentrations from 0.001 to 0.1 μg ml⁻¹. However, when C12N was used at 1 μg ml⁻¹, settlement was significantly reduced with most larvae still being alive and actively swimming. However, at 10 μg ml⁻¹, no settlement was observed and all the larvae were dead. The C16N compound was active only at 10 μg ml⁻¹ and all the larvae were dead. From these results, it appears that the most environmental solution for inhibition of cyprid settlement would be to use C12N compounds (as it is active both in solution and when incorporated into a paint formulation) at a concentration of 1 μg ml⁻¹ (as it leads to total settlement inhibition without mortality). C8N and C12N were active only when tested at 10 μg ml⁻¹ and at this concentration 100% mortality of cyprids was observed.

The EC50 values for settlement for the three amines solutions (C8N, C12N, and C16N) are presented in Table 3. The efficacy of the three amines was lower than that of TBTO. Among the three amines assayed,
it is interesting to note that C12N, which displayed the best antimicrobial activity, had the highest inhibitory activity against the cyprid larvae of *A. amphitrite*, with similar EC_{50} values to CuSO_{4}. In addition, C12N can be readily biodegraded by bacteria (Alexander 1999). These results show the potential of such compounds as active ingredients for AF coatings.

**Toxicity tests using naupliar larvae of *A. amphitrite***

The toxicity of the coatings and amines in solution was tested towards mortality of nauplii of *A. amphitrite*. The results showed that the PECXN1 coatings were as toxic towards the naupliar stages as the TBT-REF sample. No swimming larvae were observed after incubation for 24h. These results suggest that the concentration of the free tertiary amines near the coating surface is higher than the LC_{50} values reported in Table 3. The three amines in solution displayed LC_{50} values > 10 μg ml\(^{-1}\), demonstrating that they are far less toxic than either TBTO or CuSO_{4}. For the three amines tested, the therapeutic ratio, LC_{50}/EC_{50}, which expresses the effectiveness of a compound in relation to its toxicity, was much greater than 1.0, demonstrating that these compounds have a suitable profile for use in an AF coatings (Rittschof et al. 2003).

**Raft immersion in natural seawater**

Different coatings were immersed on a raft to evaluate the *in vivo* AF efficiency. Uncoated PVC panels were used as controls to assess which organisms settled on untreated substrata. The main biofouling organisms observed were encrusting species such as tubeworms, bryozoans, spirorbid worms, and brown algae. These organisms grew quickly on the surface of the uncovered PVC panels from the first month of immersion and covered the entire surface after immersion for 9 months.

The colonization of ammonium salt-based coatings by fouling organisms with immersion time is shown in the Supplementary Information [Supplementary material is available via a multimedia link on the online article webpage]. There were no macrofoulers and only non-adherent slime as seen on the ammonium salt-based coatings during the first month of immersion, whatever the tertiary amines (C8N, C12N and C16N) and their loading (Figure 5). More slime was observed on coatings containing the lower amount of C12N within the coating PEC12N0.35 compared with PEC12N1 and PEC12N2 (see Table in Supplementary Information [Supplementary material is available via a multimedia link on the online article webpage]). In addition, PEC12N1 and PEC12N2 coatings showed similar efficacy in seawater, demonstrating that an excess of tertiary amine did not enhance the AF efficiency at the early stage of immersion. This result corroborates the laboratory assays that demonstrated

**Table 3. Concentration values at which settlement of cyprids is reduced 50% (EC_{50}) and 50% of naupliar larvae are killed (LC_{50} value).**

| Amine | EC_{50} (μg ml\(^{-1}\) from TNO | LC_{50} (μg ml\(^{-1}\)) |
|-------|---------------------------------|--------------------------|
| C8N   | 4.2                             | > 10                     |
| C12N  | 0.8                             | > 10                     |
| C16N  | 5.6                             | > 10                     |
| CuSO_{4} | 0.84\(^{a}\) | 0.71\(^{b}\) |
| TBTO  | 0.02\(^{a}\) | 0.004\(^{b}\) |

\(^{a}\) From Thirionet et al. 1998; \(^{b}\) From Pinkney et al. 1989.
that C12N had antimicrobial potency. For immersion for 1 month, the concentration of the amino compounds near the surface appeared to be sufficient to inhibit the settlement of micro- and macrofoulers and it could be argued that C12N had a repellent effect (as observed in the laboratory assays) while C16N caused the mortality of larvae by surface contact (as observed in the laboratory assays). Surprisingly, even if C16N showed no antimicrobial and no anti-diatom activity in laboratory assays, its performance in the field was satisfactory whatever the loading of amines. Only removable slime was observed with time on C16N-based coatings. Red algae appeared after immersion of these systems for 6 months, but they had disappeared by 9 months immersion. No barnacles settled on the PEC16N1 and PEC16N2 coatings whereas barnacles were present on the TBT-based paint. In addition, PEC16N1 and PEC16N2 coatings displayed an oily surface as the free amine content exceeded 24wt%. This hydrophobic surface combined with the AF activity of the amine could lead to a higher AF efficiency of the corresponding coatings. In summary, all the ammonium salt-based coatings inhibited the settlement of brown algae, spirobid worms, tube-worms, bryozoans, and barnacles in comparison with PVC blank panels. Although the AF efficacy was high, the coatings had poor mechanical properties. C8N- and C12N-based coatings delaminated from the panel surface after immersion for 3 and 6 months in seawater, respectively. Only the C16N-based coatings remained intact for the longer immersion time. The ammonium salt-based coatings were too sensitive to seawater and became swollen before complete dissolution/delamination. The high polishing and poor mechanical properties of these copper-free paints make them unsuitable as AF coatings until their mechanical properties are improved. This could be achieved by, for example, adding hydrophobic co-binder and/or pigment or by decreasing the number of carboxylic acid groups on the polymer backbone.

**Conclusion**

The research focused on the screening of ammonium salt-based acrylic resins as potential binders for copper-free, chemically-active AF paints. The AF efficiency of tertiary amines and the resulting coatings was assessed. Toxicity and anti-settlement tests were performed against nauplii and cyprid larvae of *A. amphitrite* and bioassays against marine bacteria and diatoms were also investigated to define the spectrum of activity of the chemically active paints. The biocidal activity of the tertiary amines used as blocking agents in poly(meth)acrylic resins was also demonstrated against each organism. Dimethyl-octyl, -dodecyl, and -hexadecyl amines displayed comparatively low activity against marine bacteria and optimum activity was obtained for the dimethyldodecyl amine with five marine diatoms. In addition, C12N demonstrated the highest and most consistent activity against the settlement of cyprid larvae of *A. amphitrite*. Laboratory assays performed on coatings showed similar trends. Poor antibacterial and anti-diatom activity was obtained for C8N- and C16N-based coatings. In addition, these coatings were revealed as toxic towards *A. amphitrite* larvae. Results from a field trial showed that coatings containing dimethyl alkyl tertiary amines provided protection against all types of macrofouling and also against slime.

Many investigators perform laboratory assays to down-select the most optimum active compounds or formulations prior to field immersion test. However, the results presented here indicate that laboratory assays do not provide sufficient information with respect to the field performance of chemically-active paints. The range of factors determining the AF performance of biocidal paints in the field is complex and it is recommended that additional laboratory bioassays are performed on coatings to define more precisely the mechanism of action of this ion-exchange paint technology.

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