Tributyl tin is an effective antifouling agent, but is also tough on marine ecosystems. As regulations are set to ban its use, the search is on for a paint ingredient to do battle with biofouling organisms. **Dr Jonathan Matias** reveals an environmentally and economically acceptable option for the ocean.

Life on the ocean wave

Marine research demonstrates how fragile ocean ecosystems can be. Scientists working in this field have shown how readily chemicals, such as pesticides and industrial wastes, generated in the last 50 years of the chemical revolution threaten human health and earth biodiversity.

Among these persistent organic chemical pollutants is tributyl tin (TBT), a toxic heavy metal incorporated in marine paints to prevent algae, oysters, mussels, barnacles, tube worms and other fouling organisms attaching themselves to the submerged portion of the ship hull. Biofouling impedes the movement of ships and assists corrosion.

The earliest recorded attempts by mariners to prevent fouling dates back to the 5th century BC. In 1625 William Beale was the first to file a patent for a paint composition containing iron powder, copper and cement. Further advances did not come about until after the Second World War with the chemical revolution producing a range of industrial chemicals for new industries.

Photo: Harbor Branch Oceanographic Institution, Fort Pierce, FL, US

New antifouling paints could soon be protecting ships like the 'R/V Seward Johnson'

TBT provided the breakthrough

A major breakthrough in antifouling technology occurred in the '70s when paint companies began to use TBT as a toxicant. The development of self-polishing paints that continuously released TBT revolutionised the ship coatings industry and permitted longer intervals between dry-docking.

However, unbeknown to its original designers, the long-term performance of TBT-impregnated paints carried with it a heavy environmental price. By the '80s and '90s, TBT was blamed for environmental damage in marine ecosystems to such an extent that the International Maritime Organization (IMO) of the United Nations passed a resolution, banning the application of TBT-containing paints by 2003, and enforcing a total ban on the presence of TBT on all ships by 2008.

The IMO Resolution and the Biocidal Products Directive of the European Union (EU) are recent examples of tighter regulatory control. In December 2000, 122 countries signed an international treaty that banned 12 of the most toxic persistent organic pollutants.

This is a harbinger of hard times to come for the biocides industry, as different compounds are likely to be added to the list. Companies willing to take a long-term view will have to rethink how their R&D funds will be allocated and focus on identifying new biocides that will survive a more difficult regulatory environment.

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Beyond the short-term solutions

The initial reaction of the coatings industry was to develop TBT-free antifoulings. Although this has led to innovations in formulation, such changes have only provided solutions to near-term needs. Many companies have optimised their coatings formulation by increasing the copper content of their paints or including biocides to augment the reduced efficiency of cuprous oxide alone.

With these new developments came increases in cost, often to two or three times that of conventional TBT-based coatings. Zinc- and copper-based biocides are non-specific, retain toxic effects and will come under regulatory pressures. With silicone-based technologies, high price alone is not a deterrent to market entry. Such non-stick coating technologies use surfactants and oils, which are toxic to marine life, and are likely targets for future regulations.

Cuprous oxide is a toxicant and an essential component of coatings. Though necessary in trace amounts for normal cellular function, toxicity occurs when cells are overloaded with copper because there is no existing mechanism for cells to remove this heavy metal from their cytoplasm.

Cutting out copper

In response to the elevated copper load in marinas, the recent working conference sponsored by the University of California Sea Grant in September recommended copper reduction programmes and advocated promoting alternative options to copper in antifouling paints.

This conference is indicative of the move toward a pro-environment stance that may eventually lead to the eventual ban on copper in the years to come in the same way that TBT has now been banned.

While it has taken over a decade to ban TBT, it is likely that regulatory control of other heavy metals, like copper, will move at a faster pace.

The adoption of the Precautionary Principle as a guide to determine legislation against the continued use of a chemical entity presents a new paradigm. This states that action should be taken against a chemical when there is sufficient evidence to prove that it threatens human and animal health, even in the absence of conclusive evidence. The search for compounds to replace TBT and other biocides has a stricter requirement for safety than before. The high cost of the discovery programme and registration of new chemical entities have deterred companies from pursuing alternative strategies.

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Search for compounds

The search for antifouling compounds from marine organisms began in earnest in the '70s and continued through the '90s based on the observation that certain marine plants and animals naturally resist fouling.

Typical examples of these are seaweeds and soft corals, notably marine sponges. Barnacles and bryozoans do not attach to these sessile organisms while they are alive. Fouling occurs only when the organisms die and no longer produce chemical defences. This initially suggested the presence of factors produced while the animals were alive because there was no other physical mechanism for these animals to rid themselves of attached foulers.

Although the research effort has produced numerous effective antifouling agents, no chemical has entered into any commercial stage because it is not economically viable to manufacture them.

Natural compounds include renillafoulins and juncellins, which were discovered as natural antifoulants in soft corals. These substances are complex and the high cost of synthetic production makes them economically non-viable. As new chemical entities, they will have to go through the same costly regulatory process as new synthetic compounds. In addition, because large and complex molecules have multiple functions in organisms, they are likely to have other modes of action that will contribute to an unsatisfactory safety profile. It is also likely that only a portion of the molecule functions as an inhibitor of attachment. A more prudent use of resources appears to be focusing on identifying the functional group important in antifouling.

Poseidon's discovery programme to understand the structure of the functional groups that contribute to antifouling effects in these complex molecules began in the mid-80s. By the mid-90s, it began to unravel the structural conformations that appeared to produce such effects.

Poseidon used its understanding of the structural requirements and matched known natural products – terrestrial and marine – that come close to such structures. The Natural Bioproducts (NB) Screening Program enabled the organisation to select a range of biological products to mimic the target structural requirement.

The key to the success of the NB Screening Programme is its ability to screen the biological effects of candidate substances. Poseidon used the barnacle *Balanus amphritite Darwin* (see sidebar). Poseidon's NB compounds were selected based on the following criteria:

- inhibition of settlement below 0.025mg/ml
- good safety profile
- amenable to low production cost
- stable chemical structure

Only a few select compounds were found to satisfy the above criteria. Compounds that were effective but expensive to manufacture were automatically eliminated for further studies. The best in the series, for proprietary reasons, is referred to as 'NB-9514.17' ('NB17') and is profiled here briefly as an example of the characteristics of compounds discovered through Poseidon's Natural Bioproducts Screening Programme.

When subjected to the barnacle settlement assay, NB17 showed a better efficacy than juncellin, the active antifoulant found in Juncella juncea. A comparison of NB17 with other purified extracts of sponges

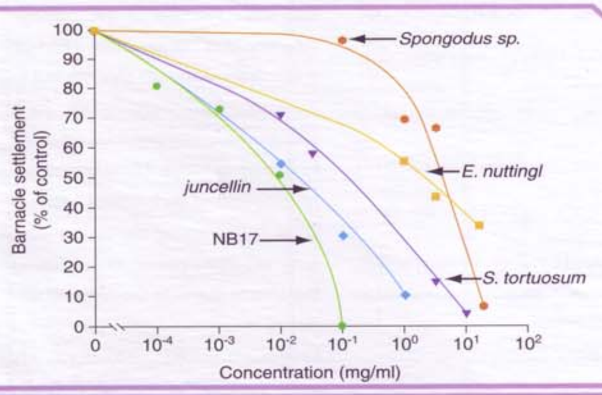

Figure 1: comparison of the inhibitory effect of NB17 on the settlement of the barnacle, *Balanus amphitrite Darwin*, against *Juncellin* and purified antifouling extracts from marine sponges in the Gulf of Mannar (India)

from the Gulf of Mannar (South India) is shown in Figure 1. NB17, a mimic of the functional group in natural antifoulants, has a good efficacy profile. The effective concentration (EC_{50}) for NB17 is 0.004mg/ml, which exceeded the standard requirement of an EC_{50} of 0.025mg/ml established by the US Navy Programme as an acceptable efficacy level for natural antifoulants.

To test the performance of NB17 under field conditions, iron panels were painted with a conventional paint composition containing cuprous oxide. In place of TBT, Poseidon introduced 2% NB17.

The experimental and control panels without NB17 were continuously submerged in San Dionisio Bay (Philippines) for 1.5 years. This protected bay area is rich in planktons and in biofouling organisms. The primary foulers are *Balanus amphitrite* communis and the rock oyster, *Crassostrea cuculata*.

Hard fouling became evident in the control panels by the ninth month. After one year, the control panels (without NB17) were totally encrusted with barnacles. In comparison, the experimental panels were practically devoid of fouling, except in minute areas where there was physical damage to the painted surface.

Booster biocides

In the near-term, copper remains an important component in marine paints. Various biocides, such as 'Sea-Nine' from Rohm and Haas or 'Zinc Omadine' from Arch Chemicals, are added as boosters to improve the efficacy of copper. While these booster biocides are effective against soft fouling (algae and bacteria), they are generally not as effective in preventing hard fouling organisms, such as barnacles and oysters.

Poseidon's NB17 may be used as a complimentary repellent against barnacles to augment the biocidal action of these compounds.

Current efforts at Poseidon are being directed toward developing the best formulations that will optimise the effect of NB17 in the absence of copper and other booster biocides.

Additional data indicated that NB17 has antibacterial activity. In a panel of 40 marine bacteria usually associated with barnacles, there was effective inhibition against Flavobacterium sp and Aeromonas sp. Other bacterial strains were moderately sensitive.

NB17 has also been shown to be inhibitory to marine microalgae such as *Dunaliella tertiolecta* and *Nitzchia sp.* Poseidon is continuing its toxicity screening to determine the safety profile of NB17 against a wider range of marine organisms.

Safety profile

Besides efficacy, toxicity is a major issue of concern. Poseidon Ocean Sciences has also examined the toxicity profile of NB17. The data shown in Table I is from a typical acute toxicity study on the effect of exposure of marine larvae to NB17.

The lethal dose to barnacle larvae lethal dose (LD_{50}) for NB17 is 0.6mg/ml. In the case of the nauplii of the brine shrimp, *Artemia salina*, as test organism, the LD_{50} was 0.75mg/ml. The LD_{50} for TBT is approximately 0.0000000034mg/ml. This shows a good margin of safety in the use of NB17 as an antifoulant considering its effective concentration was at 0.004mg/ml.

Mysids, tiny crustaceans found in all the world's oceans, are important in the marine food chain. These organisms are used routinely in toxicity tests to evaluate potentially hazardous chemicals in the environment.

When exposed to NB17, mysids demonstrated a high survival rate with a lethal dose being reached at 1.5 g per litre. In the 15-day old fry of the grouper, a priced coral fish, a much higher tolerance was observed. Even at high concentrations of 10g per litre, the grouper fry showed remarkable tolerance to NB17.

In plants and animals, there are specific pathways to biodegrade NB17. Collectively, the data so far strongly suggests that NB17 is safe to use in the marine environment.

Rittschof[2] pointed out that the industry will have to look at its antifouling R&D programme with a different point of reference. If the point of comparison for efficacy uses existing potent biocides and toxicants, natural antifoulants with low toxicity profiles cannot be expected to meet industry expectations.

Natural antifoulants that are non-toxic are likely to work through a repellent mechanism by disrupting specific cellular functions associated with attachment. Alternatively, they may work as minor irritants rendering the surface unfavourable for attachment, parameters untested or unused by existing toxicants.

The processes involved in the attachment to surfaces by diverse organisms vary. A single natural antifoulant will probably not meet industry requirements. A combination of antifoulants working through different mechanisms will provide the spectrum of responses to meet the industry's needs.

Poseidon has chosen NB17 as its primary candidate because of its efficacy and safety. The cost of manufacturing this compound is also quite low and comparable to the raw material costs for toxicants like TBT.

As the search for effective antifouling agents continues, compatibility with various paint formulations, leaching rate and long-term environmental impact need to be assessed.

A full list of references is available from our offices.

Balanus amphitrite Darwin

is a predominant biofouling organism found in nearly all harbours in the world. It is an ideal test organism because of its once-a-week reproductive cycle, rapid larval growth, standardised methods to rear them in mass cultures and a predictable settlement behaviour.

Upon reaching stage IV-nauplius, the larvae undergo transformation into cyprids, the non-feeding settlement stage. The cyprids walk over a surface using antennules, which possess numerous sense organs and are able to secrete a temporary adhesive.

Once a suitable surface is selected, the cyprid fixes itself on the surface by producing cement through its sucking disc. The cyprid moults into a pinhead barnacle and becomes firmly entrenched on the surface 24 hours later. The process of attachment is a complicated event, with several environmental cues affecting successful settlement. The barnacle settlement assay developed by Rittschof et al enabled Poseidon to pursue a rational approach to the selection of a series of naturally occurring compounds referred to as the NB Class.

Parameter	concentration
EC_{50}	0.004mg/ml
LD_{50}	0.600mg/ml

Table 2: comparison of the effective concentration and the lethal dose of NB17 on the larvae of the barnacle, *Balanus amphitrite Darwin*

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