APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. **13**, 135–143 (1999)

Review

Alternative Antifouling Biocides

N. Voulvoulis, M. D. Scrimshaw and J. N. Lester*
Environmental Processes and Water Technology Group, The T. H. Huxley School of the Environment, Earth Sciences and Engineering, Imperial College of Science, Technology and Medicine, London SW7 2BP, UK

In response to increasing scientific evidence on the toxicity and occurrence of organotin residues from antifouling paints in the aquatic environment, the use of triorganotin antifouling products was banned on boats of less than 25 m length in many countries during 1987. The use of tributyltin (TBT) products on small boats was superseded by products based on copper, containing organic booster biocides to improve the efficacy of the formulation. Available information and evidence on the occurrence, fate and toxicity of these biocides is reviewed. It is concluded that increased copper concentrations in the aquatic environment, due to the increased use of copper-based antifoulants, do not have significant effects on marine ecosystems. However, lack of validated analytical methods, limited monitoring data, and very little information about the fate and toxicity of the booster biocides in the aquatic environment, make accurate risk assessments in relation to these compounds difficult. Copyright © 1999 John Wiley & Sons, Ltd.

Keywords: copper; booster biocides; chlorothalonil; dichlofluanid; diuron; Irgarol 1051; Kathon 5287; dithiocarbamates; zinc pyrithione; TCMS pyridine; TCMTB

Received 13 January 1998; accepted 8 April 1998

INTRODUCTION

Antifouling products play an important role in the shipping industry and are of significant economic importance. It is estimated that, on average, fuel consumption increases 6% for every 100 μm increase in the average hull roughness caused by fouling organisms.[1,2] For example, tributyltin (TBT) saves the US Navy an estimated $US150 million annually.[3] Many antifouling systems have been used over the centuries, from copper and lead sheathing to paints containing compounds of copper, lead, arsenic and mercury in the 20th century. Organotins were first used in the mid-1960s, due to their acute toxicity to target organisms. Concern over the effects of organotins used in antifouling paints first arose in France, where severe problems were encountered in commercial oyster fisheries in areas where there was intense boating activity and poor tidal exchange.[4–6] Since then, the distribution, fate and effects of organotins on the marine[7,8] and freshwater environments[9,10] have been studied extensively. Research evidence of the damaging effect of triorganotins on the reproduction and growth of various marine life forms has prompted action by many countries to regulate or ban their use in antifouling products.[3,11] Following these legislative controls, TBT contamination of boat harbour water and fresh waters has decreased;[12,13] however, studies have indicated that even though regulations were effective in reducing TBT levels, contamination of sediments by organotin compounds is still widespread and has ecotoxicological consequences.[14,15] As a consequence of these restrictions, the use of these products on small boats was superseded by the use of products based mainly on copper oxides with organic booster biocides to improve the efficacy of the formulation by inhibiting the primary growth of copper-resistant fouling organisms such as algal slimes.

The available information on the occurrence, fate and toxicity of the copper-based alternatives to TBT-based antifoulants is reviewed, and evidence on the booster biocides is presented to determine whether their substitution is likely to yield any environmental benefits.

---

* Correspondence to: Prof J. Lester, Head of Env Processes, TH Huxley School, Imperial College, RSM Building, Prince Consort Rd, London SW7 2BP, UK.
E-mail: j.lester@ic.ac.uk

CCC 0268–2605/99/030135–09 $17.50

ALTERNATIVE BIOCIDES

In the absence of the antifouling potency of TBT, a copper compound such as cuprous oxide (Cu_2O), copper thyocyanate (CuSCN) or metallic copper is utilized as the principal biocide. Copper exhibits antifouling activity against organisms such as barnacles, tube worms and the majority of algal fouling species. However, several algal species (e.g. *Enteromorpha* spp., *Ectocarpus* spp., *Achnanthes* spp.) show marked physiological tolerance to copper. In order to achieve protection against these tolerant species, various booster biocides are used in conjunction with copper to control copper-resistant fouling organisms. Twelve booster biocides have been used as active ingredients in approved amateur and professional copper-based antifouling products marketed in the UK.[16] In addition, in most cases when TBT is still used (boats >25 m), TBT copolymer compositions normally include copper compounds and an organic booster as a co-biocide in order to provide effective performance against the whole spectrum of fouling organisms.[3]

Copper in the aquatic environment

Some concern about the occurrence of copper in the aquatic environment has arisen out of the assumption that further regulatory action on triorganotin-based antifouling products is likely to increase the use of copper-based products.

In the UK, the current Environmental Quality Standard (EQS) for copper in seawater is $5\ \mu g\ l^{-1}$ (expressed as an annual average). This is designed to protect all marine life and associated non-aquatic organisms (i.e. direct and indirect effects have been taken into account). However, the EQS refers to dissolved and not total copper, because copper forms insoluble complexes in seawater which effectively bind the copper and reduce its bioavailability. The UK EQS for copper is similar to those used in the USA and Denmark ($2.9\ \mu g\ l^{-1}$, acute criterion), but less stringent than the Ecotoxicological Assessment Criterion recommended by the Oslo and Paris Commission ($0.1–1.0\ \mu g\ l^{-1}$), which is used as a guide to identify areas for further monitoring.[17]

Copper is an essential element, required for the normal growth of all plants and animals, and occurs commonly in the environment.[18,19] However, high concentrations can be deleterious to algae and other aquatic biota.[20] Copper is not lipophilic and shows only a slight tendency for bioaccumulation. The most bioavailable, and thus the most toxic form of ionic, unbound copper, is the free hydrated ion, $Cu(H_2O)_6{}^{2+}$. Copper speciation is governed by pH, salinity and the presence of dissolved organic matter.[3]

Copper toxicity to algae depends upon the individual species, their physiological and environmental conditions, and the chemical forms of metal in the medium. For example, *Hydrilla verticillata* (an aquatic macrophyte) exhibited indications of toxic stress in the presence of copper at low pH (4.5), while at high pH (9.5) toxicity was considerably reduced.[21] Biological indicators differ widely with respect to copper sensitivity and a general decreasing order of sensitivity would be: micro-organisms > invertebrates > fish > bivalves > macrophytes.[22] The presence of water-soluble ligands that bind copper reduces toxicity, probably by decreasing the concentration of free ionic copper.[23] Binding of the cationic species with organic ligands results in the formation of anionic, hydrophilic and kinetically inert copper chelates.[24] Speciation studies carried out in coastal waters indicate that more than 99% of the total copper is strongly bound or chelated with organic ligands, leaving the concentration of free Cu^{2+} at levels that are non-toxic to most organisms. In addition there is evidence that strong copper chelators are synthesized and excreted by micro-organisms in response to gradual and potentially toxic increases in copper concentrations in the water column.[25]

However, the effects of some organic compounds (including booster biocides such as dithiocarbamates) and copper are additive, as lipophilic complexes are formed and synergistic effects have been observed.[26] Short-term uptake experiments with the fungicides ziram and maneb using a coastal diatom demonstrated that these Zn and Mn complexes can subsequently form lipophilic organic copper complexes in the environment that diffuse across the plasma membrane and into the cytosol of the cell. Intracellularly, copper is thought to dissociate from the transport ligand and complex intracellular ligands. On the basis of these results, it has been suggested that the presence of dithiocarbamate fungicides in surface water may inadvertently enhance the passive uptake of a variety of toxic heavy metals from the water into biota.[27]

A number of studies have tried to link changes in the environmental concentrations of copper with its use in antifouling paints. Monitoring of oysters in Arcachon bay (on the Atlantic coast of France) over the period 1979–1991 demonstrated an increase in the copper content of oysters (*Crassostrea gigas*) in the vicinity of marinas and mooring areas. This was attributed to the growing use of copper-based paints.[28] In another study, monitoring and analysis were conducted in inshore Chesapeake Bay, including three boating marinas where leaching of copper from antifouling paints was found to be significant. It was demonstrated that all the stations yielded highest mean dissolved copper concentrations during August and September. Increasing copper concentrations were observed between the inner harbour and the exit channel on falling tides, followed by a decrease as the channel broadened out into open waters.[29] In general, copper concentrations above the EQS in water are expected to be causing a range of deleterious sublethal effects in several invertebrate phyla, and even lethal effects in early life stages.[17] Of particular concern is the production of the readily absorbed lipid-soluble organic complexes with other compounds used in antifoulants (e.g. dithiocarbamates).[30] However, in the UK the Advisory Committee on Pesticides (ACP) has suggested that the contribution from antifouling products to the environmental loading of copper is not a cause for concern. Subsequently it has recommended that monitoring of copper in water and appropriate biota should continue and also be conducted in and around UK harbours and marinas to assess the risk to sediment-dwelling organisms.[31]

Organic booster biocides

Organic booster biocides that have been used as active ingredients in approved amateur and professional antifouling products marketed in the UK are shown in Table 1. Irgarol 1051 (Irgarol) is highly effective against freshwater and marine algae. It belongs to the *s*-triazine group of compounds which act as photosystem-II (PSII) inhibitors, with the inhibition of photosynthetic electron capture transport in chloroplasts as their biochemical mode of action.[32] Diuron, one of the major urea herbicides in use since the 1950s, also inhibits photosynthesis. Although utilized in antifoulants, it is predominantly used on land for general weed control on non-crop areas. However, it has been detected in saline coastal waters at concentrations higher than in fresh waters, suggesting that its use in antifouling products may be of significance.[33] Kathon 5287 is a highly effective, broad-spectrum biocide. It is an isothiazolone compound which is licensed for use as an active component in antifouling products in the UK and elsewhere. It was the first organic booster biocide to be registered for use by the USA-EPA.

Various other booster biocides are also used in antifouling applications: chlorothalonil, dichlofluanid, TCMTB, TCMS pyridine, zinc pyrithione and the dithiocarbamates thiram, ziram, zineb and maneb (Table 1). These are protective fungicides with a wide range of action against a number of organisms.

Occurrence

Booster biocides were introduced into antifouling paint formulations only after restrictions were imposed on the use of organotins. Little or no monitoring of these biocides has been carried out, possibly as these compounds were not perceived to be an environmental problem. Their relatively recent introduction, limited usage and perceived lower toxicity in comparison with TBT may have been some of the factors reducing their priority as compounds of environmental concern. Lack of established analytical methodology may also have been a contributory factor.[34] Limited monitoring data are available for Irgarol[35–38] whereas, among the other booster biocides, diuron, chlorothalonil, dichlofluanid and some of the dithiocarbamates have been studied in the marine environment, but not in the context of their use as antifouling products. Almost all of these compounds have agricultural uses (pesticides, fungicides, herbicides etc.). Therefore their presence in the aquatic and estuarine environments cannot be attributed solely to the use of antifoulants (Table 2).[16]

Water samples collected from marinas, estuaries and coastal waters along the Southern UK coast and sediment samples from the Hamble estuary in the UK were analysed by Gough *et al.*[37] Irgarol residues were present in most marine and estuarine samples, but were not detected in fresh waters. The highest concentrations were found in areas of high boating activity, particularly marinas and the Hamble estuary, indicating a correlation with its use in antifouling paints. Sediment contamination with Irgarol was found to be related to high concentrations in the water column. In another survey, selected antifouling agents were monitored in subsurface waters from the Mediterranean (Cote d'Azur) coastline, and substantial levels of Irgarol were detected in all marinas, with concentrations reaching 640 $\mathrm{ng\,l^{-1}}$.[35] Toth *et al.*,[36] assessing the contamination of different compartments of Lake Geneva (water, sediments, zebra mussels, macrophytes and algae) over a period of nine months,

Table 1. Nomenclature and structures of booster biocides currently used in antifouling paints

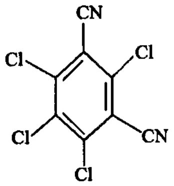
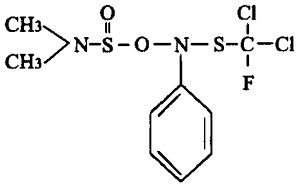
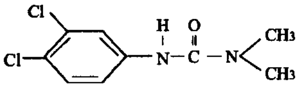
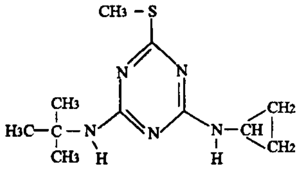
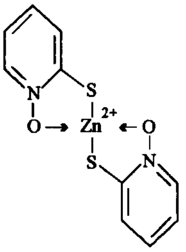
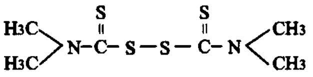
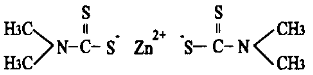
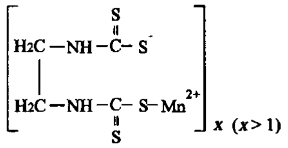
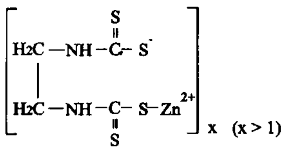
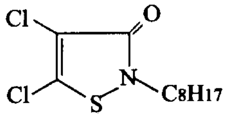
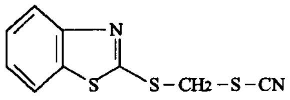
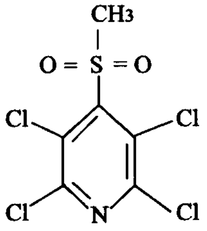
Biocide	Structure
Chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile)	
Dichlofluanid (*N*′-dimethyl-*N*-phenylsulphamide)	
Diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea]	
Irgarol 1051 (2-methylthio-4-*t*-butylamino-6-cyclopropylamino-*s*-triazine)	
Zinc pyrithione (zinc complex of 2-mercaptopyridine-1-oxide)	
Thiram [bis(dimethylthiocarbamoyl)disulphide]	
Ziram [zinc bis(dimethyl thiocarbamate)]	
Maneb (manganese ethylene bisdithiocarbamate)	
Zineb (zinc ethylene bisdithiocarbamate)	

Table 1. Continued.

Biocide	Structure
Kathon 5287 (4,5-dichloro-2-*n*-octyl-4-isothiazolin-3-one)	
TCMTB [2-(thiocyanomethylthio)benzothiazole]	
TCMS pyridine [2,3,5,6-tetrachloro-4-(methylsulphonyl)pyridine]	

found concentrations comparable with those observed by Tolosa *et al.*[35] and Gough *et al.*[37]. In a more recent study, water samples taken from Plymouth Sound (UK) were analysed for Irgarol, which was detected at all sampling sites. The highest levels were found in close proximity to areas of high boat density, especially where water flow was restricted within marinas. The highest detected value was 127 ng l^{-1} at Sutton Harbour Marina.[38]

Of the other biocides, the impact of the agricultural uses of diuron was demonstrated by a survey detecting herbicide residues in water samples from draining streams and pumping stations of the agricultural area of Thessaloniki, Greece. Considerable amounts of diuron were found to be released from the agricultural fields and transferred through rivers, draining streams and pumping stations to the coast.[39] In the UK, Environment Agency (EA) data show that one of the pesticides which most frequently exceeds the EQS of 1 mg l^{-1} in Controlled Waters is diuron, predominantly due to its use as a herbicide in agriculture, although some inputs are related to its use as an antifouling agent in paints.[33,40]

Degradation and toxicity

There are many factors that influence the degradation and persistence of these biocides in the marine

Table 2. Other applications of booster biocides

Compound	Other applications
Chlorothalonil	Agriculture (fungicide), paints, adhesives
Dichlofluanid	Agriculture (fungicide)
Diuron	Agriculture (herbicide)
Irgarol 1051	Agriculture (herbicide)
Kathon 5287	No other applications
Maneb	Agriculture (fungicide in fruits and vegetable crops)
TCMS pyridine	No other applications
TCMTB	Agriculture (fungicide), wood preservative, leather industry
Thiram	Agriculture (fungicide, leaf and seed treatment)
Zinc pyrithione	Agriculture (bactericide, fungicide), shampoos (at concentrations of around 1%)
Zineb	Agriculture (fungicide, fruits and vegetable crops)
Ziram	Agriculture (fungicide, fruits and vegetable crops)

environment. These include their chemical and physical properties as well as ecosystem-specific parameters such as the nature and concentration of microbial populations, dissolved and suspended material, temperature, etc. In a study investigating biodegradability in seawater, the breakdown of diuron, Irgarol, Kathon and chlorothalonil was monitored over eight weeks by bioassay using *Amphora coffeaeformis* (a ship-fouling diatom). The results demonstrated that abiotic pathways were unlikely to be a major route for degradation of these substances in the sea, although photolysis may be significant in the upper layers of water. Irgarol and diuron were not readily biodegraded, while chlorothalonil biodegradation became apparent after four weeks and proceeded faster in seawater supplemented by cultured marine bacteria. Kathon was the most readily biodegradable compound studied, having a half-life of 8.5 days in natural coastal seawater.[41] In another study on the fate of Kathon in the aquatic environment, it was found that its half-life was less than 1 h in both an aerobic and an anaerobic microcosm consisting of marine sediment and seawater.[42]

The toxicity of diuron has been studied in 30-day-old *Pimephales promelas* (fathead minnow) and the 24, 48, 96 and 168 h LC_{50} values were found to be 23.3, 19.9, 14.2 and 7.7 mg l^{-1} respectively. Diuron exposures of concentrations less than 78 μg l^{-1} did not significantly affect hatchability of fathead minnow eggs or fish growth (up to 60 days post-hatch). However, at higher exposure (>78 μg l^{-1}) fish survival decreased and numbers of dead and deformed fry increased. The maximum acceptable toxicant concentration was determined to lie between 33 and 78 μg l^{-1}. Diuron did not bioaccumulate to any great extent and its residues in fathead minnow tissue were quite rapidly lost (the bioconcentration factor was less than 2.0).[43] Molander *et al.*[44] investigated the combined effects of tributyltin and diuron on marine periphyton in indoor aquaria over a four-week period. A reduction in diatom species richness accompanied by increased pollution-induced community tolerance was observed.

The highest detected concentration of Irgarol (over 120 ng l^{-1}) in the study carried out in the Plymouth Sound locality[38] significantly inhibited the growth of *Enteromorpha intestinalis* (a green seaweed) spores under laboratory conditions. Photosynthetic efficiency in the adult frond of *E. intestinalis* from Sutton Harbour marina was inhibited by Irgarol in the laboratory with an EC_{50} (72 h) of 2.5 μg l^{-1}. The no-effect concentration was 22 ng l^{-1}. In a recent study, it was indicated that *Phanerochaete chrysosporium* (white rot fungus) is capable of biotransforming Irgarol. It appeared that the metabolism of Irgarol by the fungus proceeds mainly via partial *N*-dealkylation, and metabolic dealkylation occurred at the cyclopropylamino group resulting in a metabolite (stable and/or terminal product) which has been tentatively identified as 2-methylthio-4-*t*-butylamino-6-amino-*s*-triazine. No evidence of the heterocyclic ring cleavage of Irgarol was observed, thus implying a possibility that its degradation products may accumulate in the environment.[1] In investigations into the environmental fate of Irgarol, the Mackay fugacity models and the ECOSAR toxicity model were applied to assess its potential fate in the environment and effects on organisms in receiving waters. A Mackay modelling comparison with atrazine suggested that, like atrazine, Irgarol resides principally in the aqueous phase but that Irgarol would partition to sediments to a greater extent than atrazine. The Level 1 fugacity model predicted that 95% of Irgarol residues would be present in the dissolved phase of marine waters, with 4.4% partitioning to sediment. Negligible volatilization was predicted. Literature data and ECOSAR predictions indicated that Irgarol is more toxic to algae than other trophic levels. Concentrations of Irgarol reported to inhibit algal growth were 10–100 μg l^{-1}, depending on the test species.[45]

Various workers have studied the toxicity of dithiocarbamates in different environmental matrices.[26,46–53] In an investigation into the synergistic toxic effects of dithiocarbamates and copper, using *Colpidium campylum* (a ciliate), it was found that ziram was toxic above concentrations of 10 mg l^{-}. However, in the presence of Cu^{2+}, ziram concentrations an order of magnitude lower caused observable effects. For thiram the synergistic effect was more marked, with toxic concentrations being reduced from 1 mg l^{-1}, in the absence of copper, to 0.01 mg l^{-1}, with free Cu^{2+} ions present.[26] In further studies, Van Leeuwen *et al.* investigated the toxicity of ethylenebisdithiocarbamates, dialkyldithiocarbamates and a number of degradation products to *Salmo gairdneri* (Rainbow Trout).[51,54,55] All compounds were found to be teratogenic, inducing severe skeletal abnormalities at concentrations below 0.1 mg l^{-1}.

The toxicity of chlorothalonil has been studied by a number of workers. Ernst *et al.*[56] presented data on the acute toxicity of chlorothalonil to a number of aquatic organisms. Exposure of *Daphnia* to concentrations as low as 32 μg l^{-1} significantly increased the time to production of the first young, but there were no delayed effects on the number of young produced or on growth at concentrations up to 180 μg l^{-1}. Mussels initially accumulated the fungicide 10-fold, but the body concentration returned to the exposure concentration within 96 h. Spraying of ponds with chlorothalonil killed *Sigara alternata* (caged water boatmen) and *Gasterosteus aculeatus* (three-spined stickleback) but caused no significant mortality of other species of aquatic insects and fish tested. Other tests on the *in vivo* toxicity of chlorothalonil to *Ictalurus punctatus* (channel catfish) showed the chemical to be highly toxic, with a 96 h LC_{50} value of 52 μg l^{-1}.[57]

DISCUSSION

Over the period 1988 to 1993 the use of the alternative, TBT-free, antifouling biocides rose from 2% to 16% of global market share,[58] predominantly in the pleasure and coastal craft sectors as a result of the legislative controls on organotin-based products. The possibility of further regulatory action on these products and also the sensitivity of the coastal areas, in which these craft are used, make the need for reviewing these alternative biocides of significant importance.

Although there is now enough scientific evidence on the toxicity and continued occurrence of organotin residues[3,15] to suggest the need for a total ban on the use of organotin-based products, the main issue to be addressed is the availability and appropriateness of the alternative biocides. The substitution of organotins by other biocides may damage the marine environment and create other environmental penalties if all the parameters related to the performance of the antifouling coatings are not taken into account. Economic and environmental performances are closely related. New antifoulants will be required to be as effective against the fouling organisms as the organotin compounds, otherwise economic penalties in terms of fuel, maintenance, inspection and corrosion costs as well as environmental penalties such as greater fuel consumption resulting in increased SO_2 and CO_2 emissions, will be incurred. Ideally the alternative antifoulants will also be less toxic to non-target marine organisms, in comparison with organotins, otherwise there may be no net environmental benefit to offset penalties incurred through increases in operating costs and fuel emissions. Ongoing improvements in new biocide coatings[59] may narrow the performance gap that is perceived to exist between TBT-based coatings and their replacements[58] and decrease the resistance of the shipping industry to further restrictions on the use of TBT.

In a regime of waning popularity of organotin-based antifouling formulations, care must be taken to avoid their substitution with other biocides which may demonstrate no overall environmental benefit. Although the toxic effects of the replacement booster biocides to aquatic ecosystems have not yet been studied extensively, these substances have the potential to cause environmental damage, as they need to be toxic to a range of marine species to function as antifouling agents.

CONCLUSIONS

The contribution from antifouling products to the environmental loading of copper needs further investigation. Increases in copper concentrations in the water column due to the use of copper-based antifoulants are observed, but complexation by dissolved and suspended organic materials may significantly reduce its bioavailability. However, it would be unwise to conclude that copper is not causing a significant environmental impact until further work on the bioavailability of copper indicates otherwise.

Alternatives to organotin-based products are of potential concern to the environment, predominantly because of the organic booster biocides they contain. Lack of validated analytical methods, limited monitoring data, and little information about the fate and toxicity of these booster biocides in the marine environment, make accurate risk assessments of these substances difficult.

Before any changes in policies are considered, it is recommended that environmental monitoring for organic booster biocides used as alternatives to organotin products should be considered as a priority, and toxicity testing should be undertaken in light of observed environmental concentrations. The UK Department of the Environment, Transport and the Regions (DETR) has already embarked on such a programme, but it will be some time before environmental concentrations of these biocides are well established. It is important that validated risk assessments of alternative products should be carried out, while it is expected that the forthcoming EC Biocides Directive will assist in that direction.[60] Monitoring programmes on organotins and copper should also continue to assess respectively the recovery of the areas from TBT contamination and the bioavailability of copper to marine organisms.

REFERENCES

1. D. Liu, R. J. Maguire, Y. L. Lau, G. J. Pacepavicius, H. Okamura and I. Aoyama, *Wat. Res.* **31**, 2363 (1997).
2. R. L. Townsin, Development in the calculation of rough underwater surface power penalties. In: Celena 25th Anniversary Symposium, Genoa 1987.
3. S. J. de Mora, Tributyltin: case study of an environmental contaminant. Cambridge Environmental Chemistry, Cambridge University Press, Cambridge, 1996, Vol. 8, and references cited therein.
4. J. M. Ruiz, G. Bachelet, P. Caumette and O. F. X. Donard, *Environ. Pollut.* **93**, 195 (1996).
5. P. Quevauviller, O. F. X. Donard and H. Etcheber, *Environ. Pollut.* **84**, 89 (1994).
6. C. Alzieu, *Mar. Pollut. Bull.* **17**, 494 (1986).
7. E. A. Clark, R. M. Sterritt and J. N. Lester, *Environ. Sci. Technol.* **22**, 600 (1988).
8. R. J. Maguire, *Wat. Sci. Technol.* **25**, 125 (1992).
9. P. H. Dowson, J. M. Bubb and J. N. Lester, *Mar. Pollut. Bull.* **24**, 492 (1992).
10. P. H. Dowson, D. Pershke, J. M. Bubb and J. N. Lester, *Environ. Pollut.* **76**, 259 (1992).
11. M. M. M. Lau, *Arch. Environ. Contam. Toxicol.* **20**, 299 (1991).
12. P. H. Dowson, J. M. Bubb and J. N. Lester, *Mar. Pollut. Bull.* **26**, 487 (1993).
13. P. H. Dowson, J. M. Bubb and J. N. Lester, *Chemosphere* **28**, 905 (1994).
14. P. H. Dowson, J. M. Bubb, T. P. Williams and J. N. Lester, *Wat. Sci. Technol.* **28**, 133 (1993).
15. Y. K. Chau, R. J. Maguire, M. Brown, F. Yang and S. P. Batchelor, *Wat. Qual. Res. J. Canada* **32**, 453 (1997) and references cited therein.
16. Anon, Pesticides 1998, HMSO, London, 1998.
17. Environment Agency, Evaluation of copper and zinc concentrations in Suffolk and Essex estuaries, Report BLC032, 1997.
18. A. G. Lewis, Copper in Water and Aquatic Environments, International Copper Association, 1994.
19. A. G. Lewis and W. R. Cave, *Oceanogr. Mar. Biol. Ann. Rev.* **20**, 471 (1982).
20. C. Chang and T. H. Sibley, *Bull. Environ. Contam. Toxicol.* **50**, 689 (1993).
21. M. Gupta, S. Sinha and P. Chandra, *Ecotoxicology* **5**, 23 (1996).
22. Y. M. Nor, *Environ. Res.* **43**, 274 (1987).
23. M. Ahsanullah and T. M. Florence, *Mar. Biol.* **84**, 41 (1984).
24. J. R. Donat, K. A. Lao and K. W. Bruland, *Anal. Chim. Acta* **284**, 547 (1994).
25. K. W. Bruland, in: Workshop on Research Needs for Coastal Pollution in Urban Areas, National Science Foundation, Wisconsin, 1997.
26. H. Bonnemain and D. Dive, *Ecotox. Environ. Safety* **19**, 320 (1990).
27. J. T. Phinney and K. W. Bruland, *Environ. Toxic. Chem.* **16**, 2046 (1997).
28. D. Claisse and C. Alzieu, *Mar. Pollut. Bull.* **26**, 395 (1993).
29. W. S. Hall, S. J. Buschong, L. W. Hall, M. J. Lenkevich and A. E. Pinkney, *Environ. Monit. Assess.* **11**, 33 (1988).
30. J. L. Stauber and T. M. Florence, *Mar. Biol.* **94**, 511 (1987).
31. ACP, A review of the use of copper compounds in antifouling products. Advisory Committee on Pesticides, Health and Safety Executive — Pesticides Registration Section, London, 1997.
32. B. Dahl and H. Blanck, *Mar. Pollut. Bull.* **32**, 342 (1996).
33. NRA, Pesticides in the Aquatic Environment, HMSO, London, 1995.
34. N. Voulvoulis, M. D. Scrimshaw and J. N. Lester, *Chemosphere*, Accepted for publication (1999).
35. I. Tolosa, J. W. Readman, A. Blaevoet, S. Ghilini, J. Bartocci and M. Horvat, *Mar. Pollut. Bull.* **32**, 335 (1996).
36. S. Toth, K. Beckervanslooten, L. Spack, L. F. Dealencastro and J. Tarradellas, *Bull. Environ. Contam. Toxicol.* **57**, 426 (1996).
37. M. A. Gough, J. Fothergill and J. Hendrie, *Mar. Pollut. Bull.* **28**, 613 (1994).
38. A. Scarlett, M. E. Donkin, T. W. Fileman and P. Donkin, *Mar. Pollut. Bull.* **34**, 645 (1997).
39. T. A. Albanis, *Chemosphere* **22**, 645 (1991).
40. DoE, Pesticides in Water: Report of 'The Working Party on the Incidence of Pesticides in Water', HMSO, London, 1996.
41. M. E. Callow and G. L. Willingham, *Biofouling* **10**, 239 (1996).
42. A. Jacobson, L. S. Mazza, L. J. Lawrence, B. Lawrence, S. Jackson and A. Kesterson, Fate of an antifoulant in an aquatic environment. In: Pesticides in Urban Environments: Fate and Significance, ACS Symp. Ser. No. 522, Racke,, K. D. and Leslie,, A. R. (eds), Americam Chemical Society, Washington DC, 1993, pp. 127–138.
43. D. J. Call, L. T. Brooke, R. J. Kent, M. L. Knuth, S. H. Poirier, J. M. Huot and A. R. Lima, *Arch. Environ. Contam. Toxicol.* **16**, 607 (1987).
44. S. Molander, B. Dahl, H. Blanck, J. Jonsson and M. Sjostrom, *Arch. Environ. Contam. Toxicol.* **22**, 419 (1992).
45. H. R. Rogers, C. D. Watts and I. Johnson, *Environ. Technol.* **17**, 553 (1996).
46. S. Babo and P. Vasseur, *Aquat. Toxicol.* **22**, 61 (1992).
47. R. Bluzat, O. Jonot and J. Seuge, *Environ. Pollut. (Ser. A)* **29**, 225 (1982).
48. R. Bluzat and J. Seuge, *Environ. Pollut.* **31**, 133 (1983).
49. D. Dive, M. N. Fourmaux, P. Vasseur, M. Pussard, R. Pons and B. Marais, *Environ. Pollut. (Ser. A)* **36**, 121 (1984).
50. J. Seuge, D. Marchal-Segault and R. Bluzat, *Environ. Pollut.* **31**, 177 (1983).

51. C. J. Van Leeuwen, T. Helder and W. Seiner, *Aquat. Toxicol.* **9**, 147 (1986).
52. C. J. Van Leeuwen, G. Niebeek and M. Rijkeboer, *Ecotoxicol. Environ. Safety* **14**, 1 (1987).
53. M. Weigand-Rosinus, K. Haberer, U. Obst and A. Wild, *Zeitschrift fur Wasser- und Abwasser-Forschung* **23**, 98 (1990).
54. C. J. Van Leeuwen, J. L. Mass-Diepeveen and H. C. M. Overbeek, *Aquat. Toxicol.* **9**, 13 (1986).
55. C. J. Van Leeuwen, A. Espeldoorn and F. Mol, *Aquat. Toxicol.* **9**, 129 (1986).
56. W. Ernst, K. Doe, P. Jonah, J. Young, G. Julien and P. Hennigar, *Arch. Environ. Contam. Toxicol.* **21**, 1 (1991).
57. E. P. Gallagher, R. C. Cattley and R. T. Di Giulio, *Chemosphere* **24**, 3 (1992).
58. J. D. Pidgeon, Critical Review of Current and Future Marine Antifouling Coatings, Lloyd's Register Engineering Services, 1993.
59. Anon, *Chemistry in Britain* **33**, 7 (1997).
60. European Commission, Proposal for a Council Directive Concerning the Placing of Biocidal Products on the Market. In: *Official Journal of the European Communities*, No C 239/3.