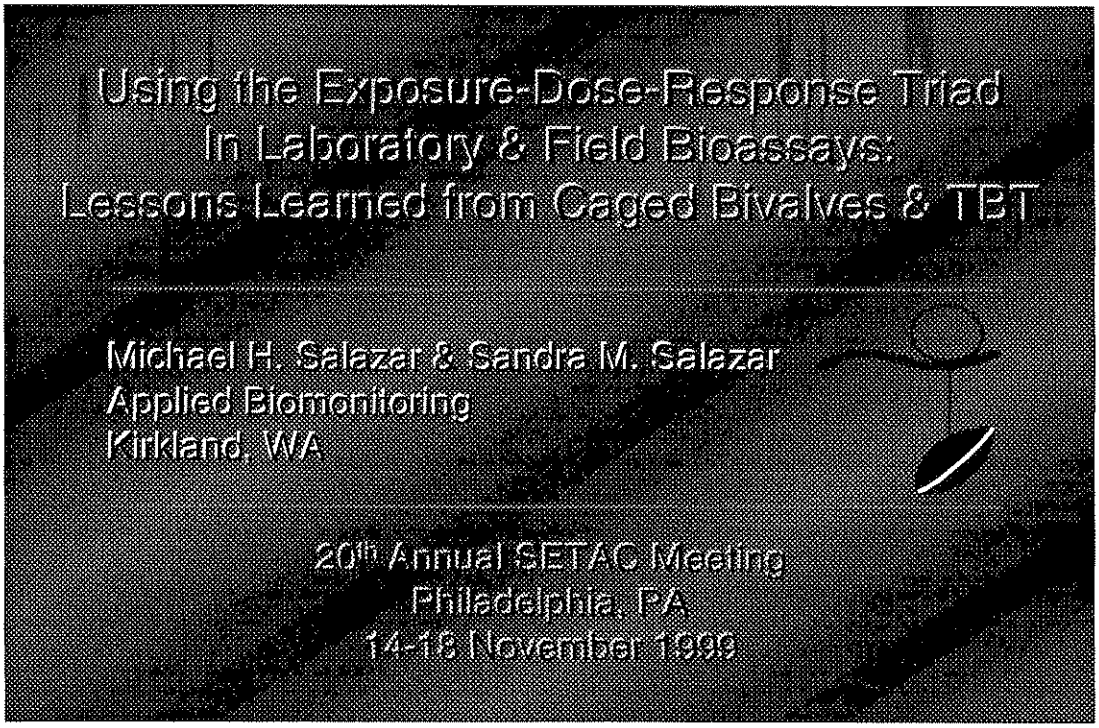
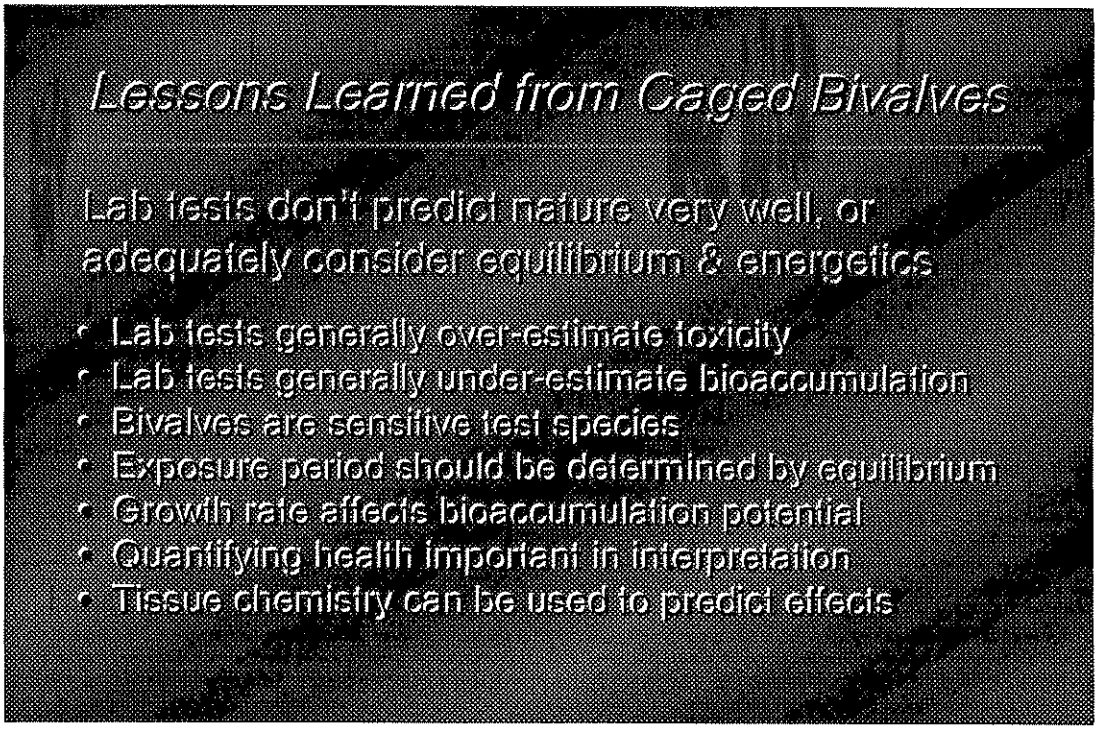
Salazar 1999



There are four concepts related to sediment management activities in Puget Sound that are addressed in this presentation: 1) Using bivalves as a monitoring tool; 2) Combining exposure and effects endpoints in a single bioassay; 3) Utility of these data in developing dose-response relationships for TBT; and 4) Monitoring and predicting effects associated with TBT based on concentrations found in water, sediment, and tissue.

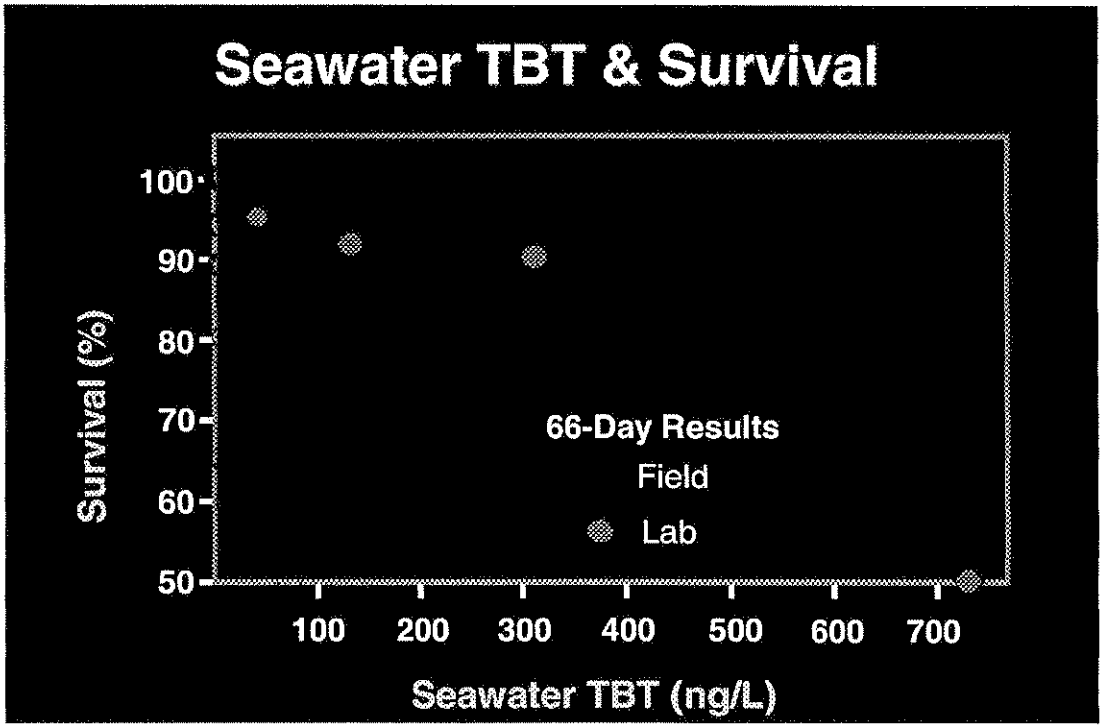
Almost 10 years ago Lynn McCarty suggested revising standard aquatic bioassay protocols to include direct measurements of exposure and effects. However, standard laboratory protocols that include synoptic measurements of exposure and effects in the same animal at the same time have not yet been developed. We believe that the continued use of separate toxicity and bioaccumulation tests results in a significant loss of information and a lack of understanding of the processes affecting bioaccumulation and growth. This also limits the utility of these tests as a predictive tool for real-world applications. In sediment testing for example, standard protocols are available for the Macoma and Nephtys bioaccumulation tests, but the environmental significance of these data is often unclear. Considering the effort and cost associated with setting up these bioaccumulation tests and performing the chemical analysis, the addition of effects endpoints, such as tissue weights, is relatively minor and yet adds potentially significant information. Similarly, standard protocols exist for Neanthes toxicity tests using the growth endpoint, but actual exposure at receptors of concern is unknown.

This paper will identify the following: 1) Problems in interpreting the environmental significance of results from standard laboratory tests for toxicity and bioaccumulation; 2) Examples of the advantages in making synoptic measurements to reduce uncertainty in the data; and 3) Suggestions on revisions to standard protocols that could be used to move toward a unified approach for predicting toxicity based on water and sediment chemistry, tissue chemistry, and associated biological effects.



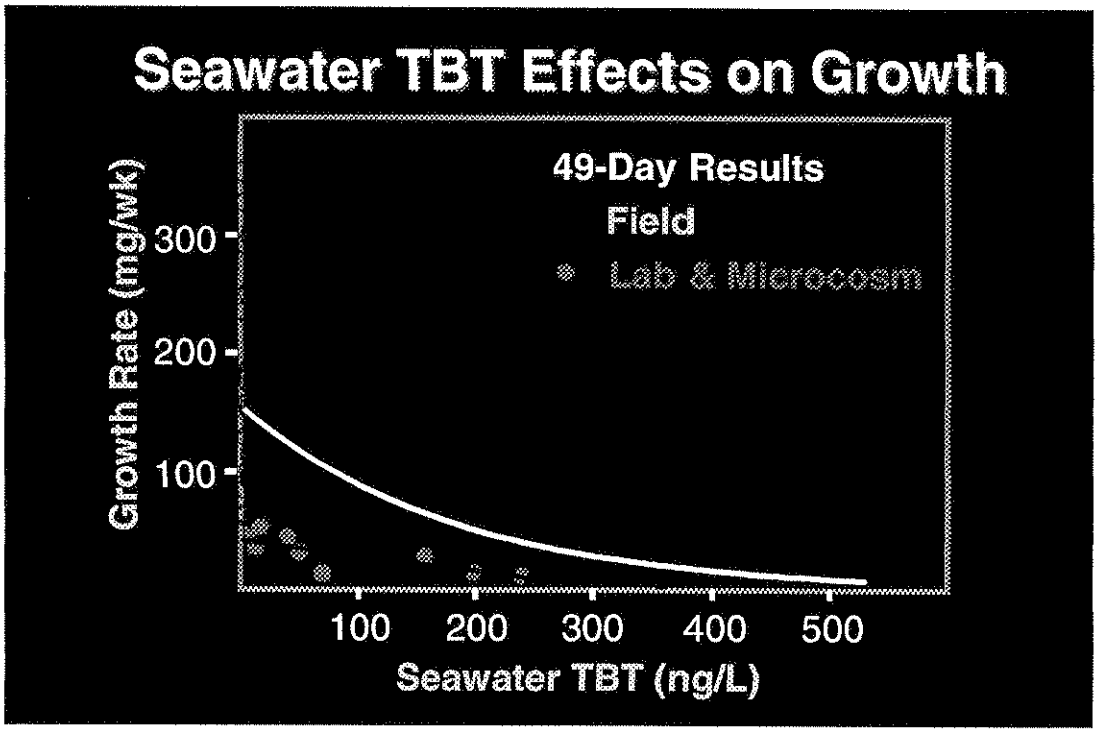
Perhaps the most important lesson learned from our work with caged bivalves under natural conditions is that lab tests do not predict nature very well, because they don't adequately consider equilibrium or energetics. Most of the following discussion is related to bivalves although the concepts may be related to other species as well. In general, we have found that lab tests with bivalves tend to over-estimate toxicity because bivalves are particularly sensitive to stress associated with laboratory holding conditions, such as temperature, nutrition and water flow rates. This laboratory-induced stress tends to make the bivalves more sensitive to chemical stressors. Laboratory-induced stress also tends to reduce the ability to accumulate test chemicals because accumulation is related to animal health and growth rates. Growth rates in the laboratory seldom, if ever, achieve those measured in the field under natural conditions. Since bivalves are commonly used as indicators of chemical exposure by measuring chemicals in their tissues, it is generally believed that they are insensitive test species. An important distinction needs to be made between resistance to chemical exposure and sensitivity as measured by chronic sublethal endpoints.

Another important lesson is related to determining appropriate exposure periods for test animals, whether the tests are conducted in the laboratory or the field. In the early stages of developing protocols for our caged bivalve methodology, we conducted experiments in the field to determine the exposure period necessary to achieve steady-state conditions. Repetitive sampling of caged mussels showed that steady-state for TBT was reached between 60 and 90 days. We selected an 84-day exposure period for convenience. It appears that exposure duration for most laboratory toxicity tests were selected primarily for convenience rather than the time necessary to achieve steady state. We also learned that quantifying animal health was important to a successful test because stressed animals tended to have slower growth rates and accumulate lower concentrations of chemicals in their tissues. Finally, perhaps the most important contribution of our work as a whole is that we can make reasonable predictions of where effects will occur by calculating where the change in relationship between accumulation and external chemical concentrations begins to change. We have shown this with our work with seawater and tissue TBT to show and work by others with sediment and tissue TBT to show that we can predict where effects will begin to occur.



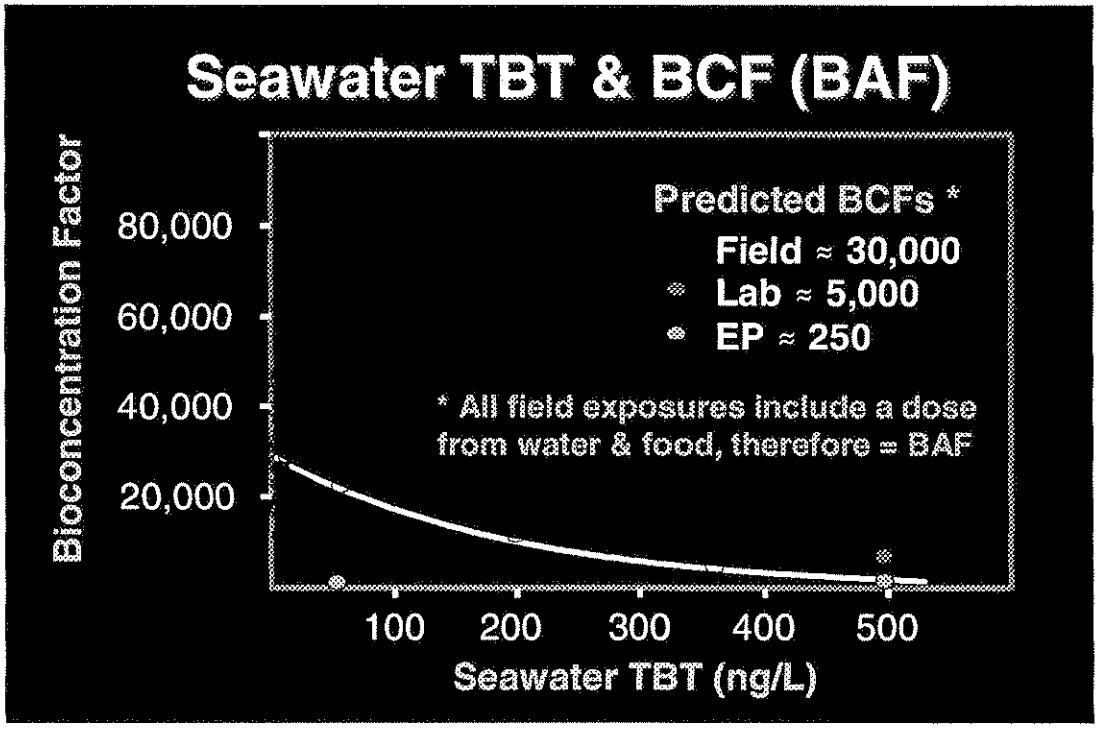
One of our important early discoveries was that caged mussels deployed in the field generally had higher survival than those under laboratory conditions. The above graph shows a clear and significant relationship between seawater TBT and survival for lab tests but no relationship between seawater TBT and survival in field tests. Even at the highest mean concentration of TBT ever measured during a 66-day exposure period (ca. 500 ng/L) survival was 100%. This too was significantly higher than predicted by the laboratory tests. The graph also shows that 100% survival was observed in approximately 75% of the individual transplant studies conducted. Only about 14% of these transplants had survival less than 90% and this toxicity was undoubtedly attributable to other factors because the concentrations were all below 100 ng TBT/L.

There are two other interesting observations that occurred during these laboratory and field tests. First, Shelter Island Yacht Basin, one of our transplant sites and the most TBT-contaminated yacht basin in San Diego Bay, also had the highest concentrations of many other chemicals as well. This is important because test animals exposed to TBT were also exposed to these chemicals and still had higher survival than test animals exposed to TBT alone under laboratory conditions. Many advocates of laboratory testing have also assumed that the extremes in environmental conditions in nature would be more stressful than the relatively constant physical and chemical conditions in controlled laboratory conditions. Our work suggests that even the most extreme field conditions that include variations in temperature, salinity, food, and various chemicals are not as stressful as laboratory conditions. The other interesting observation is that all of the largest animals died during the laboratory exposures. This supports the belief, and other data, that larger and older animals are more susceptible to stress than smaller and younger animals.



Another important early discovery was that caged mussels deployed in the field generally had higher growth rates than those in laboratory or microcosm tests. The above graph shows a statistically significant relationship between seawater TBT and growth rate for combined lab and microcosm tests as well as between seawater TBT and growth rate in field tests. The significance of these relationships, as with the survival data, is that the laboratory exposures over-estimated chronic toxicity as measured by mussel growth rates. When we placed caged mussels at the seawater intake, they grew about 3.5 times faster than mussels in the microcosm control tanks. Several important lessons were learned from these studies with caged bivalves. Environmental realism cannot be assumed on the basis of flow-through conditions or exposure to the sun. Caged bivalves were used to validate the environmentally unrealistic conditions of the microcosm flow-through tanks. Caged bivalves could be used routinely to validate the realism of laboratory or microcosm exposure conditions. It was as a result of these validation tests that we concentrated our efforts on the use of caged bivalves rather than microcosm tests.

It should also be emphasized that the survival and growth studies were part of the same field test. For both endpoints, the caged mussels were exposed to the extremes of field conditions as well as a number of other chemicals in addition to TBT. Nevertheless, as with survival, caged mussels deployed in the field grew faster than mussels exposed to TBT in the laboratory. This observation also has implications for ecological risk assessment. Advocates of probabilistic risk assessments suggest that risk is best predicted by calculating probabilistic exposure and toxicity and determining whether or not there is an overlap. This assumes that the estimates of chemical exposure and associated biological effects are accurate. Based on the survival and growth data presented here, at least for bivalves, questions can be raised regarding the ability of laboratory exposures to adequately predict survival or growth effects that would occur in the real world.



Having shown that laboratory exposures tend to overestimate toxicity of TBT to bivalves, it is important to evaluate the ability of laboratory tests to predict bioaccumulation. The above graph shows predicted bioconcentration factors (BCFs) for TBT under field conditions, in the laboratory and from equilibrium partitioning theory (EqP). BCFs predicted from field studies are about a factor of six higher than those predicted from laboratory studies and more than an order of magnitude higher than those predicted from EqP. Furthermore, the field studies show a concentration dependence for bioaccumulation that is not predicted from either laboratory studies or EqP.

Strictly speaking, it may not be appropriate to compare the results from field studies with EqP because EqP primarily predicts exposure at equilibrium from water while the predictions from field studies also include bioaccumulation from the food exposure pathway and should be referred to as a bioaccumulation factor (BAF). We believe that this is a shortcoming of EqP in that it does not adequately consider accumulation from food. It is also possible that many field conditions are not at steady-state and deviate from the EqP model. Similarly, we believe that the laboratory exposures under-estimate bioaccumulation because of the same laboratory-induced stress that affected mussel survival and growth rates when compared to field exposures.

Collectively, these results have potentially significant implications for traditional assessment methods and the utility of caged bivalves to accurately predict exposure and effects that might occur under real-world conditions.

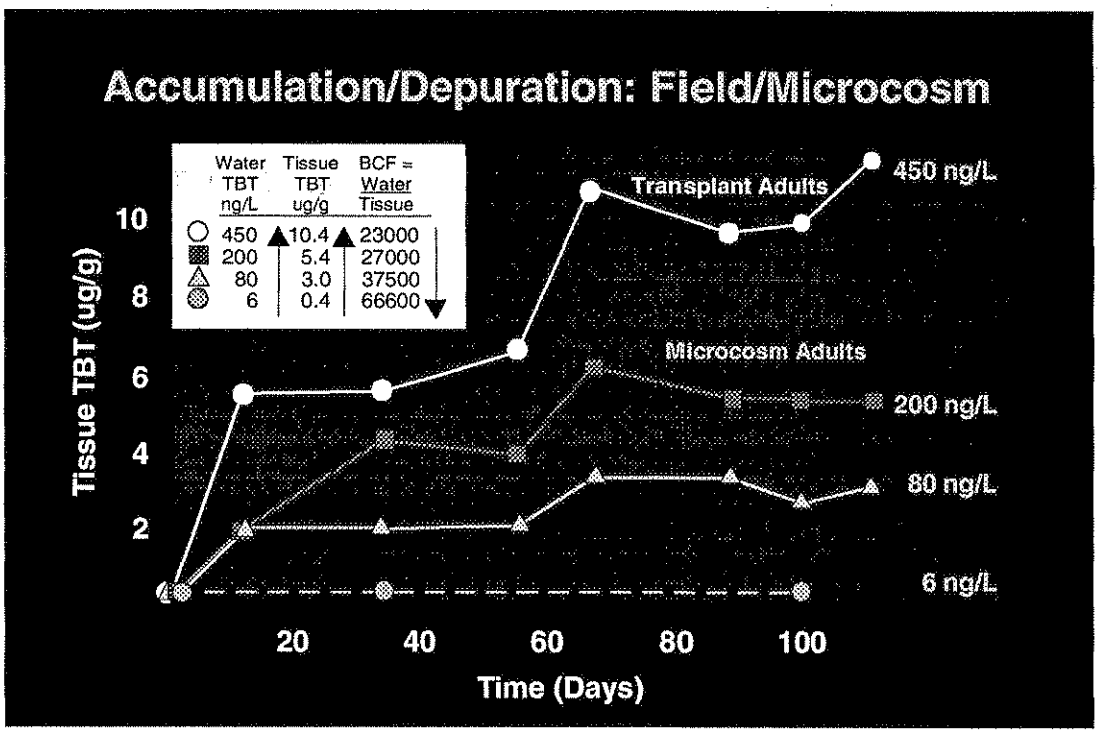
Relative Sensitivity of Bivalves

Bivalve Species	Species Compared	Exposure	Endpoint	Sensitivity
Anodonta grandis (Giant Floater)	Daphnia, Fathead Minnow, Rainbow Trout	Municipal Effluent	LC-50	Equal
Anodonta imbecillis (Paper Pondshell)	Daphnia	Pulp & Paper Mill Effluent	10-d vs 7-d mortality	More
Anodonta imbecillis (Paper Pondshell)	Daphnia, Midge, Fathead Minnow	Metals	7-d mortality	Equal
Musculium trans. (Fingernail Clam)	17 different species	Ammonia	20-d mortality	More than 16
Mercenaria mercenaria	2 Amphipods, Microtox	Sediment	7-d growth, 10-d mortality	More
Caged *Mercenaria* more sensitive than lab *Mercenaria*				
Mulinia lateralis	Amphipod	Sediment	7-d growth, 10-d mortality	More
Mytilus galloprovincialis	Amphipod	In-situ water column	84-d growth, 10-d mortality	More, [tissue TBT]

It is generally believed that because bivalves are so widely used in bioaccumulation studies that they lack the needed sensitivity for effects testing. In fact, there are some ASTM protocols that suggest the species commonly used for bioaccumulation testing are too insensitive to be used for effects testing. The table above represents all studies we could find with comparative data. This table shows that bivalves were as sensitive or more sensitive to chemicals than most commonly used test species. In fact, the EPA ambient water quality criterion document suggests that the freshwater fingernail clam Musculium transversum is the second most sensitive species to ammonia of all species tested. A critical review of these data suggests that these clams may have suffered from the same laboratory-induced stress observed in other studies. While M. transversum may be sensitive to ammonia, our recent in-situ field study with caged bivalve suggests, along with other studies, that bivalves exposed in the field are less sensitive to chemical exposure and they are able to tolerate higher concentrations of ammonia than predicted by laboratory testing.

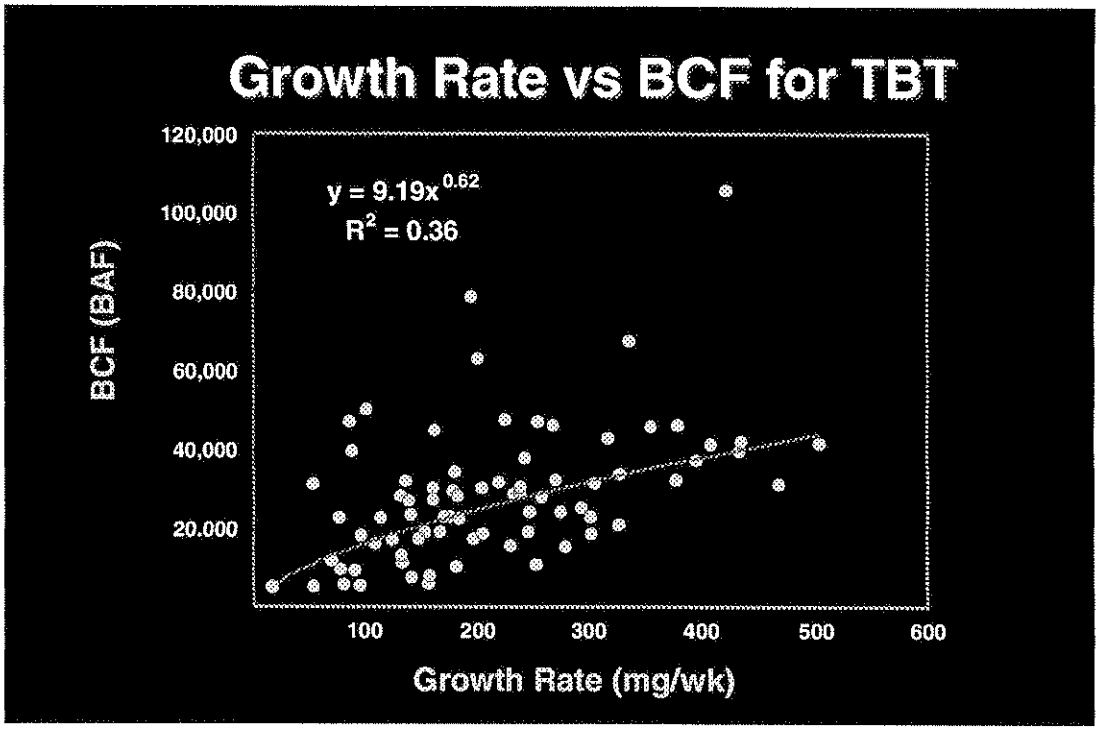
One of the most interesting comparisons is that between the clam Mercenaria mercenaria (7-day growth) and two species of amphipods (10-day mortality), in which the growth endpoint was more sensitive than the 10-day mortality endpoint. Further, results from caged M. mercenaria studies showed that this endpoint more closely resembled benthic community endpoints than any laboratory exposures.

A comparison of M. galloprovincialis and amphipods shows that for TBT, on a tissue residue basis, mussels are more than an order of magnitude more sensitive than amphipods. We do not believe that mussels are more sensitive than amphipods, but that the 84-day growth endpoint used for mussels is more sensitive than the 10-day amphipod mortality endpoint. These differences are also consistent with theory suggesting, based on QSAR relationships that the acute toxicity endpoints should be about an order or magnitude higher than the chronic endpoints.



The graphic above shows results from initial field and mesocosm studies to assess accumulation and depuration kinetics and to develop an appropriate exposure period for caged mussel testing. The graph shows a general leveling off of tissue TBT concentrations between 60 and 90 days of exposure. The table shows a relationship between increasing concentrations of TBT in seawater and increasing concentrations of TBT in mussel tissues as expected. Somewhat surprisingly we found an inverse relationship between these increases and decreases in bioconcentration factor (BCF) or bioaccumulation factor (BAF).

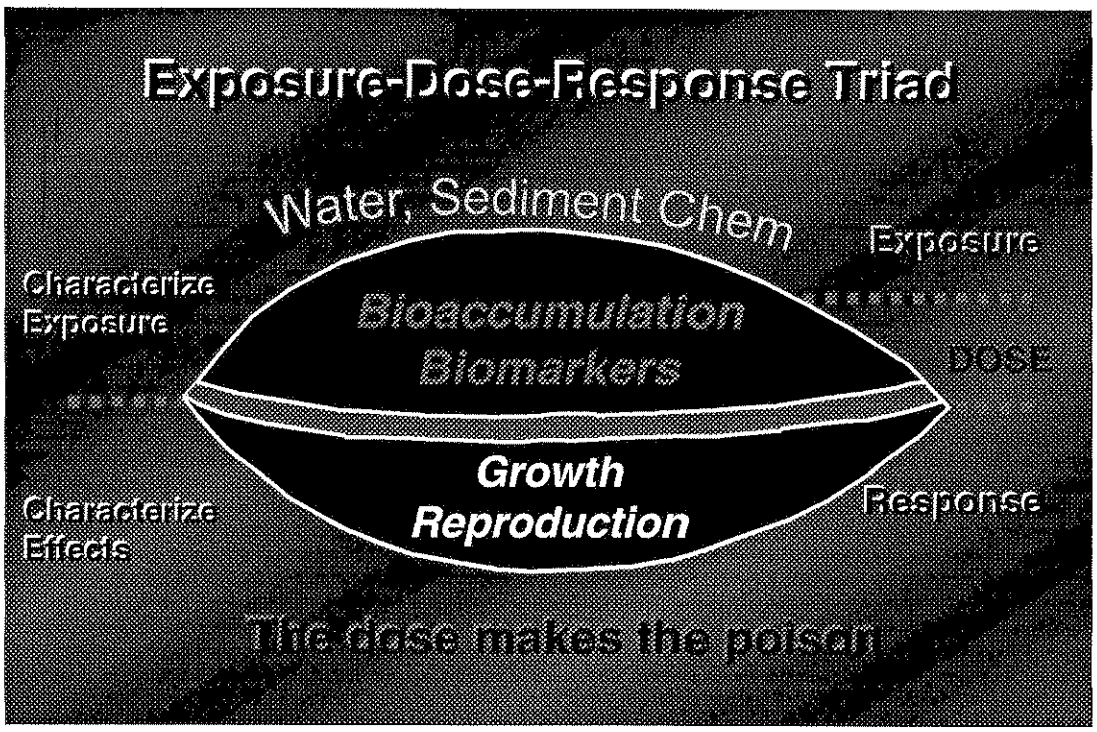
We believe that this concentration dependence is a general response that has been observed for other chemicals and other species. Our generic explanation is consistent with the explanation for the differences in survival and growth observed between laboratory and field exposures. At higher concentrations of chemicals, TBT in this example, test animals become increasingly stressed and begin to reduce their metabolic processes, such as filtration in bivalves as a way to combat this increasing stress. The net result of metabolic reduction is reduced chemical accumulation. A simpler explanation is that bivalves under increasing stress remain closed for longer periods of time, filter and feed less, and thus accumulate less because of reduced exposure.



The graph above demonstrates the relationship between juvenile mussel growth rate and bioconcentration factor based on 9 mussel transplant studies conducted in San Diego Bay between 1987-1990. The graph shows that bioconcentration factor generally increases with increasing growth rate. This is also consistent with the theory that healthier test organisms will tend to accumulate higher concentrations of TBT.

Considering the most extreme examples, one would not expect dead animals or animals close to death to accumulate much TBT. Conversely, healthy test animals that are feeding normally (filter- or deposit-feeding) will tend to accumulate more TBT. The problem is that there needs to be a quantification of animal health in the bioaccumulation test protocols that currently does not exist.

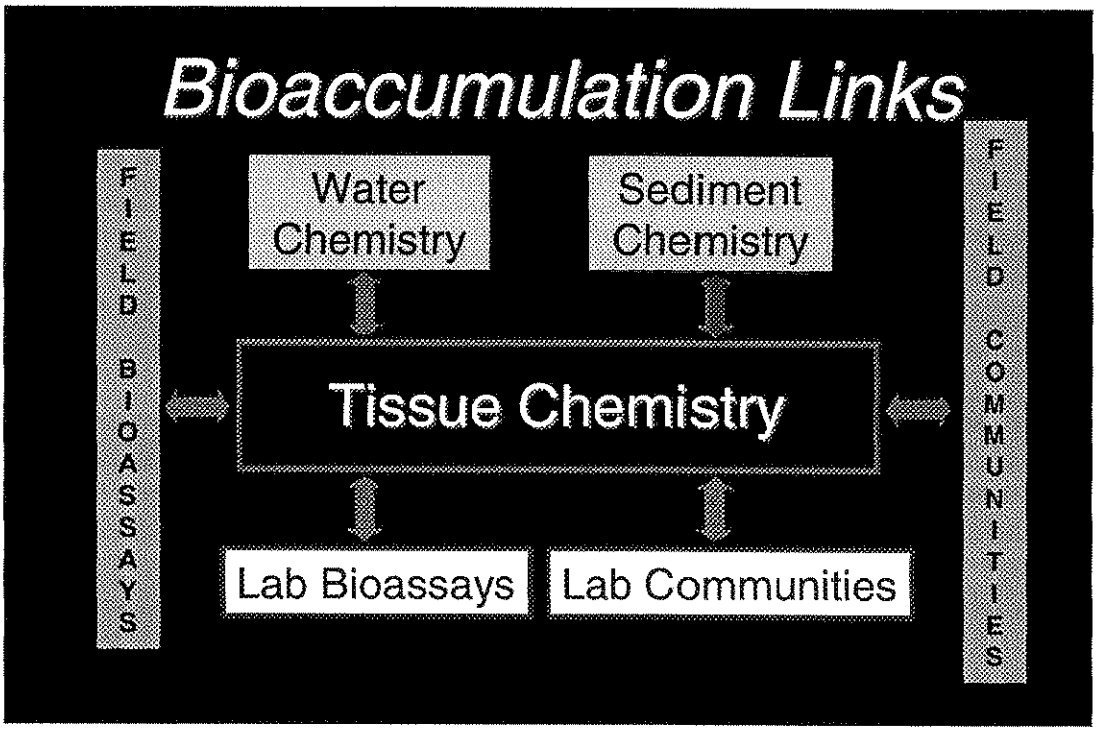
While the 80% survival criterion seems reasonable, a criterion for <20% loss in tissue weight for control animals during the exposure period also seems appropriate. This is based on laboratory tests where starved mussels lost approximately 10% of their tissue mass after 4 weeks and approximately 20% of their tissue mass after 8 weeks. Similar results would have to be demonstrated for Macoma in static and flow-through tests.



After years of caged mussel monitoring, we suggested an Exposure-Dose-Response (EDR)Triad to incorporate the major elements of a risk assessment format and those from the sediment quality triad. The major differences between our approach and the triad is the inclusion of tissue chemistry and in-situ bioassays as required elements.

Exposure is traditionally characterized by measuring the concentrations of chemicals of concern in water and sediment. However, these measurements only represent external exposure and the chemicals measured may not be biologically available. Exposure should also be characterized by measuring the concentrations of chemicals in mussel tissues, closer to the actual biological receptors of concern.

In our triad using bivalves, exposure is characterized by measuring water and sediment chemistry (exposure) as well as accumulated chemicals of concern (dose). Effects are characterized by using growth (response). Although we have traditionally used bioaccumulation and growth as the key elements of the EDR triad, we are currently working with other investigators to incorporate biomarkers and reproductive endpoints to better characterize exposure and effects.



The diagram above shows all elements of the Exposure-Dose-Response triad in its generic form and how tissue chemistry can be used to form links between various elements of the triad for predictive purposes.

Links for characterizing exposure are established by combining measurements of the 2 external exposure elements (water & sediment chemistry) with the dose element (tissue chemistry).

Links for characterizing effects are established by combining the dose element (tissue chemistry) with response element (single species bioassay and community endpoints). These bioassay and community endpoints are further divided into those measured in the lab and those measured in the field.

Revise Bioassay Protocols?

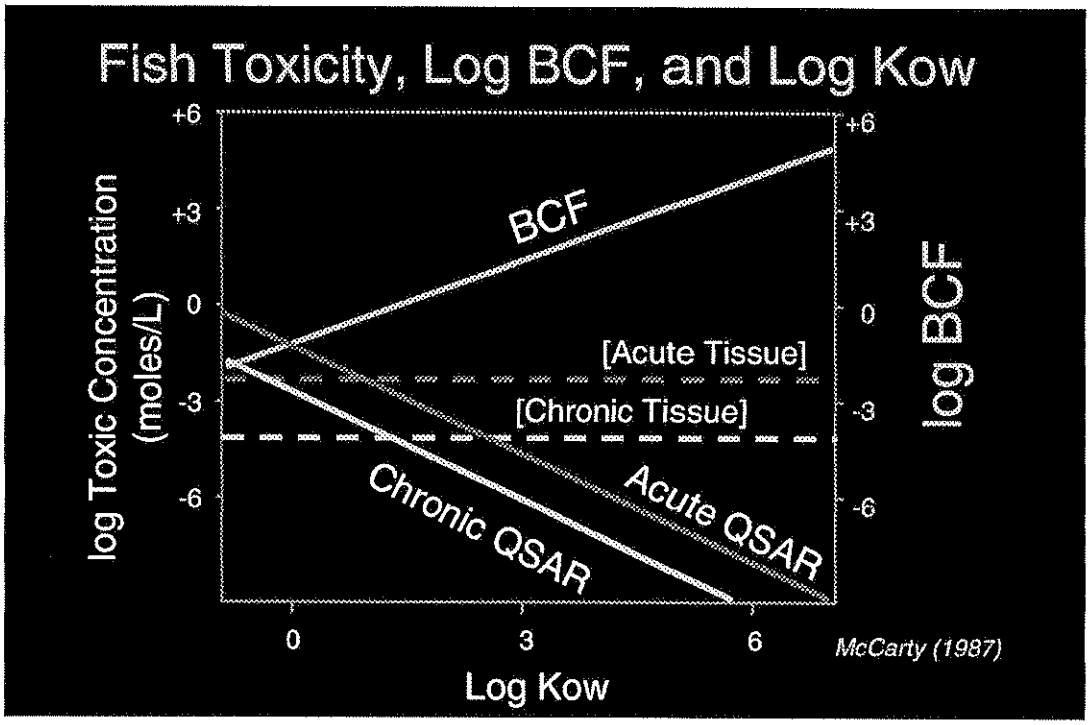
"The ultimate goal is the development of a single bioassay methodology, where the kinetics of bioconcentration to a given body or tissue level are linked with an understanding of the toxicological significance of that tissue residue level. Thus the nature and time course of external exposures can be linked with related processes in the body of exposed organisms."

McCarty (1991)

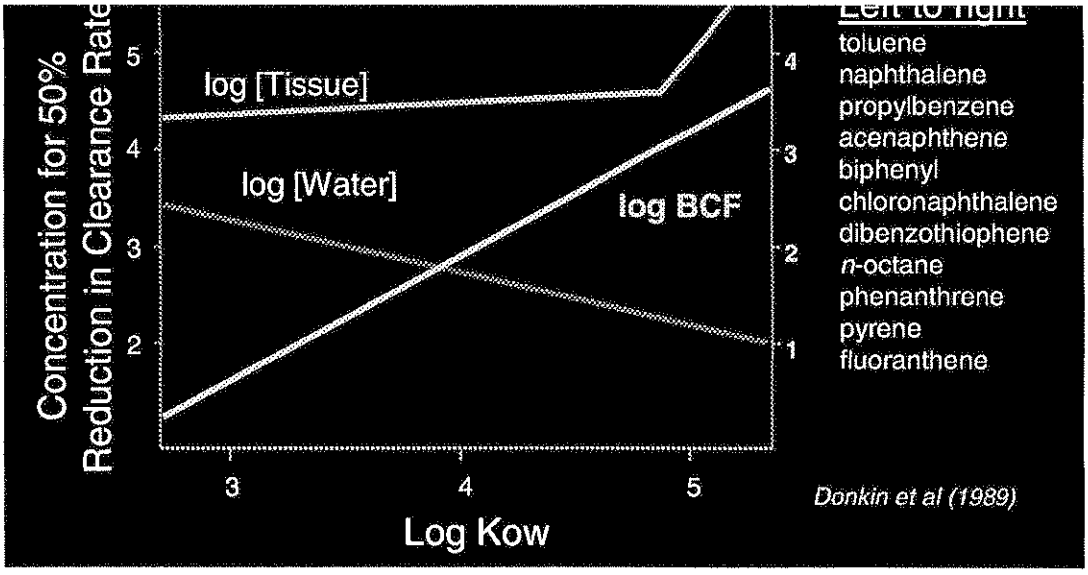
Why In-situ Tests?

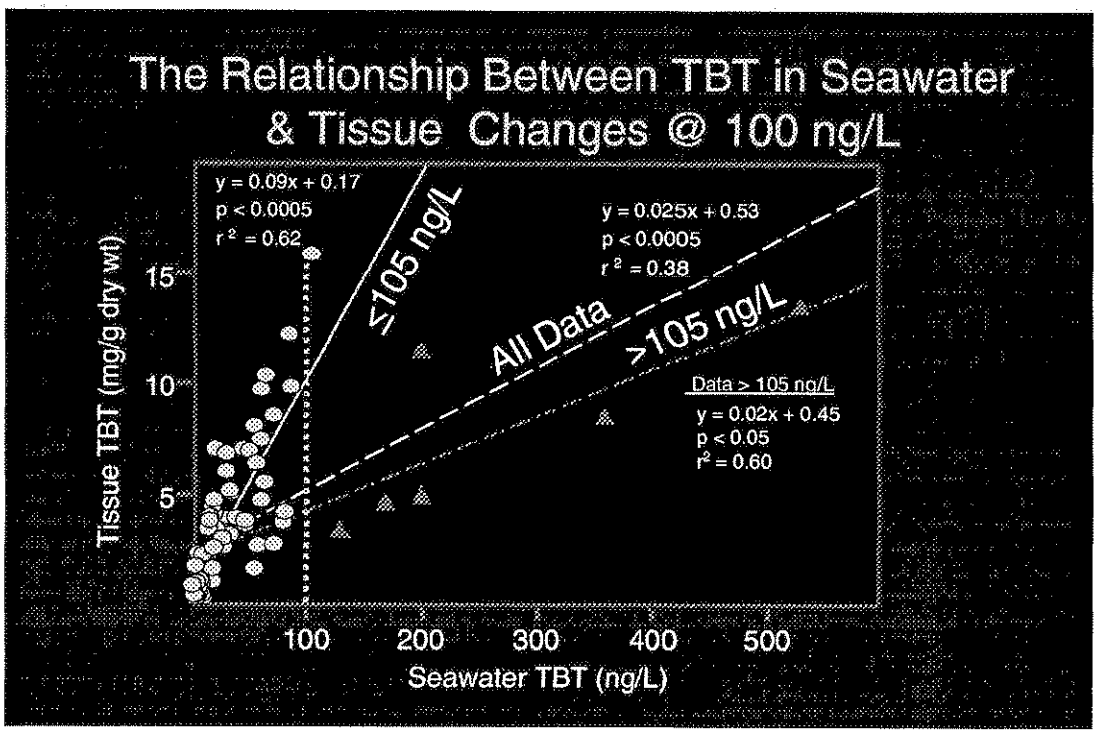
"The shift from aquaria to microcosms to field studies is not concerned with toxicity; it is concerned with the real variable in hazard assessment, the exposure assessment."

Parrish et al., 1988

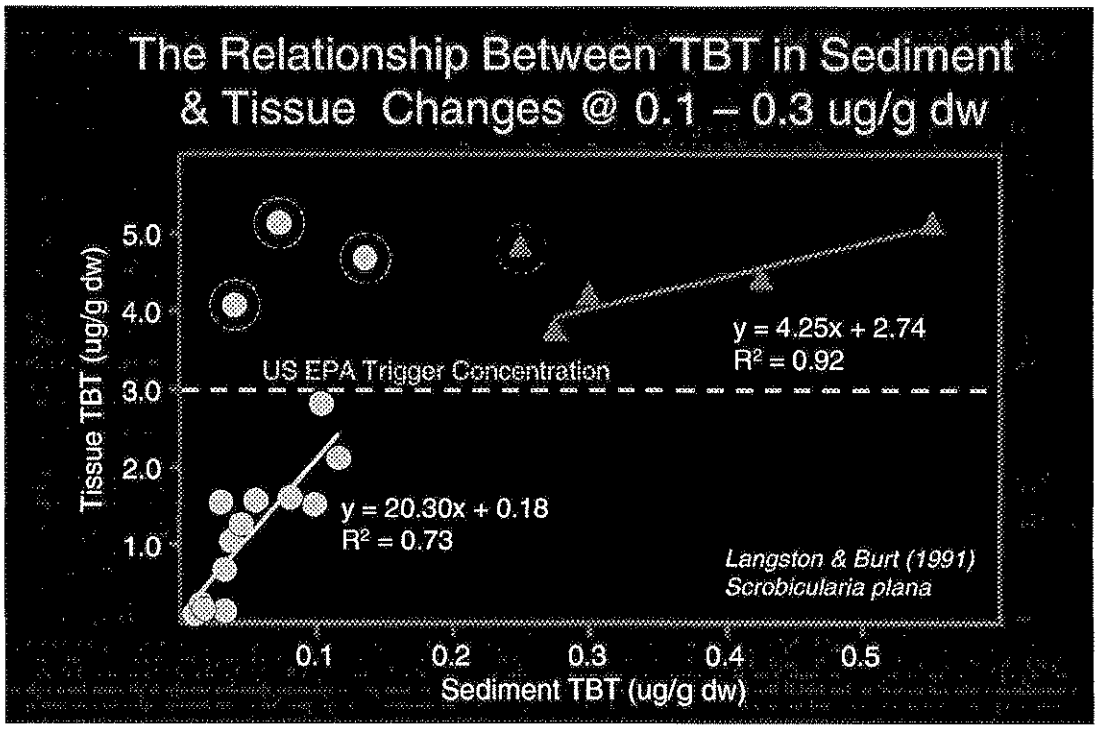

Fish Toxicity, Log BCF, and Log Kow
log Toxic Concentration (moles/L)
+6
+3
0
-3
-6
BCF
[Acute Tissue]
[Chronic Tissue]
Chronic QSAR
Acute QSAR
log BCF
0
3
6
Log Kow
McCarty (1987)


Water, Tissue, BCF vs Mussel Feeding
6
5


Concentration for 50% Reduction in Clearance Rate
log [Tissue]
log [Water]
log BCF
5
4
3
2
4
3
2
1
3
4
5
Log Kow
Left to right
toluene
naphthalene
propylbenzene
acenaphthene
biphenyl
chloronaphthalene
dibenzothiophene
n-octane
phenanthrene
pyrene
fluoranthene
Donkin et al (1989)



The significance of the dramatic changes in the relationship between exposure, dose, and response is illustrated by the studies conducted with caged mussel between 1987-1990. This graph shows how the relationship between tributyltin (TBT) in seawater and TBT in mussel tissues (Mytilus galloprovincialis) changes near seawater concentrations of 100 ng/L. TBT bioaccumulation is concentration dependent. The highest bioconcentration factors occur at the lowest seawater TBT concentrations. These data indicate that mussels were processing accumulated TBT differently, depending on exposure conditions. From these data we were able to identify the water and tissue TBT concentrations where effects may be expected.



Since it has been suggested that there was no relationship between concentrations of TBT in sediment and bivalve tissue concentrations we reviewed the literature to find the best available field study that evaluated this relationship. Those data came from Langston & Burt (1991) for Scrobicularia plana collected from 23 estuaries in the UK. Although the authors plotted their results on a log scale, we used a normal scale in an attempt to distinguish the fine structure of the relationships. We found a change in the relationship that was surprisingly similar to what we had discovered between seawater and tissue TBT in mussels. We have deleted the three points in circles from the regression analyses since seawater concentrations at those sites were very high and undoubtedly contributed to the concentrations accumulated in clam tissues. It appears a similar concentration dependence occurs for accumulation of TBT in sediment as we found for seawater. As mentioned previously, this phenomenon has been observed for a number of different species and a number of different chemicals.

Unfortunately, Langston & Burt have few data in the range between 0.1 and 0.3 ug/g dw, but based on the change in the relationship we believe that this is where effects begin to occur. This prediction is also consistent with other recent data. For example, EPA Region X has recently established 3.0 ug/g dw in tssues as the bioaccumulation trigger level requiring additional testing. If this trigger is extended across the graph it crosses the extended curve somewhere between 0.1 and 0.3 ug/g dw in sediment as predicted. Meador (1999) has also recently shown that these concentrations in sediment are associated with decreased growth rates in the polychaete worm Armandia brevis.

Philosophical Differences in Approach

Lab Toxicity Approach

- Bioaccumulation maybe
- Bioaccumulation not an effect
- Field validation impossible/unnecessary
- Compliance driven

Field Bioaccumulation Approach

- Bioaccumulation required
- Predict with bioaccumulation
- Field validation an integral part
- Risk assessment driven

Summary & Conclusions

Need for combined exposure & effects endpoints, more emphasis on the following:

- Understanding processes
- Bioaccumulation
- Field studies

Develop effects endpoints for Macoma & Nephtys, exposure endpoints for Neanthes

Summary & Conclusions

Need for combined exposure & effects endpoints, more emphasis on the following:

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- Field studies

Develop effects endpoints for Macoma & Nephtys, exposure endpoints for Neanthes

Hello Everyone,

Attached please find a copy of our SETAC platform presentation entitled "Using the Exposure-Dose-Response Triad in Laboratory & Field Bioassays: Lessons Learned from Caged Bivalves & TBT." The attachment is a PDF file that can be read with Acrobat Reader.

The crux of this paper is that most of our data from caged bivalves exposed to TBT strongly suggests that laboratory tests tend to over-estimate toxicity and under-estimate bioaccumulation. The reason for this is that bivalves seldom if ever grow as rapidly in the lab as in the field and since growth rate is related to bioaccumulation, reduced feeding by laboratory-induced stress can lead to unhealthy test animals that are incapable of accumulating chemicals the usual way. This problem is exacerbated with the presence of toxic chemicals in sediment since bivalves have the ability to close for extended periods to avoid exposure. We have reduced this problem in the field by conducting tests for 60 to 90 days to force exposure. Another problem with laboratory flow-through exposures is that facultative deposit-feeders like Macoma have the ability to switch between deposit- and filter-feeding over relatively short temporal scales. This is the reason for a shift from laboratory testing to microcosms and field testing; to ensure that the natural pathways of exposure are available.

This issue of exposure is becoming increasingly important in Puget Sound where regulators have revised traditional approaches to a bioaccumulation-based tissue trigger level for requiring additional testing. It is important to remember that there have been only three sets of laboratory bioaccumulation tests conducted using this new approach and they have all been conducted by the same laboratory. Another important difference is that most of the other suitability determinations have been based more on a preponderance-of-evidence approach using a combination of the sediment quality triad and AETs. Therefore, uncertainty is elevated because the lack of baseline data from 45-day exposures with Macoma in flow-through tests and the fact that regulators are now relying on a single number based on only a few tests from one laboratory. Considering the arguments raised in the attached paper and questions raised by other studies, there should not be much confidence in the TBT bioaccumulation tests conducted to date. The results have been ambiguous and conflict with other laboratory and field studies.

APPLIED BIOMONITORING

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HAVE MUSSELS, WILL TRAVEL