ENVIRONMENTAL SIGNIFICANCE AND INTERPRETATION OF ORGANOTIN BIOASSAYS

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ABSTRACT

Factors affecting the interpretation of organotin bioassay results with respect to environmental significance were reviewed. It was determined that a thorough understanding of organotin chemistry and bioavailability is essential to this interpretation but neither are well characterized in the laboratory or the field. Analytical limitations enhance this problem. The bioavailability of organotins is affected by suspended particulates, bottom sediment and dissolved organics, but most bioassays do not include or measure these parameters. It is concluded that environmental impact cannot be predicted with the data available from organotin bioassays and field monitoring.

INTRODUCTION

A bioassay is generally defined as the measurement of a biological response that is used to assess the effects of toxic substances or environmental factors either separately or in combination (1). In their review, White and Champ (2) found considerable variation in results and drew the following conclusions about bioassays: 1) they are not comparable among laboratories; 2) they do not accurately simulate the natural environment; 3) they are not good predictors of ecological consequences; and 4) their use by regulators as a predictive tool is questionable.

For these reasons, the interpretation of laboratory bioassay results and extrapolation from the laboratory to the field is subjective, even for well characterized contaminants. It is not surprising therefore, that the interpretation of organotin bioassays and their alleged environmental significance have inspired such controversy. The chemistry and bioavailability of organotins in nature are not well characterized and the relationship between chemical measurements and biological responses has not been clearly established.

Toxicity and bioaccumulation are dependent on bioavailability. A toxicant must be available in a form that the organism can absorb and metabolize to elicit a biological response. Existing analytical methods cannot precisely distinguish bioavailable organotins, and most bioassays do not include or measure factors that could affect bioavailability. Few bioassays are environmentally relevant and there has been no field experimentation to corroborate laboratory results. Bioassay results are currently being used to predict the potential environmental impact of organotin antifouling coatings in harbors and estuaries. Yet estuaries are among the most heterogeneous and variable of aquatic environments due to inputs from associated wetlands, freshwater/saltwater interactions, tidal cycles and seasonal changes.

The unique characteristics of organotins and estuaries complicate measurement and interpretation. Simulating a natural organotin environment and monitoring these conditions in laboratory bioassays is difficult. Existing levels of organotins in a number of harbors and estuaries have been reported (3), but the relationship between field measurements and laboratory measurements has not been clearly established.

This paper explores various factors that affect the interpretation of organotin bioassays with respect to environmental significance. Since the tributyltin cation (TBT) is generally accepted as the most deleterious to organisms (4, 5), this organotin species will be emphasized. Estuaries are emphasized since the highest levels of TBT have been measured in harbors and estuaries with poor flushing (3, 6).

ORGANOTIN BIOASSAY INTERPRETATION

Physical/chemical factors and their interactions influence all bioassays. Results can be shifted by several orders of magnitude by altering these test conditions. The following additional factors were found to be the major sources of variation in bioassays: age, sex, genotype, acclimation and exposure (2). Only decreased sensitivity with age and increased effects with increasing duration of exposure have been reported for TBT (7, 8).

To be meaningful, the frequency of chemical measurements must be commensurate with the variability of the environment (9). This is true in the laboratory and the field and particularly with
organotin bioassays. Significant fluctuations in test concentrations have been reported (10, 8). Such variations may be environmentally realistic in some cases (11) they complicate interpretation. Most bioassays are not designed to examine the effects of variable concentrations on test organisms and therefore inadequately address this issue. Using statistical means of variable test concentrations to estimate lethality may be inappropriate. Some filter feeding bivalves have the ability to sense contaminants and disperse them in the water near the sediment (12, 13). Further, at low TBT concentrations some bivalves accumulate TBT differently than at higher concentrations (14).

In addition to TBT measurements, all the physical/chemical parameters affecting bioassay results must be measured, particularly those affecting bioavailability. The difficulty in interpreting TBT bioassays is compounded by the uncertain relationship between organism response and chemical measurements of bioavailable TBT. Most bioassays do not include factors that could affect bioavailability, like suspended particulates and bottom sediment. Without these factors present and quantified, bioassay interpretation becomes even more speculative.

**Bioavailability**

Bioavailability may be affected by a variety of physical/chemical factors and especially those that influence TBT complexation and adsorption. TBT binding is the most enigmatic and perhaps the most important factor to understand when interpreting the bioavailability of TBT to organisms. Although there is conflicting evidence on the bioavailability of dissolved and complexed metals to certain organisms, most of the bioassay evidence indicates that TBT associated with suspended particulates are an important TBT source for suspension feeders (4, 15, 10). The key to bioavailability may be the combined role of organic ligands, suspended particulates, and bottom sediment.

**Organic Ligands**

It has been suggested that TBT strongly binds to dissolved organic material and that there are many potential organic ligands in the estuarine environment (4, 5). Similarly, many organically bound metals have been identified in nature (16). The predominant form of certain metals in natural waters is an organic complex (17, 18, 19). These complexes reduce the bioavailability and toxicity of metals to phytoplankton and zooplankton (20, 21, 22, 23, 24, 25, 26, 27). However, these same complexes may enhance the bioavailability and toxicity of metals to filter feeding bivalves (28, 29, 30, 31).

Bioavailability is also dependent upon ligand strength and type. Lipid-soluble ligands increased while water-soluble ligands decreased metal availability and toxicity to a crustacean (32). Strong chelators complex metals so tightly they could be unavailable to a filter feeding bivalve (33). Depending on type and strength, naturally occurring organic ligands can facilitate or inhibit transport across membranes (34).

Microlayer measurements also suggest dissolved organic associations. In freshwater samples, TBT in the surface microlayer was measured at concentrations more than three orders of magnitude higher than subsurface water (35). Dissolved organics concentrated in the surface microlayer bind heavy metals and perhaps TBT as well. If such complexes exist with TBT, this could account for the higher concentrations of TBT in the surface microlayer and suggest that organic ligands could affect TBT bioavailability. Some bioassays with contaminated sediment suggest that organics associated with the sediment may reduce TBT bioavailability and toxicity to some crustaceans (36, 37).

**Suspended Particulates**

TBT probably binds very strongly to suspended particulates (4, 5). Most suspended particulates are coated with organics (38), and organics on the surface of sediment particles enhance metal adsorption (39, 10). Bioavailability is also affected by the type of particulate binding (41). Some studies imply that organics attached to particles can be utilized as food or that particles enhance food utilization (42, 43).

Laboratory studies have shown that TBT rapidly binds to suspended sediment particles, algal cells, and bacterial cells (4, 44, 45, 46). In suspended sediment studies, TBT adsorption was related to grain size and organic content (46, 36, 37). Binding was not metabolically dependent in bacteria since freshly killed cells bound twice as much TBT as living cells (44). Additional studies also suggest that binding is an adsorption process since over 95% of the TBT was associated with the bacterial cell wall (45).

The role of particulate binding in TBT bioaccumulation is implied in several bioassays. Based on limited chemical measurements, Henderson (15) suggests that TBT did not bind to phytoplankton. However, in the same test, two species of coral had significantly higher mortality when exposed to TBT with phytoplankton present than without (15). Laughlin (4) found that phytoplankton enhanced TBT accumulation in mussels. Humic acids and kaolin, commonly used to bind heavy metals, did not increase accumulation over dissolved TBT. However, clay particles with associated TBT were rapidly trapped by gill mucus and later rejected as pseudofaeces. If bivalves can discriminate between food particles and non-food particles and reject the latter as pseudofaeces, enhanced bioaccumulation would not be expected.

Walldock and Thain (10) showed that suspended sediment was a significant positive factor and TBT was a significant negative factor for growth of oyster spat. When compared to seawater controls, oyster spat exposed to TBT grew more rapidly with suspended sediment than without. When compared to the clean suspended sediment control, sediment...
particles and TBT reduced growth more than TBT alone. Further, oyster spat accumulated TBT from contaminated sediment in suspension. These data suggest that TBT associated with sediment particles is more bioavailable to oyster spat than unbound TBT and that they may act synergistically.

The three bioassays discussed (15, 4, 10) provide evidence that suspended particulates enhance TBT bioaccumulation in suspension feeders like coral and bivalves. Bioavailability of TBT associated with non-food particles is still unclear and may be species dependent.

The bioavailability of particles is also related to feeding and particle size. Most filter feeding bivalves utilize an effective filtration system in concert with mucus to trap particles as small as 0.1 μm (47). Many zooplankton species have a coarse filtration system and rely heavily on raptorial feeding. They generally do not retain particles less than 0.5 μm. Feeding type may explain a reduction in bioavailability of metal complexes to zooplankton and enhancement of bioavailability to filter feeding bivalves. It may also explain molluscan sensitivity to TBT.

**Bottom Sediment**

The amount of TBT adsorbed on sediment particles is related to grain size and organic content (46, 36, 37) as it is for many contaminants. Grain size and organic content also influence toxicity and bioaccumulation of metals (48). Changes in depuration rates with microcosms and particulates (15) indicate that some organotin compounds may be more strongly bound than others and implicate organic ligands. Since the epifauna was affected more by TBT than the infauna in these experiments, some sediment dwellers may be more resistant to TBT, or the sediment-bound TBT may have been less bioavailable. Similar results were obtained using TBT-contaminated sediment in other bioassays (36, 37).

Mysids exposed to TBT survived better with sediment than without sediment (36, 37). These data also show that bottom sediment was a significant positive factor for mysid survival and TBT a significant negative factor, as reported for oyster spat growth with suspended sediment (10).

The three bioassays discussed (15, 36, 37) provide evidence that TBT binds to bottom sediment. Further, this bottom sediment and feeding type influence bioavailability.

**ENVIRONMENTAL SIGNIFICANCE**

In harbors and estuaries, TBT associations with suspended particulates, bottom sediments, dissolved organics and organisms could be similar to the associations with metals and other toxic organics. Estuaries are particularly efficient traps for many contaminants. Field measurements have shown that sediments and organisms accumulate TBT at concentrations three and four orders of magnitude above water column concentrations, respectively (3, 49, 12). The environmental significance of this bioaccumulation is unclear, although effects on growth have been implicated in laboratory studies (10). Environmental impact cannot be predicted until bioaccumulation measurements in the laboratory and the field have been associated with some biochemical measurements or sublethal responses in nature to clearly demonstrate a cause-and-effect relationship (50).

The fate of particulate-bound contaminants is of particular significance in estuaries because major biological energy flows involve consumption of detrital particles. Particles have the ability to scavenge metals and toxic organics from solution and sequester high concentrations in suspended particulates and bottom sediments (51, 52). They probably do the same with TBT. Few data are available on TBT partitioning. At environmentally realistic phytoplankton concentrations, more TBT was associated with phytoplankton than in solution (4). In another experiment with environmentally realistic concentrations, approximately 30% of the TBT in solution was associated with suspended sediment (10). However, in nature these particles would be contacting with many others for TBT adsorption and it is not certain how much TBT would be available for each fraction at a particular concentration.

Field measurements suggest only 3 to 17% of measurable TBT is associated with suspended particulates, with only a small portion bound to microorganisms (53). However, only particles larger than 0.4 μm were analyzed. The relative significance to organisms of the smaller particles is unknown. Particles smaller than 0.4 μm may be just as important or more important to many filter feeding bivalves with the ability to retain 0.1 μm particles (47). Considering the potential importance of small particles, the accepted definition of suspended particulates and dissolved materials must be re-evaluated. Regardless of the TBT associated with suspended particulates on a per-weight basis, the amount available is large when considering the filtration capacity of suspension feeders.

Impacts upon estuarine ecosystems of particle-bound contaminants with a well-characterized chemistry are difficult to predict. It is even more difficult for TBT. The complex interaction of physical, chemical and biological factors in estuaries enhances the difficulty in designing representative bioassays to predict the fate and effects of TBT.

It has been suggested that the marine environment has not been adequately sampled in most field monitoring studies to account for cyclical and random variability (9). This is true for TBT as well, although some general trends have been measured. A gradual increase in TBT concentration has been measured over a 3-year period (12). Some measurements have demonstrated a seasonal fluctuation for TBT, with the highest concentrations near 2 ppb occurring in the summer (12, 10, 6). TBT concentrations have also been shown to vary by more than an order of magnitude
with tidal cycle outside a marina with high TBT concentrations (11).

A safety limit of 0.05 ppb TBT has been proposed for the estuarine environment (54). The most sensitive laboratory bioassays indicate biological effects attributable to TBT near 0.1 ppb (55, 7, 56) and the estimated safety limit. For extrapolation to natural conditions, these laboratory values require careful scrutiny considering TBT bioavailability and other problems previously discussed.

In general, variability occurred in the bioassays, variability occurs in estuaries, and there has been no field validation to demonstrate a clear relationship between bioassays and natural conditions. Clear relationship concentrations were extremely variable and measured infrequently. It is also significant that all three tests used planktonic forms in which complexed and particulate-bound TBT might not be bioavailable.

In the final analysis, bioassays remain the effective screening tool they were originally intended to be. Sometimes their utility is overextended. More discriminating experiments in TBT partitioning, animal feeding and particulate interactions will help to understand the fate and effects of organotin behavior in nature. This is not only a bioassay problem, but a chemistry and field monitoring problem as well.

CONCLUSIONS

1. Bioavailability is affected by suspended particulates, bottom sediment and dissolved organics and therefore must be included and measured in meaningful organotin bioassays.

2. The interpretation and environmental significance of organotin bioassays is dependent upon a complete understanding of bioavailability, which includes measurements of bound TBT and its relationship to feeding.

3. Field validation of quantifiable animal responses associated with meaningful chemical measurements of TBT are required for prediction of environmental impact.

4. Environmental impact cannot be predicted with the data available from organotin bioassays and field monitoring.

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