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CHARACTERIZING EXPOSURE AND EFFECTS OF TBT IN BIVALVES USING TISSUE CHEMISTRY AND SUBLETHAL ENDPOINTS

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Background

Recent Superfund-related studies in Puget Sound utilizing the sediment quality triad have shown a lack of correlation between sediment concentrations of tributyltin (TBT), laboratory toxicity tests, and benthic community structure. While some high concentrations of sediment TBT have been associated with apparently altered benthic community assemblages, little toxicity has been measured in laboratory bioassays with commonly used species like amphipods. This lack of correlation between amphipod toxicity and sediment TBT has also been shown for studies conducted in San Diego Bay (Fairey et al, 1998). These same San Diego Bay studies failed to show a significant correlation between amphipod toxicity and the concentration of any of the chemicals measured. The most likely explanation for this lack of toxicity includes insufficient exposure time to reach chemical equilibrium and insensitivity of the amphipod mortality endpoint (Meador, 1997; Meador et al., 1997; Salazar and Salazar, 1998).

Based on uncertainties in the relationships between TBT in sediment, TBT in tissues, and associated effects, the Dredged Material Management Program (DMMP) agencies decided that the porewater approach would be a better alternative to regulating TBT-contaminated sediments than the current Apparent Effects Threshold approach (Michelsen et al., 1996). While some researchers and laboratories support the porewater measurement approach, others have suggested that it represents an unnecessary and inappropriate deviation from existing protocols. We suggest that it would be easier to characterize and understand the processes of exposure, uptake, and effects by combining exposure and effects endpoints in a single bioassay and extending the exposure period to better represent steady-state conditions (Salazar and Salazar, in review). Current bioassay protocols are not designed to address persistent chemicals like TBT, or to provide synoptic information on exposure and effects that is critical for managing TBT-contaminated sediments. Combining exposure and sublethal effects endpoints in a single bioassay and extending test duration could provide the agencies the data needed to better evaluate TBT-contaminated sediments.

The purpose of this paper is to document the need for revising standard laboratory testing protocols and begin a scientific dialogue regarding the feasibility of proposed modifications. This paper will demonstrate (1) why it is necessary to modify existing laboratory procedures to include synoptic measures of exposure and effects, (2) how effects endpoints can be used to develop dose-response relationships, calibrate bioaccumulation, and quantify animal health to establish test acceptance standards; and (3) how the pattern of the relationship between seawater, sediment, and tissue TBT can be used to predict the threshold concentrations where effects will begin to occur.

Why Existing Protocols Need Revision

The utility of synoptic measurements of exposure and effects in bivalves has been demonstrated over the past two decades in both laboratory and field studies. The standard American Society for Testing and Materials (ASTM) and Puget Sound Dredge Disposal Authority (PSDDA) laboratory bioassay protocols would be more consistent with US Environmental Protection Agency's (EPA's) risk assessment paradigm (US EPA 1997, 1998) if they used the exposure-dose-response triad as a conceptual model (Salazar and Salazar, 1998). It has been almost 10 years since McCarty (1991) recommended combining toxicity and bioaccumulation testing. In the context of ASTM and EPA protocols, he suggested that the ultimate goal is the development of a single bioassay methodology linking bioaccumulation and effects endpoints. The net result would be the ability to link external exposures, internal tissue chemistry, and associated effects in exposed organisms. This is related to both water and sediment testing and is a first step toward establishing critical body residues (CBRs) as a regulatory tool.

Exposure and effects endpoints should be used together in an integrated strategy for assessing and managing contaminated sediments. This exposure-and-effects approach has been used successfully for caged bivalves (Salazar and Salazar, 1991,1996,1998) and should be used in laboratory bioassays (Salazar and Salazar, In review). A combined exposure and effects assessment strategy utilizing bivalves and other species would allow the agencies to make management decisions based on a preponderance-of-evidence from sediment chemistry, tissue chemistry, and associated effects in a variety of species. In practice, this would mean adding effects endpoints to bioaccumulation tests with *Macoma* and *Nephtys* and exposure endpoints to toxicity tests with *Neanthes*. Methods currently exist for using this approach in laboratory tests.

The Exposure-Dose-Response Triad

The exposure-dose-response triad (Figure 1) is a preponderance of evidence approach that includes characterizing external exposure with chemical analysis of water and sediment, and the internal exposure, or dose with chemical analysis of animal tissues. All three environmental compartments are necessary for an accurate characterization of total exposure. Since internal chemicals are closer to organism's receptors of concern, bioaccumulation may be a better predictor of effects and the use of CBRs is becoming more widespread. Using bivalves, growth represents a response in the exposure-dose-response triad that is easily measured and easily understood. In our caged bivalve approach, the best predictions of adverse effects associated with site-specific environmental conditions are made through synoptic measurements of total exposure (internal and external) and sublethal effects endpoints. The exposure-dose-response triad approach using caged bivalves has been used in San Diego Bay for assessments of water-associated TBT (Salazar & Salazar 1991,1996,1998) and at the Harbor Island Superfund Site in Puget Sound for sediment-associated TBT (Salazar et al. 1995).

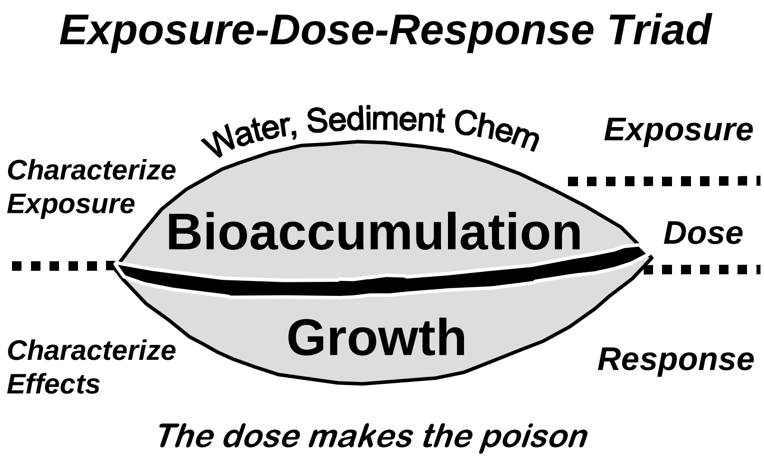


Figure 1 – *The exposure-dose-response triad using bivalves showing that characterizing exposure requires quantifying the external exposure and the internal dose as well as some biological response like growth to characterize effects*

Why Characterize Exposure?

Characterizing exposure is probably the single most important element in EPA's risk assessment paradigm (US EPA, 1998) and sediment evaluations in the Superfund process (US EPA, 1997). Effects occur as a result of chemical exposure and associated biological uptake. If exposure is not properly characterized, the effects measurements may not be meaningful or reproducible, and the conclusions based on those characterizations may be misleading. This may be the single largest uncertainty associated with laboratory toxicity tests, the sediment quality triad, the apparent effects threshold (AET), and current PSDDA approaches: exposure is assumed from the results of effects measurements. Tissue chemistry data can be used to establish a link between external exposure and effects as well as other commonly used effects-assessment endpoints (Figure 2). Tissue chemistry and effects data are most meaningful when they are collected from the same organism at the same time, as in a bivalve test for bioaccumulation that includes effects endpoints like growth. The protocols currently being used in bioaccumulation tests with *Macoma* for example, only include the exposure endpoint (US EPA, 1993). It was not designed to measure effects. In the context of agency regulation of TBT-contaminated sediments, potentially useful information is being lost with every sediment evaluation.

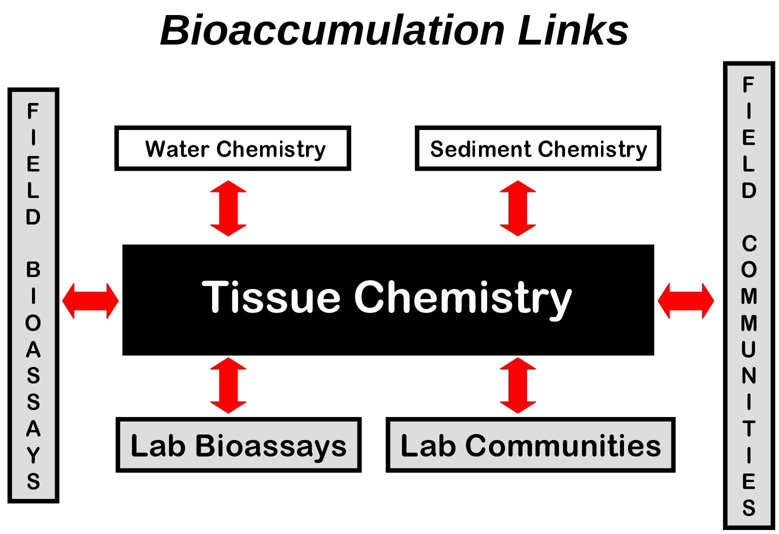


Figure 2 – *Establishing links with water and sediment chemistry as well as various effects endpoints in lab and field assessments using tissue chemistry*

Why Characterize Effects?

There are three reasons for quantifying effects: 1) to provide an effects endpoint for dose-response relationships, 2) to calibrate bioaccumulation, and 3) to establish test acceptance criteria. Characterizing effects is the second most important element in EPA's risk assessment paradigm (US EPA, 1998) and is often considered the primary environmental concern. The focus on effects data from laboratory toxicity tests has led to shortcuts in the assessment process and bioaccumulation testing is no longer required in every sediment evaluation. An over-reliance on laboratory toxicity tests, the sediment quality triad, and AETs has evolved over the years. These approaches do not adequately characterize chemical exposure and generally do not include internal exposure estimates from bioaccumulation data. These and other uncertainties have been identified as significant obstacles in attempting to use traditional laboratory toxicity tests for accurately predicting the effects of TBT (Salazar, 1986). Similarly, internal exposure is seldom included in evaluations of the benthos.

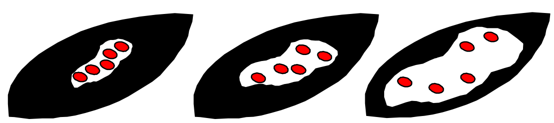
As an Effects Endpoint—Growth represents an integration of all internal processes and is easily measured and understood. Bivalve growth can be quantified as a dose-response, related to population effects, and used to predict other potential effects. The pairing of effects measurements with tissue chemistry data is particularly useful since it provides an estimate of the relative potency of chemicals within organisms. These paired exposure-effects data are the basis for the tissue residue-effect databases currently being developed by the EPA and the US Army Corps of Engineers (ACOE). At the recent National Sediment Bioaccumulation Conferences sponsored by the EPA and ACOE, it was suggested that CBRs could be utilized on a national basis within the next decade.

Calibrating Bioaccumulation—A second reason for quantifying effects is to assist in interpreting and "calibrating" bioaccumulation data. Evidence for other chemicals suggests measured concentrations of chemicals in tissues could either over- or under-estimate actual bioavailability if test animals are stressed and lose tissue mass. The ramifications of tissue loss on TBT bioavailability measured in laboratory bioaccumulation tests is unclear.

A schematic representation of why it is important to calibrate bioaccumulation results based on measured changes in tissue weights during the test is provided in Figure 3. If the chemical mass remains constant, any change in tissue mass will result in a corresponding change in tissue chemical concentrations. Without knowing if the tissue mass has changed significantly during a bioaccumulation test, it is impossible to state with confidence that the measured concentration of chemicals in those tissues are a result of chemical bioavailability or loss of tissue mass. Chemical dilution due to increases in tissue mass and chemical enhancement due to decreases in tissue mass are two phenomena that are often not considered when interpreting tissue chemistry data (Luoma, 1985; Meador, In review).

Calibrating ulation Changes Weight

Bioaccum with in Tissue



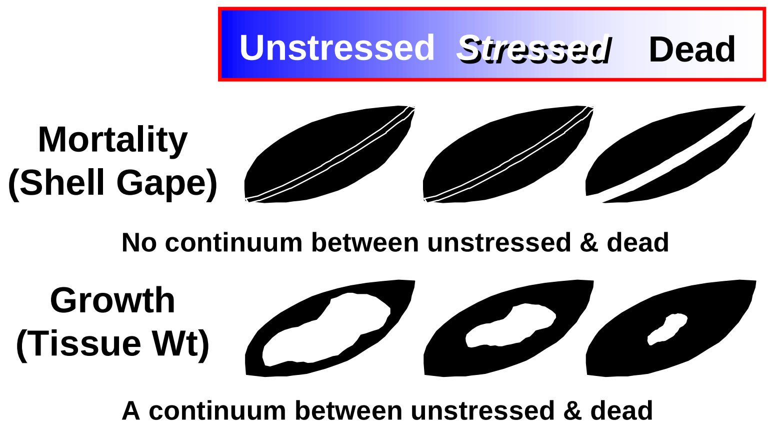
Chemical Mass	**5 ug**	**5 ug**	**5 ug**
Tissue Mass	**2.5 g**	**5 g**	**10 g**
Concentration	**2 ug/g**	**1 ug/g**	**0.5 ug/g**

If chemical mass remains constant,
Δ 4x tissue mass = Δ 4x tissue concentration

Figure 3 – *Calibrating bioaccumulation by using changes in tissue weight explain measured tissue concentrations*

Test Acceptance Criteria—A third reason for quantifying effects is to demonstrate that the bioassay results have met reasonable acceptance criteria. Mortality is not an appropriate indicator of animal health or an appropriate criterion for accepting bioaccumulation test results (Figure 4). As shown by the shaded bar, there is a continuum in bivalve health between unstressed, stressed, and dead test animals. However, mortality is too insensitive to quantify health because there is no continuum between a closed shell and a gaping shell that provides any indication of animal health. It is also difficult to determine how much of a gape and/or lack of response indicates death or poor health. However, effects endpoints like growth (i.e., changes in whole animal, shell, or tissue weight) can be used to assess animal health because there is a quantifiable continuum between unstressed, stressed, and dead animals.

Even if test animals meet the survival criterion of 80%, the test may not have been successful if results are not properly interpreted. While the 80% survival criterion seems reasonable, a criterion for <20% loss in tissue weight for control animals during the exposure period also seems appropriate. This is based on based on laboratory tests where starved mussels lost approximately 10% of their tissue mass after 4 weeks and approximately 20% of their tissue mass after 8 weeks (Ernst et al., 1991). This criterion was also used in a recent caged bivalve study at a depth of 70 meters in Port Valdez and has been incorporated into proposed ASTM protocols currently under review (Salazar and Salazar, 1997).



Quantifyin g Bivalve Health

Figure 4 – *Quantifying bivalve health by measuring changes in tissue weight*

Predicting TBT Effects from Bioaccumulation Processes

The following examples are provided to show that effects can be predicted from (1) changes in the relationship between TBT concentrations in water and tissue, and (2) changes in the relationship between concentrations of chemicals in sediment and tissue.

Caged Mussels in San Diego Bay

Caged juvenile mussel studies (*Mytilus galloprovincialis*) conducted in San Diego Bay between 1987 and 1990 resulted in a large enough data set to critically evaluate the relationships between TBT in water and tissues and their effect on growth (Salazar and Salazar, 1991,1996,1998). Through visual examination and repetitive regression analysis, it was possible to identify the critical exposure concentration, i.e., the concentration where the relationship between exposure and dose changed most significantly. For seawater, the critical exposure concentration was near 100 ng/L (Figure 5). These San Diego Bay data, as well as data from other studies, indicate that mussels uptake TBT without apparent stress at concentrations below 100 ng TBT/L. However, as the exposure concentration exceeds 100 ng TBT/L, normal processes appear to slow down and the relative amount of TBT accumulated decreases. This is shown by a significant decrease in slope of the regression. When the single outlier is excluded from the seawater TBT data >105 ng/L, the significance of the regression improves significantly (R^2 = 0.99). The change in relationships also provide an estimate of the TBT concentrations in seawater and tissues where adverse effects would be expected.

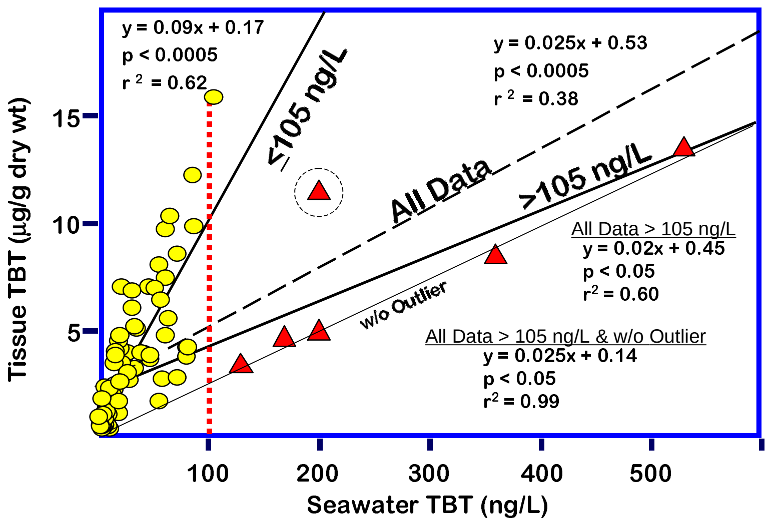


Figure 5 – *The relationship between TBT in seawater and tissue changes dramatically at seawater concentrations of 100 ng TBT/L and suggests that this may be a threshold concentration for effects*

The Relationship Between TBT in Seawater & Tissue Changes @ 100 ng TBT/L

Natural Populations of Clams in the UK

We have seen a similar pattern of a dramatic change in the relationship between exposure and dose for several different chemicals and several different bivalves. Significant changes in the relationship between sediment concentrations of TBT and tissue concentrations of TBT in clams are clearly seen when the data from Langston and Burt (1991) are plotted using different symbols to emphasize the difference in the regression lines (Figure 6). There appears to be a dramatic change in the relationship between sediment and tissue TBT that occurs at a sediment concentration around 0.2 ug TBT/g dw. From these data, the effective tissue concentration in *Scrobicularia* is around 3.0 ug/g dw. Similar dramatic changes in exposure-dose relationships have been shown by Salazar and Salazar (1996) for water and tissue concentrations of TBT in mussels (Figure 5). The critical sediment-effects threshold concentrations derived from the Langston and Burt (1991) data are consistent with those reported for adverse effects on *Armandia* using TBT-contaminated sediments from Puget Sound (Meador, in review). Furthermore, the effective TBT tissue concentration associated with the dramatic change in the exposure-dose relationship for *Scrobicularia* is almost identical to EPA Superfund's recently proposed tissue trigger level of 3.0 ug/g dw for EPA's West Waterway project. The four data points shown in circles were deleted from these analyses because they appeared to be outliers. Three of the four cases seem to be adequately justified for removal due to relatively high concentrations of TBT in the water (>66 ng TBT/L). It appears that both water and sediment were significant TBT pathways of exposure for *Scrobicularia* bioaccumulation. This is another reason for quantifying the amount of TBT in water, sediment, and tissues and better understanding the processes involved in exposure-dose relationships.

The Relationship Between TBT in Sediment & Tissue Changes @ 0.2 ug TBT/g dw

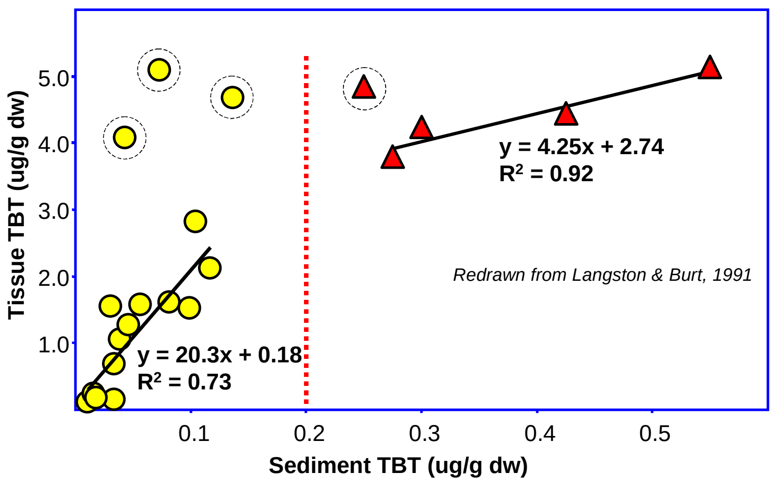


Figure 6 – *The relationship between sediment TBT and tissue TBT in UK clams changes dramatically near 0.2 ug TBT/g dry weight and suggests that this may be a threshold concentration for effects*

Langston and Burt (1991) found a statistically significant correlation (R^2 = 0.69, $p<0.001$) between TBT concentrations in sediment and clam tissues. The data come from 25 estuarine locations in England and Wales where deposit-feeding clams (*Scrobicularia plana*) and sediment samples were collected synoptically. They found that these bivalves consistently had higher concentrations of TBT in their tissues than other benthic organisms and interpreted this as an indication of sediments being an important exposure pathway for TBT. Langston and Burt (1991) also conducted kinetic studies which showed that chemical equilibrium between TBT in sediment and TBT in clam tissues is reached in approximately 40 days. These data are consistent with equilibrium predictions by Meador (in review) for amphipods and those for the filter-feeding mussel *Mytilus galloprovincialis* by Salazar and Salazar (1996). These and other data support the suggestion to increase exposure time in laboratory bioassays to approximately 45 days for amphipods, clams and worms and include exposure and effects endpoints in those tests.

Summary and Conclusions

We need to change the way we think about current assessment and management strategies with equal emphasis being given to exposure and effects This is best achieved by measuring exposure and effects endpoints in the same organism at the same time rather than using different tests and different species. The new emphasis by EPA and the DMMP agencies on better characterizing and understanding processes associated with bioaccumulative chemicals of concern in the environment reflect a growing regulatory understanding that these types of chemicals are more persistent, and the potential effects are more significant. It also underscores the need for longer exposure times, more sensitive tests, and more emphasis on bioaccumulation testing.

From our research and that of others, we have concluded that TBT is not unique among bioaccumulative chemicals of concern or even organometallic compounds, and that we must characterize and understand the underlying processes associated with these chemicals before moving forward with monitoring and prediction. As other chemicals undergo the same scrutiny as TBT, we may discover that even some of the basic elements of managing contaminated sediments in Puget Sound such as the AET may not be appropriate and that currently accepted bioassay protocols are not appropriate in terms of either exposure or effects endpoints. Clearly, we could learn more about the processes governing chemical bioavailability and organism response if effects endpoints were included as part of both bivalve and polychaete bioaccumulation tests.

Recommendations

1. Conduct a pilot study to determine the feasibility of measuring *Macoma* and *Nephtys* tissue weights as indicators of effects and calibrating bioaccumulation data.
2. Evaluate the data to determine if the results are useful and if additional modifications to the method are needed such as pairing length and weight measurements to establish regressions between length and tissue mass, and reducing the size range of test animals.
3. Establish survival and growth criteria for a successful bioaccumulation test and provide guidance in data interpretation.
4. Evaluate other potential effects (health) endpoints and identify how they could be used in a preponderance of evidence approach for regulatory decision-making.
5. Adopt a preponderance of evidence approach that includes direct measurements of exposure and effects in laboratory and field testing, and sediment-water-tissue partitioning as assessment tools for contaminated sediments.

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