Bull. Environ. Contam. Toxicol. (1996) 56:500-504

Bulletin of Environmental Contamination and Toxicology

Relationship Between Tissue Concentrations of Tibutyltin and Shell Morphology in Field Populations of *Mytilus edulis*

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Received: 21 October 1994/Accepted: 28 August 1995

While tributyltin (TBT) -containing antifoulants have seen widespread use in the past, their use has now been restricted in many countries due to evidence of adverse effects on marine life (Waldock et al. 1987; Anon 1994). For example, TBT has been found to be acutely toxic to many species of marine invertebrates at very low water concentrations in the µg/L - ng/L range (Maguire 1987; Snoeij et al. 1987; Joshi and Gupta 1992). Concern about the adverse effects on marine life of TBT leaching from treated surfaces has resulted in controls on its use in France, the UK, the USA and elsewhere. (Waldock et al. 1987). Even though regulation has reduced environmental levels of TBT (Waite et al. 1991; Anon 1994), localized sources of TBT such as shipyards and commercial ship traffic remain potentially important. Consistent with the widespread use of *Mytulis edulis* as a sentinel organism for various pollutants, TBT concentrations in mussels have been reported for various locations (Grovhoug et al. 1986; Quevauviller et al. 1989; Wade et al. 1990; Page and Widdows 1991). Tributyltin contamination has also been associated with shell deformity in the Japanese oyster, *Crassostrea gigas* (Waldock and Thain 1983; Alzieu et al. 1986) and in the Portuguese oyster, *Crassostrea anguluta* (Phelps 1990). Measurements of shell thickening in *C. gigas* have been used as a bioindicator of TBT contamination (Stephenson et al. 1986). *Mytilus edulis* from a marina area have also been reported to exhibit shell thickening (Stephenson et al. 1986), making shell thickening a potential bioindicator of TBT contamination in this species as well.

In the present study, *Mytilus edulis* was used as an sentinel organism to determine the extent of TBT inputs at various sites on the Maine (USA) coast and to relate the TBT concentrations in mussel tissues to an overall indicator of stress. While Maine is a state with a low population density and a relatively unpolluted coastline, localized activities such as shipyards, marinas, commercial ship traffic, and wood and fishing net treatment remain as potential sources of TBT.

MATERIALS AND METHODS

Mytulis edulis, were sampled from 6 coastal areas in Maine at 15 sites between October 1987 and November 1989. All sampling sites were sheltered intertidal areas of comparable wave exposure and height in the intertidal zone.

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Adult mussels (15-25) of comparable size (ca. 5 cm shell length) were collected from each sampling site and frozen until analyzed for TBT and dibutyltin (DBT). Shell thickness index (TI) was determined on the shells of the animals collected from each site for alkyltin analysis. Thickness index was determined by measuring the greatest length and the greatest depth of the entire mussel shell with the valves closed. The depth of the shell was measured perpendicular to the plane of shell closure across both halves. Shell thickness index (TI) = shell depth/shell length * 100. Whole mussel tissue homogenates were analyzed for butyltins by the gas chromatographic method described in detail elsewhere (Page 1989). In summary, the method consists of a wet extraction of a tripentyltin chloride (recovery standard) spiked whole mussel tissue homogenate with methylene chloride. The dried concentrated extract was reacted with hexyl magnesium bromide to form the hexyl derivatives of any extractable organotin species present. The derivatized extract was cleaned up by silica gel liquid chromatography followed by the addition of a butyltripentyltin spike (quantification standard). The final concentrated extract was quantified against known alkyltin standards using capillary GC/FID. Based on replicate analyses of field and laboratory samples and standard addition experiments, the analytical precision and accuracy of the method was generally better than ± 10% each (see Page 1989).

The sampling areas are described below:

- Kittery: an industrialized estuary having a major naval shipyard facility and ocean-going ship activity associated with oil terminals and other industry.
- Portland: the largest city in Maine, has a major oil terminal for large tankers, marinas and other commercial vessel activity.
- Freeport: a small enclosed bay with a major marina facility providing moorings for 200+ boats of all sizes during the summer months.
- Boothbay: a major resort area with large and small pleasure craft, small boatyards, and commercial fishing facilities.
- Searsport, Long Cove: an isolated small harbor that accepts dry cargo and petroleum products from ocean-going vessels.
- Deer Isle. Hezzie's Point: a pristine remote location.

RESULTS AND DISCUSSION

Table 1 gives the results of alkyltin analyses and thickness index measurements. The relationship of mussel tissue TBT concentration to the overall degree of TBT contamination given by Page and Widdows (1991) provides a basis for placing these results in context, where: 0 - 0.5 ug/g dry tissue weight corresponds to none to low TBT input; 0.5 - 2 ug/g corresponds to moderate TBT input; and greater than 2 ug/g corresponds to heavy TBT input. In the present study, the tissue TBT concentrations ranged from 0.04 ug/g for a pristine reference location to 4.31 ug/g for marina/commercial harbor site. During the sampling period, 5 of the locations had mussels with tissue TBT concentrations indicative of moderate to heavy TBT inputs. The highest tissue TBT concentrations were in Boothbay Harbor near a small shipyard (4.31 ug/g) and near a lobster fisherman's cooperative facility (3.56 ug/g) reflecting the possible use of TBT as a wood trap and/or net preservative. These TBT concentrations are well above the ca. 2 ug/g threshold of physiological effect level, as defined by negative scope for growth, observed for adult mussels (Page and Widdows 1991).

Table 1. Results of TBT and DBT analyses in the tissue samples over all sampling locations and the corresponding thickness index values. The tissue concentrations of TBT and DBT are given in ug/g dry tissue weight as TBTCl and $DBTCl_2$, respectively. Values for TBT and DBT are given as the average of duplicate analyses ± the standard error (n=2) with the exception of (*) which was a pooled duplicate. Each thickness index value is the average of measurements of the 15-25 individuals in a sample ± the standard deviation (n=15-25).

Date	Location	ug/g TBT	ug/g DBT	Thickness Index
10/2/87	Kittery Bridge	0.77 ±0.01	0.25 ±0.04	0.46 ±0.03
10/2/87	Kittery Reference	0.11 ±0.00	0.08 ±0.00	0.42 ±0.03
10/19/87	Freeport Inner	0.73 ±0.04	0.25 ±0.01	0.46 ±0.05
10/19/87	Freeport Outer	0.50 ±0.01	0.21 ±0.03	0.43 ±0.03
9/16/87	Portland West End	1.75 ±0.13	0.47 ±0.08	0.48 ±0.04
9/16/87	Portland Reference	0.60 (*)	0.27 (*)	0.43 ±0.04
9/11/87	Freeport Marina Dock	1.75 ±0.04	0.48 ±0.03	0.48 ±0.03
8/23/88	Deer Isle Reference	0.04 ±0.00	0.04 ±0.01	0.39 ±0.03
10/7/88	Long Cove, Searsport	0.04 ±0.01	0.40 ±0.02	0.42 ±0.03
7/9/89	Deer Isle Reference	0.04 ±0.00	0.04 ±0.01	0.39 ±0.03
6/27/89	Boothbay, Shipyard I	4.31 ±1.05	3.28 ±0.77	0.53 ±0.05
6/27/89	Boothbay, Inner Harbor	1.66 ±0.25	0.71 ±0.09	0.50 ±0.02
6/27/89	Boothbay, Lobster COOP	3.56 ±0.25	2.35 ±0.14	0.56 ±0.04
6/27/89	E. Boothbay, Shipyard	0.27 ±0.00	0.16 ±0.04	0.48 ±0.04
6/27/89	Boothbay, S. Bristol	0.45 ±0.00	0.24 ±0.08	0.45 ±0.03

Figure 1 shows a linear relationship ($r^2 = 0.77$) between TI and log(tissue TBT concentration) constructed from the data in Table 1. It is interesting to note that a major outlier (log[TBT] = -0.57; TI = 0.48: $r^2 = 0.80$ less this point) in the data set corresponds to a shipyard in E. Boothbay (Table 1) at which TBT use had been discontinued; the ambient exposure to TBT had decreased, but the effect on shell morphology remained. The relationship between tissue TBT concentration and TI indicates that shell deformation may be a bioindicator of TBT exposure in *Mytilus edulis*, given care in sampling and data interpretation.

In applying an overall stress indicator such as thickness index, the following things should be kept in mind:

- Variables other than TBT should be minimized. These variables include physical factors such as wave exposure and height in the intertidal zone which are known to affect shell shape in *Mytilus edulis* (Seed 1968).

- Measurement of a biological effect should be coupled with a chemical measurement on a subsample of the same mussel population sampled at the same time.

- Changes in TBT inputs can result in higher or lower tissue TBT concentrations than would be consistent with a given thickness index in mussels. It is important to be aware of the history of a given sampling location whenever possible.

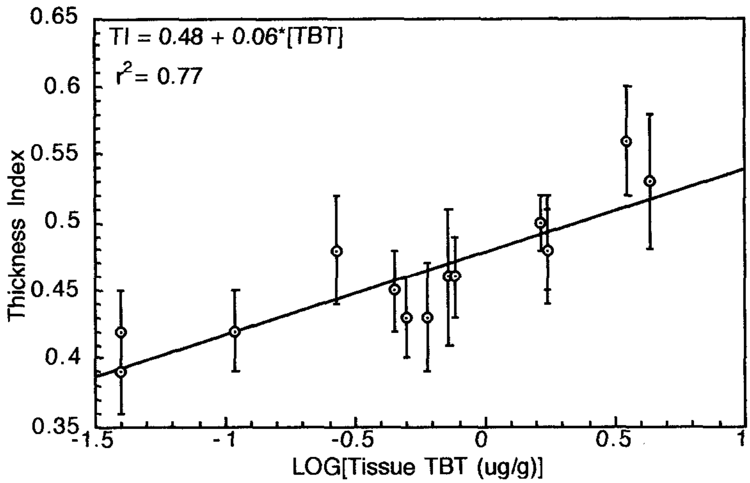

Figure 1. Thickness index (TI) vs log TBT concentration (ug/g dry wt. of whole animal homogenate) for *Mytilus edulis* from the field locations given in Table 1.

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