

USING CAGED BIVALVES TO CHARACTERIZE EXPOSURE AND EFFECTS ASSOCIATED WITH PULP AND PAPER MILL EFFLUENTS

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ABSTRACT

Routine environmental effects monitoring (EEM) should include field bioassays with caged bivalves—chemical exposure is characterized by measuring the concentration of chemicals in tissues and biological effects are characterized by measuring growth. This field bioassay combines the experimental control of laboratory bioassays with the environmental realism of field monitoring. Transplanting caged bivalves in the immediate vicinity of discharges ensures maximum exposure; if bioavailable chemicals are present in the effluent they will be accumulated within the tissues, and if the concentrations are sufficiently high to be deleterious, the effect of the exposure can be quantified. The transplant methodology described herein can be used to identify the following: (1) site-specific differences; (2) short-term and long-term trends; (3) temporal and spatial variability; (4) source identification; and (5) *exposure-dose-response* relationships. These results will help answer one of the most commonly asked questions asked by industry, government, and the public: “Are conditions getting better, worse, or staying the same?” Further, using the bivalve transplant approach as part of an integrated program will help reduce uncertainties associated with more traditional approaches like fish monitoring, laboratory bioassays and benthic community assessments.

KEYWORDS

Caged bivalves; environmental effects monitoring; field bioassay; mussels; pulp and paper; transplants

INTRODUCTION

There is increasing support for using more integrated approaches in environmental assessment programs (Chapman, 1996; Hall, 1996). However, this integration should be based on approaches best suited to answer the questions posed by the monitoring model. The risk assessment framework provides a very focused approach to environmental assessment and monitoring because it includes characterizations of both exposure and effects. The recently proposed *exposure-dose-response* (EDR) triad (Salazar and Salazar, 1995a, in review) facilitates those characterizations (Fig. 1).

With the EDR triad approach, exposure is characterized through the chemical analysis of environmental media (i.e., water and sediment) and biological tissues. Effects are characterized through bioassays and community structure studies, both of which are conducted in the lab and in the field. Using caged bivalves facilitates the field

bioassay element of the EDR triad. Through synoptic measurements of bioaccumulation and growth, uncertainties associated with exposure and effects can be reduced.

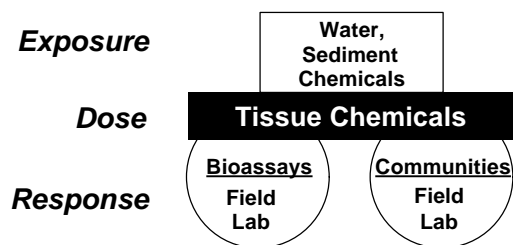


Figure 1. The EDR triad model identifying the major three elements. Exposure = chemicals in environmental media; dose = chemicals in biological tissues, and response = biological effects from bioassays and community studies in the field and in the laboratory.

Transplant studies with caged bivalves should be included in environmental effects monitoring (EEM) programs for the pulp and paper industry. The concept is not new. In 1980, reduced growth and reproduction were measured in caged marine mussels adjacent to a pulp and paper mill outfall in Canada (Wu and Levings, 1980). These results were correlated with a previous study showing reduced densities of natural mussel populations near the outfall (Levings and McDaniel, 1976). In Finland, caged bivalves have been routinely used since 1984 to monitor exposure from freshwater pulp and paper mill effluents (Herve, 1991; Herve *et al.*, 1996). Recently, caged bivalve monitoring for exposure has been required at a pulp and paper mill in the USA as part of their NPDES permit (EPA, 1994). The problem with most of these studies is that they have not properly integrated the exposure and effects elements. The work by Wu and Levings (1980) only included effects measurements and the other monitoring studies only included measurements of exposure. The methods for using field bioassays with caged bivalves have been refined to facilitate synoptic bioaccumulation and growth (Salazar and Salazar, 1995b). Growth is the recommended effects endpoint; in bivalves it is easily measured and understood. Growth represents an integration of all internal biological processes and can be quantified as a *dose-response*. Bivalve growth data can be readily extrapolated to potential population effects. Surprisingly, the initial basic plans for environmental effects monitoring (EEM) at pulp and paper mills in Canada included a caged bivalve requirement with estimates of bioaccumulation and growth (Parker *et al.*, 1991). It is not clear why this approach was abandoned.

Bivalves are commonly used as biological indicators of exposure because of their ability to concentrate and integrate chemicals from water and sediment in their tissues (Metcalf and Charlton, 1990; Phillips and Rainbow, 1993) and the utility of caged bivalve transplants in monitoring (de Kock and Kramer, 1994). Field bioassays with caged bivalves combine the advantages of experimental control from standard laboratory bioassays with the environmental realism from traditional field monitoring. Strategic placement of caged bivalves along chemical gradients facilitates more environmentally representative descriptions of chemical exposure over space and time than water or sediment samples. The integrating power of bivalve filtration helps to normalize the variability associated with quantifying pulp and paper mill effluents and their receiving waters. These factors include intermittent and variable discharges, variability in the direction and velocity of water currents, and natural factors such as storm events, episodic sedimentation, and runoff. All of these factors affect chemical exposure and associated biological effects and have been addressed previously (Beck, 1996; Whitfield and Wade, 1996). A single chemical analysis of bivalve tissue provides an integrated record of bioavailable chemicals that cannot be defined with thousands of water or sediment samples. Chemicals in bivalve tissues, which will be referred to as the "dose," provide a direct link between chemical exposure and associated biological effects. It also provides a way to compare the results of bioassays and population or community responses in the field.

This paper will provide an overview of the caged bivalve methodology and a framework for incorporating this approach into existing EEM programs. Selected results from a multi-year study to evaluate tributyltin (TBT)

antifouling coatings in San Diego Bay, California, USA, will be provided as an example of how this approach can be used to help characterize chemical exposure and associated biological effects. Long-term studies provide information regarding temporal and spatial trends that can be used to predict effects.

METHODS

A complete description of the caged bivalve methodology is provided in Salazar and Salazar (1995b). Refinements over other methods include: 1) minimized size range, 2) defined exposure period, 3) measurements of bioaccumulation and growth in the same test animals; and 4) multiple growth metrics.

In the San Diego Bay TBT study, nine individual transplant studies were conducted at 18 sites over a three-year period between 1987-1990. Mussels (*Mytilus galloprovincialis*) in the 10-12 mm size range were used in each test. Only two sites (SI and SID) in one of the most TBT-contaminated yacht basins (Shelter

Island Yacht Basin) will be discussed here to demonstrate the utility of the caged bivalve methodology. SI, 1 meter below the surface, and SID, 1 meter above the bottom are separated by only 3 meters vertical distance. Results presented here represent a significant part of the U.S. Navy's ecological risk assessment for TBT.

Cages with individual compartments were used to hold the mussels so that measurements could be made on each mussel during the test period. This increased the statistical power. The following metrics were used to estimate growth: whole-animal wet-weight (WAWW), shell length, shell weight, and end-of-test tissue weight (EOTW). Shell weight and tissue weight could only be measured at the end of the study because these are destructive processes. At the start of the test and at selected intervals throughout the defined exposure period, WAWW and shell lengths were measured for each mussel. Growth rate regressions were determined from these multiple measurements. Only tissue growth (EOTW) will be presented here. For the mussels transplanted to SI and SID, initial shell weights and tissue weights were estimated from a representative sample of mussels. Each study utilized an 84-day exposure period. This provided sufficient time for chemicals to come to equilibrium in mussel tissues and mussels to demonstrate significant growth responses under stress. Chemical analysis of seawater provided data on external exposure conditions while tissue chemistry was used to estimate the actual dose at internal mussel receptors.

RESULTS

The results of the mussel transplant studies can be used to demonstrate: 1) site-specific differences, 2) short- and long-term trends, 3) temporal and spatial variability, 4) source identification; and 5) *exposure-dose-response* relationships. These types of results can be used to support an ecological risk assessment for any chemical introduction, including chemicals from pulp and paper mills. The advantages of using caged bivalves instead of natural populations is demonstrated by the fact that none of the comparisons made here would have been possible using natural mussel populations, because mussels were not found at that depth.

Site-specific Differences

There was a statistically significant difference in exposure, dose, and response, when SI and SID were compared across tests (Fig. 2). Tissue TBT concentrations were derived from a pooled sample measured at the end of each test and, therefore, could not be statistically compared by test. The multiple WAWW and shell length measurements were used to develop growth rate regressions but only the end-of-test tissue weight data (EOTW) are presented here. Statistical analysis of these tissue growth data indicated that growth rates were significantly lower for the mussels at SI. This was surprising since the two sites were separated by only 3 meters vertical distance. It was also surprising that in many comparisons, tissue growth based on a single measurement of tissue weight was just as discriminating as multiple measurements of whole-animal wet-weight. Seawater TBT concentrations were significantly higher at SI in every test but two. The ability to detect these differences demonstrates the statistical power of the methodology.

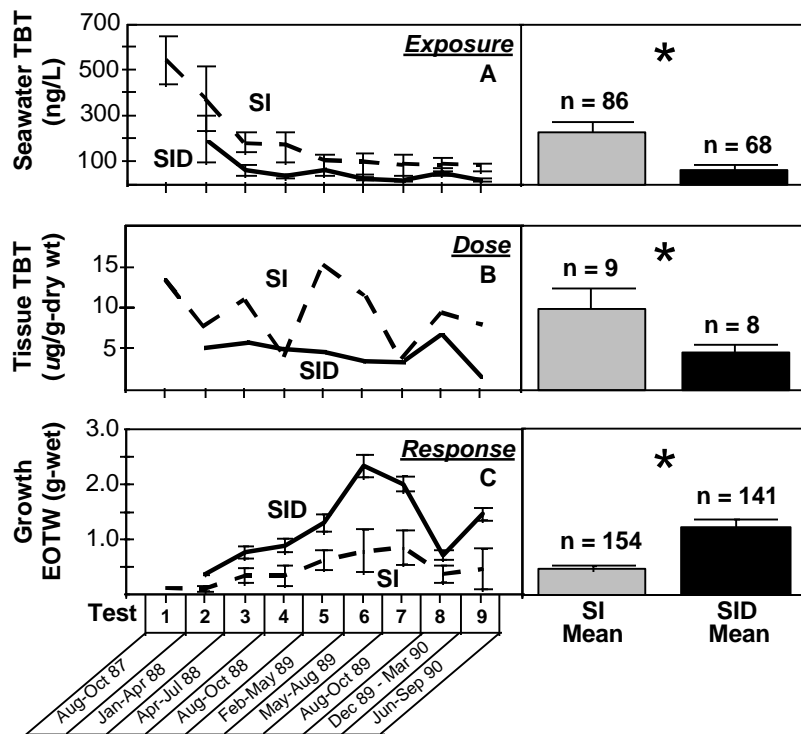


Figure 2. Synoptic measurements in the EDR triad. Exposure (A) = seawater TBT concentration. Dose (B) = tissue TBT concentration. Response (C) = growth estimated by end-of-test tissue weight (EOTW). SI = surface site; SID = bottom site. Data points for seawater TBT concentrations represent 12-week means (± 2 standard errors). * = statistically significant difference ($\alpha = 0.05$).

Short- and Long-term Trends

The seawater concentration of TBT (exposure) decreased significantly at both sites in the Shelter Island Yacht Basin between 1987-1990 due to the restrictions on the use of TBT antifouling coatings. The concentration of TBT in mussel tissues (dose) decreased similarly during the first four tests but then showed a dramatic increase, even as seawater concentrations decreased or remained constant. Growth (response), based on EOTW, increased steadily at both sites through Test 6 with a significant decline in Test 8 which was followed by another small increase in Test 9. Growth had an apparent effect on bioaccumulation.

Temporal and Spatial Variability

Seawater TBT concentrations and WAWW varied by a factor of six on a weekly basis during the nine tests. This variability is one of the reasons for measuring tissue chemistry; i.e., to normalize exposure concentrations. The variability in tissue measurements may be attributable, at least in part, to the following: the use of wet-weights in tissue analyses; inadequate replication, and differences in animal health that affect tissue concentrations. The concentrations of chemicals in tissues can also be calibrated by taking into consideration changes in tissue weights during the exposure period. Higher seawater TBT concentrations reduced growth and uptake of TBT. There was greater variability in the higher concentrations of TBT in seawater and tissue TBT at the surface. Conversely, there was greater variability in mussel growth (EOTW) at the bottom site where growth rates were significantly higher. Variability in seawater and tissue concentrations and growth increased and decreased with the magnitude of the measurements as expected.

Source Identification

The source of the TBT was confirmed through these caged mussel studies. The significantly higher concentrations of TBT in both surface water and mussel tissues confirmed that boat hulls were the primary source of TBT and not the bottom sediments. Even though sediments are the primary sink for TBT, the release of TBT from sediment-bound particulates to the water column is slow. Source identification would not have been possible through the monitoring of resident populations of fish, crabs, or mussels. The mobility of animals like fish and crabs generally preclude associating tissue residues with site-specific chemical sources. In many cases, resident populations are not always present in the area of concern. In the Shelter Island Yacht Basin, mussels were not naturally found at SID during the study period.

Exposure-Dose-Response

Exposure-dose-response relationships were developed using field data for SI and SID from serial transplants with repetitive measurements of seawater TBT, tissue TBT, and mussel growth. Although conditions during field bioassays are more variable than in laboratory bioassays, the results are more environmentally realistic and it is possible to provide first-order approximations for water concentrations and tissue concentrations associated with adverse effects on tissue growth (EOTW). These results are shown in Fig. 3. Data from tests 8 and 9 were excluded because temperatures below 15°C and above 21°C were shown to reduce growth. Although these graphs represent a subset of data from all 18 test sites, the relationships are similar. The *exposure-dose* regressions show that the relationship changes significantly above 100 ng TBT/L. At these high concentrations, bioaccumulation is reduced. The *exposure-response* relationship also changes above 100 ng TBT/L. This is the predicted effects level for adverse effects on mussel growth. Similarly, the predicted effects level for tissue TBT concentrations in the *dose-response* relationship is 4µg TBT/g dry weight. Since some relationships improved with only Shelter Island data, these results show the importance of using site-specific data to characterize chemical exposure and biological effects.

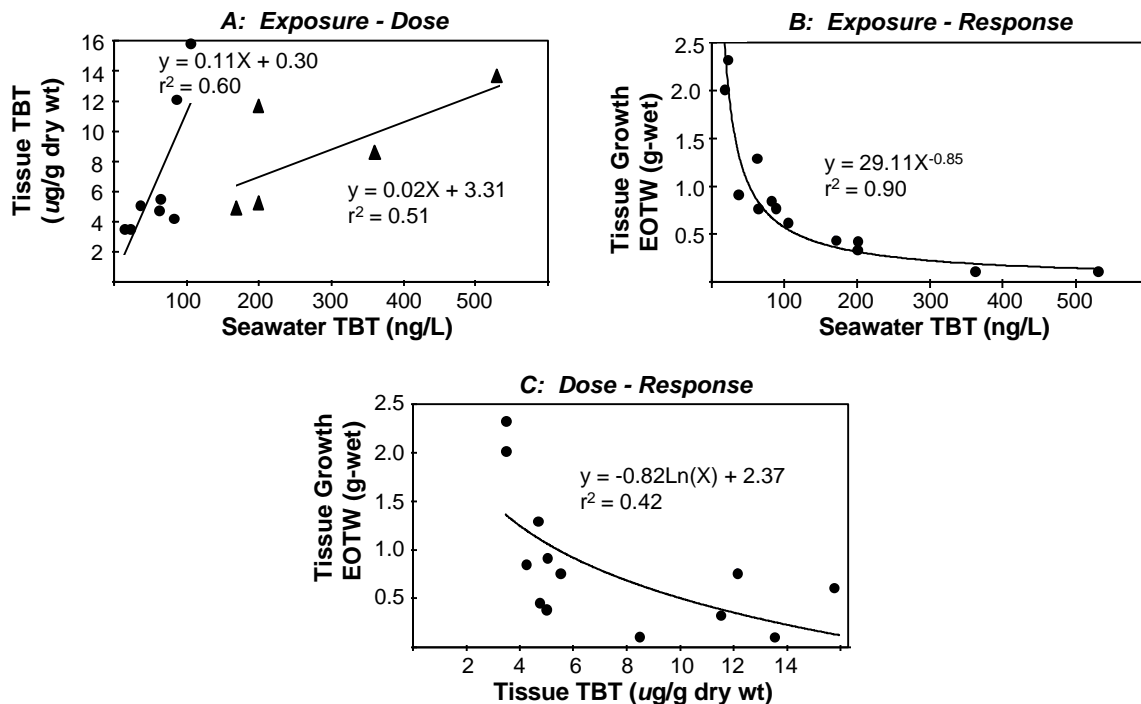


Figure 3. EDR triad showing A: *Exposure-dose*, B: *Exposure-response*, and C: *Dose-response*. Shown here are the relationships between seawater TBT and tissue TBT; mussel tissue growth (EOTW) and 1) seawater TBT and 2) tissue TBT for a surface (SI) and bottom (SID) site. All regressions are statistically significant ($\alpha = 0.05$).

DISCUSSION

It has been proposed that routine environmental effects monitoring (EEM) should include field bioassays with caged bivalves. The *exposure-dose-response* (EDR) triad has been presented as a monitoring and assessment framework for characterizing exposure and effects. Measurement of tissue chemistry (dose) is suggested as a primary link between environmental exposure and organism effects. The dose also provides a link between responses of single-species bioassays and community studies, conducted in both the lab and field. A tributyltin (TBT) assessment has been presented as an example of how these types of data can be used in a retrospective risk assessment by identifying site-specific differences, short-term and long-term trends, temporal and spatial variability and chemical sources (Salazar and Salazar, 1995a,b). The *exposure-dose-response* relationships from the EDR triad can provide the basis for predictive assessments that further link other laboratory and field studies (Salazar and Salazar, in review). While field validation of laboratory bioassays is commonly discussed, perhaps it is equally appropriate to view the EDR triad approach as a paradigm shift toward laboratory validation of field data. This integration includes chemical measurements of water, sediment, and animal tissues that are associated with biological responses in the lab and in the field. With these data it is possible to make predictions by combining various elements from exposure, dose, and response. The statistical significance of these relationships can be used to help understand the exposure and response mechanisms involved. This is also a good example of how monitoring data can be used to validate the proposed monitoring model and to make necessary adjustments when appropriate.

Traditional methods used to monitor pulp and paper mill effluents have a relatively high degree of uncertainty associated with quantifying temporal and spatial changes in chemical exposure and associated biological effects (Colodey *et al.*, 1991; Hodson, 1996). Many of the fish tissue samples that are analyzed reveal no exposure, and no adverse effects are often seen in the laboratory assessment of effluents. Fish biomarkers typically show a response, but the relationship with population effects is unclear. Uncertainties associated with benthic community structure are even greater due to the subtle effects of natural factors such as grain size and organic content. Collectively, results from all these types of studies do not necessarily implicate one method as being more sensitive or more environmentally meaningful than another. They clearly demonstrate that no single methodology or measurement endpoint can provide the answers to questions such as whether conditions are getting better, worse, or staying the same. However, the use of field studies with caged bivalves can help reduce the uncertainty seen with the traditional methods.

Uncertainties associated with monitoring biomarkers include fish movements that affect the intensity and duration of exposure, seasonal and species differences, the inability to find appropriate species in the area of concern, undefined time scales of exposure and response, and an unclear relationship between biomarker response and population effects (Hodson, 1996). While caging fish can solve several of these problems several remain, and others are introduced. Although caging fish defines the exposure position and duration, it modifies pathways of exposure in that fish are not able to feed in a natural way and are generally removed from sediment exposure. Caging bivalves is much easier than caging fish, it does not alter their natural pathways of exposure, and it is possible to use both water column and infaunal species.

Uncertainties associated with laboratory bioassays are equally problematic (Salazar, 1986). It is often difficult to even speculate whether laboratory tests underestimate or overestimate potential effects. It could be argued that laboratory test animals are generally under more stress because existing exposure methodologies cannot adequately simulate nature, and there is evidence supporting this argument. In the context of pulp and paper mill effluents this is an issue because of nutritional benefits provided by some constituents of the effluent. It also could be argued that exposure periods are unrealistic and therefore laboratory bioassays tend to underestimate effects in nature. In the context of pulp and paper mill effluents for example, the environmental significance of a 10-day laboratory exposure is questionable if it takes 30 days for tissues to reach chemical equilibrium in the laboratory exposure vessels. One of the advantages of field bioassays is reducing several uncertainties associated with predictions from laboratory bioassays that do not include all of the site-specific factors that could affect both exposure and response. The caged bivalve methodology can be used to quantify exposure and effects before, during, and after changes in technology used to reduce chemical loading in pulp and paper mill effluents. These data, in conjunction with other elements of the EDR triad, can be used to answer the question of whether environmental conditions are getting better, worse, or staying the same.

Another benefit of using the EDR triad is to help maintain a focused monitoring and assessment program that serves as a reminder of the importance in understanding these relationships between exposure, dose, and response in a risk assessment format (Mearns, 1985). For example, with an equal emphasis on water, sediment, and tissue chemistry, it is difficult to avoid the question of chemical equilibrium in laboratory and field studies. The time required to reach chemical equilibrium then becomes an important issue in determining appropriate exposure periods, and in the design and conduct of both laboratory and field studies. If tissue residues are then measured in both laboratory and field studies, it provides a method for comparing the effects data from more natural and more controlled conditions. The tissue residue approach has been suggested by a number of investigators as being more realistic than attempting to use water or sediment concentrations (McCarty and Mackay, 1993). Tissue bioaccumulation measures only biologically available chemicals. Since bivalves lack the capacity to metabolize many of the chemicals that are readily metabolized by fish, this is another advantage of using bivalves over fish. In summary, the application of integrated monitoring approaches to characterize pulp and paper mill effluents is strongly encouraged. Using field bioassays with caged bivalves to estimate chemical exposure and biological effects represents a cost-effective approach that could reduce uncertainties associated with any integrated monitoring program.

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