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Butyltin Compounds in the Oyster, *Saccostrea cucculata,* from the West Coast of India

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Oganotins, especially tributyltin (TBT) compounds, are used in a wide range of applications including stabilizers in the PVC industry, plastic additives, industrial catalysts, insecticides, fungicides, bactericides, wood preservatives and antifouling paints (Hwang et al. 1999; Hoch 2001). As an antifouling paint additive it is used exclusively on ship and boat hulls, sewage pipe systems, docks, fishnets and buoys to prevent the growth and attachment of barnacles, mussels, tube worms, algae and other marine fouling organisms. Such large scale utilization has resulted in the occurrence and high abundance of TBT and other organotins in many aquatic and marine environments (Hoch 2001; Champ 2000). TBT is one of the most toxic compounds to be introduced deliberately into marine environment by man (Goldberg 1986). Even at very low concentration (<10 ng/l), TBT can cause several detrimental effects on non target organisms, including high larval mortality, severe malformation of shells and reduced reproduction in oysters (Alzieu 1991, 2000), imposex in dogwhelk populations (Law et al. 1998; Evans et al. 2000), growth retardation in mussels (Salazar and Salazar 1991) and microalgae (Beaumont and Newman 1986). Because of these environmental effects many countries have formulated laws to control the usage of TBT.

India is one of the most rapidly developing countries in South-Asia. In India, TBT compounds have been used as an antifouling agent in marine paints. The usage of organotins is not controlled in India. Further, little is known of organotin concentrations in the marine environments of India. In this report we present the results of the first survey of butyltins in oysters from the Indian waters. To elucidate the levels of butyltins, TBT and its degradation product, DBT were determined in the edible oyster *Saccostrea cucculata* collected from the Dona Paula Bay, west coast of India over a period of 15 months from May 1999 to July 2000.

MATERIALS AND METHODS

Samples of the oyster *Saccostrea cucculata* were collected at monthly intervals during May 1999 to July 2000 from a location in Dona Paula Bay

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(15.31^0 N, 73.59^0 E). Samples were immediately transported to the laboratory where the soft tissues were removed using clean stainless steel spatula and lyophilized. The lyophilized material was powdered using mortar and pestle and stored at -20^{0}C pending analysis.

Butyltin compounds were extracted from the oyster tissue following the method of Thomas et al. (2000). A known aliquot (500 mg) of the lyophilized tissue sample was weighed and transferred to a clean tube to which was added 10 ml of 0.1 % NaOH in methanol followed by 645 ng of tripropyltin chloride (TPrT) as an internal standard. At this point a mixed standard of TBT, DBT and TPrT was prepared by adding 605 ng of TBT, 1015 ng DBT and 645 ng TPrT to 10 ml of 0.1 % NaOH in methanol. A blank containing 10 ml of 0.1 % NaOH in methanol and 645 ng of TPrT was prepared. The sample, standard and blanks were vortexed for one hour to extract butyltin compounds. Then 5 ml hexane and a 50 mg of sodium borohydride were added to all the tubes and the reaction mixture was vortexed for 15 minutes. The hexane layer containing the derivatised compounds was removed with a pipette and the methanol layer was re-extracted twice with hexane. All three hexane extracts containing the butyltin compounds were pooled. The hexane extract was dried over anhydrous sodium sulphate and concentrated to 500 µl using a gentle stream of nitrogen.

Separation and quantification of butyltin compounds were performed using a capillary gas chromatograph (Agilent HP 6890 Series plus model) with a flame photometric detector (FPD), a tin specific filter (610 nm) and a HP-1 capillary column (5 m x 530 µm x 2.65 µm). A 1µl sample or standard mixture was injected using a programmable on column injector with an initial oven temperature of 60^0 C. After 2 minutes the oven temperature was programmed to 230^{0}C at 20^{0}C min^{-1} and held at this temperature for 8 minutes. Nitrogen was used as a carrier gas (1 ml min^{-1}). The injector was operated in track oven mode, and FPD detector was maintained at 250^{0}C with hydrogen and air flowing at 80 and 105 ml min^{-1}, respectively. Quantification of each peak in a sample was done using the data handling system installed in the instrument.

Precision of the analytical method based on six replicates varied from 8 to 10 % for both DBT and TBT. The average recovery rates for DBT and TBT spiked into the sample, and passed through the entire analytical procedure were between 82 to 96 % for each compound.

Organic carbon was estimated using the method of Parsons et al. (1984). In order to extract lipid, oyster tissue was refluxed with 6% methanolic potassium hydroxide in a boiling water bath for 4 hrs. After cooling, the sample was extracted three times with dichloromethane. The extracts were pooled and dried over anhydrous sodium sulphate, and analysed for lipid content following the method of Parsons et al. (1984).

RESULTS AND DISCUSSION

Seasonal changes in the concentrations of butyltin compounds were evident in the oyster *Saccostrea cucculata* during the period May-1999 to July-2000 (Table 1). Concentrations of TBT and DBT ranged between 25 to 368 ng/g dry wt and 33 to 87 ng/g dry wt, respectively.

Table 1. Seasonal variation of organic carbon, lipid and butyltin compounds in oyster, *Saccostrea cucculata* from Dona Paula bay during May-1999 to July 2000.

Sampling period	OC mg-C/g	Lipid mg/g	Butyltin Compounds (ng/g) DBT	TBT	Total	DBT / TBT
May-99	386.4	16.0	42.7 ± 5.5	38.7 ± 3.2	81.3	1.10
Jun-99	281.8	14.5	41.7 ± 3.5	25.7 ± 1.1	67.3	1.62
Jul-99	333.6	15.0	44.4 ± 7.7	43.4 ± 2.7	87.9	1.02
Aug-99	395.9	15.6	49.7 ± 3.2	55.3 ± 5.5	105.0	0.90
Sep-99	408.1	11.4	33.8 ± 3.9	118.7 15.9	152.5	0.28
Oct-99	349.9	11.9	61.7 ± 2.5	138.3 ± 6.9	200.0	0.44
Nov-99	357.2	5.0	63.8 ± 7.5	248.9±17.1	312.8	0.25
Dec-99	347.9	10.5	79.9 ± 5.5	285.9 ± 3.2	365.8	0.28
Jan-00	311.5	9.2	70.2±10.8	209.1 ± 5.3	279.3	0.33
Feb-00	332.5	9.6	87.8±11.9	223.3 ± 5.5	311.1	0.39
Mar-00	327.6	8.9	ND	368.5 ± 1.7	368.5	ND
Apr-00	408.8	7.9	55.4 ± 4.7	107.9±15.6	163.4	0.51
May-00	441.7	13.0	56.7 ± 2.5	233.2 ± 8.5	290.0	0.24
Jun-00	341.6	13.8	66.9±10.9	161. ± 13.9	228.4	0.41
Jul-00	311.5	10.4	59.3 ± 5.3	173.8 17.0	233.1	0.34

ND – Not detected.

Generally, the concentrations of both TBT and DBT increased from late May 1999 until March 2000. Thereafter, the values of TBT and DBT decreased marginally with the exception of the value observed for the month of April 2000. Concentrations of butyltin compounds were generally high in winter and summer (November to May) and low during the monsoon period (June to September). TBT was the most abundant constituent (38 to 100 %) of total butyltins.

Seasonal changes in TBT levels have been frequently reported for water samples (Hoch 2001; Champ 2000; Evans and Huggett 1991). These changes have been related to seasonal changes in boating activities in the respective area. Page and Widdows (1991) found temporal variations of TBT levels in *Mytilus edulis* collected from Lynher River, UK, and related this to seasonal inputs from recreational boat launching in spring and early

summer, and reduced flushing of the estuary in spring and summer. Skarphedinsdottir et al. (1996) also reported strong seasonal variability in the concentrations of TBT and DBT in *M. edulis* and dogwhelk *Nucella lapillus* collected from Reykjavik harbour, Iceland. They did not find any correspondence between the intensity of ship traffic and the accumulation of butyltins in these animals since there was no seasonal variation in these parameters in this locality, and pleasure craft are rarely used. Nevertheless, they related the seasonal variations in TBT and DBT with seasonal feeding and resting activity of dogwhelk and feeding activity of the blue mussel.

Relatively higher TBT concentrations were observed during winter (December) and early summer (March), while lower concentrations were observed in late summer (April/May) and monsoon season (June/September). Poor growth and spawning is generally observed during the monsoon period (June/September). Thus, low levels of TBT were associated with poor growth condition period. Growth, gonad development and spawning activity is generally observed for the remaining times of the year (*Krishnakumari, personal communication*). This indicates some correspondence between the abundance of butyltin compounds and growth and development. However, ship traffic in the adjacent Marmugao harbour as well as in the Bay is at a minimum during the monsoon season (June/September) and starts increasing from October and generally is high for the winter (November to February) and summer (March to May) periods. Similarly, the traffic of recreational boats at the study site increases from October to May. Therefore, increase in the intensity of ship traffic also compares well with an increase in TBT abundance in the oyster. This suggests the role of ship traffic in controlling TBT concentrations in the oyster. Thus, from our data it would appear that the concentration of TBT in oyster tissues may be influenced by both growth patterns and the intensity of shipping traffic.

In order to predict if the contamination is recent or not, it is useful to calculate the butyltin degradation index. An estimation of the fate of TBT can be obtained by calculating the ratio of the degradation product DBT and the parent compound TBT. The DBT/TBT ratio or the degradation index is shown in Table 1. For most of the study period the DBT/TBT ratio is less than 1 indicating recent inputs of TBT. These values also suggest that the degradation process is very slow in these animals. Relatively higher DBT/TBT ratios in May, June and July may indicate that abiotic or biotic degradational processes of TBT were occurring in oyster tissues.

There was a drop in the levels of TBT in the tissue of the oyster collected in April. This may suggest depuration by the oyster. Experimental studies have shown that many organisms can depurate quickly. Zuolian and Jensen (1989) observed 50% of organic tin was depurated after 40 days in clean seawater, while Laughlin et al. (1986) found depuration of 50% of

tissue burden after 14 days. Osada et al. (1997) reported that in the oyster, *Crassostrea gigas*, TBT levels rapidly declined when transferred to fresh seawater.

Organic carbon and lipid concentration in oyster tissues also showed seasonal changes during the period of study (Table 1). However, there were inverse correlations between the concentrations of butyltin compounds and the organic carbon ($y = -1.624 X + 797.54$; $r^2 = 0.3056$; $P \leq 0.05$) and lipid concentrations ($y = -35.18 X + 644.95$: $r^2 = 0.6869$; $P \leq 0.001$) in oyster tissues. Butyltin compounds are hydrophobic in nature and because of this we would have expected a positive relationship with lipids. The observed inverse relationship between TBT compounds and the lipids suggest that some other processes were perhaps involved in the accumulation of the TBT in oysters.

The seasonal fluctuations in the body burden of TBT imply that the timing of monitoring TBT may be critical. Our results indicate that the appropriate time for sampling oysters for the estimation of maximum burden of TBT is winter or early summer. Stephenson (1991) reported TBT values ranging from ~34 to 4000 ng/g with most of the values being <500 ng/g in *Crassostrea gigas* collected from 13 locations in the coastal waters of the California, US. Wade et al. (1991) observed an average concentration of 49-3559 ng/g for the oyster *Crassostrea gigas* collected during 1986 to 1989 from the Gulf of Mexico. When these authors repeated the observations in the year 1990, the TBT values showed some decrease and varied from 14-1718 ng/g. The concentrations of TBT varied from 5 to 17 µg/g wet weight in the Sydney Rock oyster *Saccostrea commercialis* sampled from the Hawkesbury river estuary, Australia (Hardiman and Pearson 1995). Concentrations of TBT measured in the oysters collected from the Dona Paula Bay, west coast of India are comparable to those reported by the above authors.

Bioconcentration of TBT by living organisms is facilitated due to its lipophilic nature. The bioconcentration factor (BCF) can be calculated using TBT concentration in animal and water sample (BCF = TBT ng/g tissue/ TBT ng/ml). An average TBT concentration of 162 ng/g dry wt in oyster tissues was observed in the present study (Table 1), whereas in the surface waters of Dona Paula Bay (Anonymous, NIO/TR-3, 2003), it was 35 ng/l. Using these TBT levels a BCF value of 4628 was calculated. This suggests that the oyster has a high BCF. Seafood consumption is the prime source of human dietary exposure to butyltin compounds. In India ~ 9 g/day/person of seafood is consumed (Kannan et al. 1995). Using this value a conservative estimate of TBT intake of 1458 ng/person/day was calculated. This value is comparable to those reported for fish consumption in many developing countries but lower than that observed for the developed countries (Kannan et al. 1995). The tolerable daily intake (TDI) level of butyltins has not been established so far in India.

Nevertheless, the observed levels of TBT in this edible oyster may be matter of concern for human health. This is because butyltins are known to be associated with the suppression of immune system (Hoch 2001).

In summary, TBT concentrations in the oyster showed strong seasonal variations. The observed levels of TBT and DBT are fairly high and suggest the contamination of this edible oyster. This indicates that there is a potential for long term chronic effects of TBT in the Dona Paula Bay. In view of this, there is a need for further research to assess the fate of organotin compounds in the Dona Paula Bay.

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