Research on tributyltin in Australian estuaries

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Tributyltin (TBT) from marine antifouling paints has been shown to have a major impact on the oyster industry in eastern Australia. Current research projects are examining the impact of TBT on Australian estuaries, assessing the response of sensitive biota to recently imposed bans and determining whether a continuing use of TBT on large vessels is an environmental concern.

Keywords: Tributyltin, estuaries, Australia, hydride generation, atomic absorption, gas chromatography, copper

INTRODUCTION

The majority of Australia's population resides on its coastal fringe, with population centres usually on estuaries. These estuaries have seen increasing numbers of moored boats, and in some waterways these are posing a threat to existing oyster industries because of the usage of tributyltin compounds (TBT) as the active biocide in marine antifouling paints. This problem has been recognized worldwide, and studies, particularly in France,[1–3] revealed the toxicity of TBT to embryonic and larval stages of the Pacific oyster, *Crassostrea gigas*, as well as indicating reduced growth and shell thickening in mature oysters. Concern for these effects has now led to the banning in many countries of the use of TBT-based paints on pleasure boats.

Oyster culture, particularly in eastern Australia, is a multi-million dollar industry. In New South Wales, the preferred commercial species is the Sydney rock oyster, *Saccostrea commercialis*. In 1970, oyster farmers in Sand Brook Inlet on the Hawkesbury River (NSW) first noted shell deformities and reduced growth rates in cultivated oysters. Experiments by Scammell in 1987 showed that TBT induced curling in the shell extremities of *Saccostrea commercialis*, and it was also possible to correlate the incidence of oysters with deformities and the presence of moored boats.[4]

The first Australian research on TBT commenced in 1986 at the CSIRO Centre for Advanced Analytical Chemistry at Lucas Heights (NSW), with investigations into improved methods of TBT analysis. In 1987, in collaboration with the NSW State Pollution Control Commission (SPCC), measurements were made on water samples from Sydney Harbour (NSW) and the nearby Georges River estuary. These data, obtained at a time of heightening concern for TBT in Australia, were presented at a Conference on Organotins in the Marine Environment in Lismore (NSW)[5] in February 1988. They showed concentrations in water ranging from 90 to 150 ng Sn dm^{-3} in areas of high boating activity, including a naval base, while in the majority of samples concentrations were below 45 ng Sn dm^{-3} and nearer 10 ng Sn dm^{-3} in uncontaminated sites. Although it was possible to predict, using a tidal prism model, that the ambient concentration of TBT based on boat numbers, painted areas, etc., should be below 12 ng Sn dm^{-3} (in agreement with the findings), water analyses of single samplings must be considered a poor estimate of the true impact of TBT. A total picture of the TBT flux in the system is required and especially measurements on a biological integrator of this flux.

A survey was therefore undertaken of tributyltin in Sydney rock oysters from leases in the Georges River, and as well as from samples from the poorly flushed Sand Brook Inlet site and from more pristine sites further north.[6] While the former had tissue concentrations below 2 ng Sn g^{-1}, values between 80 and 130 ng Sn g^{-1} were obtained in areas of high boat density (Table 1). A set of oysters from Sand Brook Inlet

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Table 1 Tribyutyltin in Australian aquatic biota

Species	Site	TBT[a] (ng Sn g^{-1} fresh wt)
Saccostrea commercialis	Upper Georges River, NSW	40–128
	Lower Georges River, NSW	15–44
	Coba Bay, Hawkesbury River, NSW	7
	Sand Brook Inlet, Hawkesbury River NSW	350
	Wallis Lake, NSW	2
	Botany Bay, NSW	15
Crassostrea gigas	Upper Georges River, NSW	175
Ostrea angasi	Port Phillip Bay, VIC	<1
Mytilus edulis	Cockburn Sound, WA	18
	Near slipway, Cockburn Sound, WA	166
Pecten alba	Port Phillips Bay, VIC	5

[a] The results are typical of a number of samplings at each location, and each figure was obtained from three pooled animals. Where a range is indicated, values between these numbers were obtained from selected sites along the River (see ref. 6).

registered 350 ng Sn g^{-1}. These data added strength to the case for government action to restrict TBT usage. A ban on the sale and usage of TBT-based antifouling paints on boats under 25 m in length was instituted in NSW in 1989, and similar bans are now in place in most States in Australia.

Prior to the banning, a number of research projects were initiated to assess the extent of the TBT problem and the impact of TBT in the Australian estuarine environment. This paper describes these studies, some of which are continuing and will be the subject of future publications.

EXPERIMENTAL

Sample collection and storage

Water samples were collected in 2-dm^3 polycarbonate containers and stored at 4°C prior to analysis. Sediment samples were collected using grab or core samplers, and were frozen for storage. Homogenized wet samples were used for analysis with moisture content being determined on a separate aliquot. Shellfish samples were chilled after collection and the flesh removed from the shells as soon as possible after receipt at the laboratory. The separated tissue was washed with distilled water and excess water removed with a filter paper prior to storage in polystyrene containers at 4°C.

Surface microlayer samples were collected using a rotating drum collector constructed in our laboratory to a design developed by the National Water Research Institute, Burlington, Canada. This design was modified to incorporate a polycarbonate rather than a ceramic drum, to avoid adsorptive losses of TBT.

Analytical procedures

Whilst earlier analyses for TBT in our laboratory used capillary column gas chromatography of extracted butyltin hydrides, better precision and lower detection limits were obtained using a purge and trap method similar to that described by Donard *et al.*[7,8] For water samples, 500-cm^3 aliquots are first acidified by the addition of 5 cm^3 of concentrated HCl. Alkyltin species together with inorganic tin are extracted twice with 0.05% tropolone in hexane (25 cm^3 and 10 cm^3) and back-extracted into 5 cm^3 of 0.05 M-nitric acid, with the solvent being removed by evaporation. For sediment and oyster samples tropolone in dichloromethane is preferred and, as described previously,[6] samples of oyster tissue homogenate (0.2 g) or wet sediment (0.5 g) are first ultrasonicated with concentrated HCl (5 cm^3) and methanol (5 cm^3). The extracted species are then converted to hydrides by reaction with sodium borohydride, and the hydrides are displaced from the reaction vessel by helium and trapped on a

Teflon column filled with 3% OV-101 on Chromosorb G-AW and cooled in liquid nitrogen. The trapped hydrides are thermally desorbed with the application of a heating ramp and are atomized, in the presence of hydrogen and oxygen, in a heated quartz furnace positioned in the burner mount of an atomic absorption spectrometer. The absorbance of 224.6 nm is recorded and the concentrations of tin species obtained by peak area integration.

For water samples, 500-cm^3 aliquots were taken and a detection limit of 0.6 ng Sn dm^{-3} was obtained. For sediment and oyster samples a detection limit of 1.2 ng Sn g^{-1} was obtained using 0.2-g samples.

Copper was determined in water samples by anodic stripping voltammetry and in oyster and sediment samples, after nitric acid digestion, using inductively coupled plasma emission (ICP) spectrometry.

RESULTS AND DISCUSSION

TBT in Australian waters

Results have now been obtained in water samples collected in most States of Australia, and a selection of these are presented in Table 2. In most instances, concentrations below 20 ng Sn dm^{-3} have been obtained, unless samples had been collected in close proximity to large surface areas of TBT-antifouled boats. Since the banning in NSW in 1989, previously sampled sites where concentrations near 45 ng Sn dm^{-3} were being measured, now show much lower values.

Sediments have been examined from similar sites. Whilst high concentrations of TBT (2–40 μg Sn g^{-1}) have been obtained from sampling close to marinas, it was typical to find values nearer 1 ng Sn g^{-1}) have been obtained from sampling close to marinas, it was typical to find values nearer 1 ng Sn g^{-1} (on a dry weight basis) in sandy sediments and 50 ng Sn g^{-1} in silty material. There is some doubt as to whether the very high numbers might not include paint flakes from the hydroblasting of paint on marina slipways where waste waters were not being contained.

There have been varying times reported for the half-life of TBT in estuarine sediments. We have found TBT in sediments to a depth of 15 cm in Sydney Harbour which is not inconsistent with the findings of De Mora *et al.*[9] who for the Tamaki Estuary, Auckland, New Zealand, observed TBT to a depth of 30 cm. From their data, they were able to calculate a half-life of 1.85 years, which is considerably longer than the reported time of sixteen weeks.[10] This greater persistence is important in considering the impact on sediment-feeding biota.

Table 2 Tributyltin in Australian waters

Site	Description	TBT[c] (ng Sn dm^{-3})
Georges River, NSW	Oyster growing area	8–40
Kogarah Bay, NSW	Near slipway	100
Garden Island, NSW	Naval dockyard	190
Rushcutters Bay, NSW	Large marina	112–220
Manly, QLD[a]	Enclosed area near marina	109
Swan Bay, QLD[a]	Fish sanctuary	14
Southport, QLD[a]	Near marina	45
Great Keppel Island, QLD[a]	Uncontaminated area	1
Lakes Entrance, VIC[b]	Port of Melbourne Authority slipway	249
Clifton Springs, Port Phillip Bay, VIC[b]	Shellfish farming area	23
Mornington, Port Phillip Bay, VIC[b]	Shellfish farming area	3
South Australia	Marina site	198
South Australia	Swimming beach	<1

[a] Data from Division of Fisheries and Wetlands Management, Queensland Department of Primary Industries.
[b] Data from Victorian Environment Protection Authority.
[c] These results are for single samples, and are typical of results measured over a number of samplings at the particular site. More details on the data for NSW are given in Ref. 5.

TBT and gastropods

Bryan *et al.*[11] have shown, from both laboratory and field tests, that the incidence of imposex in the population of the dogwhelk *Nucella lapilla* in south-west England could be attributed to TBT. In Australia, the Australian Research Council is currently funding a project to examine the use of gastropod species as an indicator of TBT pollution in Australian waters. This work is a collaboration between the Australian Nuclear Science and Technology Organization's Environmental Biology Section, the SPCC Centre for Environment Toxicology, and our Centre for Advanced Analytical Chemistry.

Two species, *Thais orbita* and *Morula marginalba*, have been sampled in a number of NSW estuaries, and the degree of imposex has been shown to correlate well with boating activity. Experiments have been undertaken to determine unambiguously the concentrations of TBT at which imposex is induced, by using polycarbonate tanks which do not concentrate TBT on their walls to which the animals are attached. In initial studies, evidence of male characteristics in female whelk species was found at TBT concentrations above 4 ng Sn dm^{-3}.

TBT in Australian bivalves

Data have now been obtained for TBT in oysters, scallops, and mussels from a range of Australian estuaries. Selected results are given in Table 1. In the case of bivalves growing in similar TBT environments (similar sediment and bulk water concentrations), there was a marked difference in TBT content between intertidal oysters and subtidal species such as, scallops (*Pecten alba*) and mud oysters (*Ostrea angasi*) (Table 1). In the former, tissue TBT reached 100 ng Sn g^{-1} (wet weight), while in the latter it rarely exceeded 15 ng Sn g^{-1}. Mussels grow under both conditions and were able to accumulate large concentrations of TBT in the vicinity of high contamination (Table 1).

A clear difference between the behaviour of these species is the likely exposure of intertidal oysters to the surface microlayer, which is likely to be enriched in TBT which will concentrate in the presence of hydrophobic constituents such as gasoline (from outboard motors), oil and algal films. Oysters sense the receding tide and therefore pump faster. Given such feeding patterns, it is likely that as the tide rises and falls the surface film will play an important role in TBT uptake by the oyster. Initial measurements have shown enrichments of over 20-fold in the surface film compared was subsurface samples. These measurements were made after banning of TBT usage in NSW, and pre-banning measurements have indicated that enrichments as high as 10^4 are possible.[12] Similar investigations are planned to determine whether problems with scallop recruitment may also be attributable to TBT enriched in the surface microlayer where the free-swimming scallop spat may feed in search of algae.

Impact on oysters of TBT removal from an estuary

Whilst there has been clear evidence produced for the impact of TBT on oysters, the sensitivity of oysters to small concentrations of TBT and the effects of removal of the source of TBT have yet to be demonstrated. At Wapengo Lake, a pristine lake on the southern NSW coast, a unique opportunity to study this sensitivity presented itself. Two boats were introduced to and moored in the lake, one for a period of 11 months and the second, freshly painted, for four weeks. The boats were then removed from the lake, with oysters being sampled one and seven days after removal of the boats. Samples of oysters from 30 leases in the lake were examined for shell deformities. Adult oysters (*Saccostrea commercialis*) displayed shell deformities when the tissue TBT concentration exceeded 40 ng Sn g^{-1} (Table 3).[13]

The proportion of TBT and its degradation products, dibutyltin (DBT) and monobutyltin (MBT), in the oyster tissue revealed an interesting trend. Oysters with deformed shells contained more TBT than DBT and MBT, while oysters with no shell deformations contained more MBT than TBT and DBT. Seven days after removal of the boats, oysters contained more of the breakdown products DBT and MBT than TBT. Given that the half-life of TBT in estuarine waters is generally agreed to be around six days,[14] oysters could be responding to the increasing concentration of degradation products in the lake waters. Although this seems a rapid response, it is likely that the half-life of TBT in the surface layers or associated with phytoplankton may be shorter than this time. Alternatively, within the oyster, metabolic processes may lead to a rapid breakdown of TBT. This appears unlikely given the

Table 3 Butyltins in Wapengo Lake oysters

Site no.	Distance from boats (m)	Shell curls	Before boat removal TBT (ng Sn g^{-1})	DBT	MBT	After boat removal TBT (ng Sn g^{-1})	DBT	MBT
1	0	6+	40	8	4	19	46	3
2	20	2–3	21	13	2	11	22	67
3	340	1–2	27	13	1	10	12	<0.2
4	240	1–2	27	14	35	32	11	0.5
5	150	0–1	13	15	0.7	4	30	2
6	60	0–1	12	14	6	<0.5	48	3
7	480	0	<1	5	75	7	5	96
8	1150	0	2	8	66	2	16	15

disproportionate concentrations of TBT over its degradation products in most oysters, but is being examined in oyster transplant experiments.

Copper and TBT uptake by *Saccostrea commercialis*

An examination of the composition of commonly used marine antifouling paints has shown that most are based on mixtures of TBT and copper [as the oxide in these formulations but as copper(I) thiocyanate in the latest paints]. This is based on the fact that whilst TBT is an effective biocide, it is less effective against plant growth, for which an additive such as copper is needed. Paint contents, on a dry weight basis, included mixes containing 43% Cu, 2.3% Sn and 30% Cu, 1% Sn, as well as 25% Cu only and 3.1% Sn only.

Analyses for copper on all of the oyster samples analysed for TBT revealed a significant correlation between the presence of both elements (Fig. 1), implicating a similar source, antifouling paints. Despite potential inputs from stormwater runoff and other sources, antifouling paints remain the important source of copper. This is in itself a valuable finding as some NSW oysters had been displaying high copper concentrations.

It is possible that the presence of both elements could have synergistic effects on their uptake by oysters and, if so, this could have implications for the effect of copper alone as the alternative to TBT. To examine this, oysters were exposed to sets of four sticks each painted with either of two paints, one based on TBT only (Epiglass DRP, Healing Industries Pty Ltd, NSW) and the other copper only (Vinyl Long Life Copper Coat, International Paints Pty Ltd, NSW), whose respective contents are as referred to above. The sets comprised (1) four copper, (2) three copper and one TBT, (3) two copper and two tin, (4) one copper and three tin, and (5) four tin painted sticks, and (6) four unpainted sticks as a control. In each duplicated experiment, sets of translocated oysters were placed 25 cm from each set of sticks for an exposure period of three months.

Whilst the uptake of TBT was found to be relatively rapid, the uptake of copper was not. The results (Fig. 2) show that the presence of TBT substantially increasses the tissue levels of copper, but only where the TBT concentration is high, but at lower concentrations no synergism

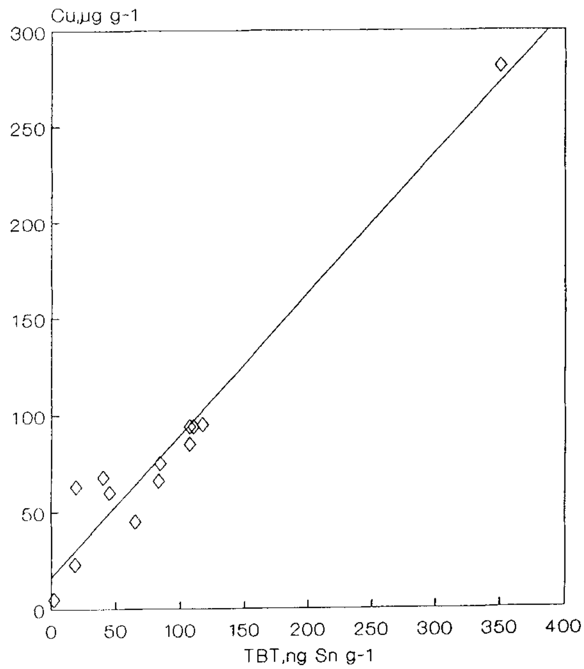

Figure 1 Correlation between copper and TBT contents of *Saccostrea commercialis*.

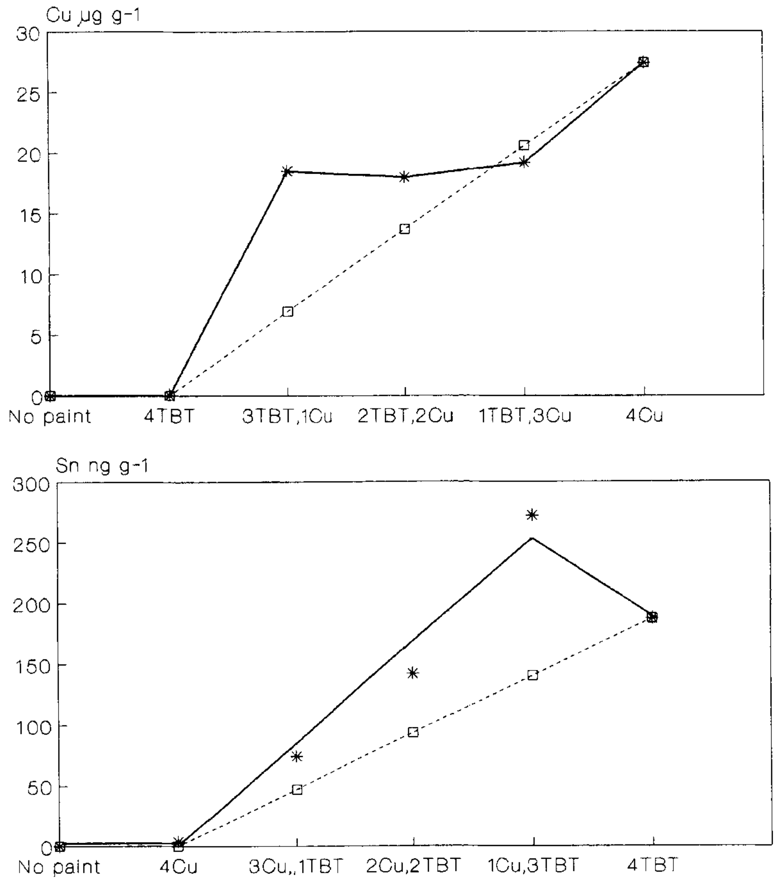

Figure 2 Effect of (a) copper and (b) TBT, on the uptake of TBT by *Saccostrea comercialis*: *, practical; □, theoretical.

was evident. On the other hand copper had a clearer effect on TBT, with tissue levels being 1.8 times greater in the presence of TBT. It seems likely therefore that, in the absence of TBT, the uptake of copper by oysters could be reduced. Further experiments to confirm this are in progress.

Fate and transport of TBT

The general question of the fate and transport of TBT has been addressed in a number of overseas studies.[10,14] In Australia, as in most overseas countries, although the impacts of TBT on oyster culture will be alleviated, and already locations in NSW are showing a recovery, TBT-based paints will continue to be used on large vessels. In major harbours, such as Sydney Harbour and Port Phillip Bay near Melbourne, TBT will continue to have an impact on biota. The problem is exacerbated by freshly painted boats, from which the initial leach rate may be as much as 20 times the final recommended leach rate of 4 μg TBT cm^{-2} day^{-1} (USA EPA).

CONCLUSIONS

Australian research on TBT has confirmed the need for the currently imposed bans to protect sensitive local shellfish industries, although there is a need for continuing research on replacement biocides. In determining the suitability of alternative paint formulations, it will be important to understand the ways by which harmful biocides such as TBT are accumulated by the different shellfish species so that their impact can be minimized. Current research should provide these answers. Despite the banning of TBT on small craft, TBT may still pose a threat to biota in bays having major ports or naval bases. Research will also define the environmental management action required to reduce the impact on non-target organisms from TBT associated with larger vessels.

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REFERENCES

1. His, E and Robert, R *Int. Counc. Explor. Sea. Committee Meeting (Mariculture)*, 1980, 27: 1
2. Alzieu, C, Thibaud, Y, Heral, M and Boutier, B *Rev. Trav. Inst. Pech. Marit.*, 1980, 44: 301
3. Alzieu, C, Heral, M, Thibaud, Y, Dardignac, M J and Feuillet, M *Rev. Trav. Inst. Pech. Marit.*, 1981, 45: 101
4. Scammell, M S Honours Thesis, University of Sydney, 1987
5. Batley, G E, Mann K J, Brockbank, C I and Maltz, A *Aust. J. Mar. Freshwater Res.*, 1989, 40: 39
6. Batley, G E, Chen Fuhua, Brockbank, C I and Flegg, K J *Aust. J. Mar. Freshwater Res.*, 1989, 40: 49
7. Donard, O F X, Rapsomanikis, S and Weber, J H *Anal Chem.*, 1986, 58: 772
8. Randall, L, Donard, O F X and Weber, J H *Anal. Chem.*, 1986, 184: 197
9. De Mora, S J, King, N G and Miller, M C *Environ. Technol. Lett.*, 1991, in press
10. Clark, E A, Sterrit, R M and Lester, J N *Environ. Sci. Technol.*, 1988, 22: 600
11. Bryan, G W, Gibbs, P E, Hummerstone, L G and Burt, G E *J. Mar. Biol. Assoc.* UK, 1986, 66: 611
12. Maguire, R J, Chau, Y K, Bengert, G A, Hale,. E J, Wong, P T S and Kramer, O *Environ. Sci. Technol.*, 1982, 16: 698
13. Scammell, M S, Batley, G E and Brockbank, C I *Archiv. Environ. Contamin. Toxicol.*, 1991, in press
14. Maguire, R J *Appl. Organomet. Chem.*, 1987, 1: 475