

## **USING CAGED BIVALVES FOR ENVIRONMENTAL EFFECTS MONITORING AT PULP AND PAPER MILLS: RATIONALE AND HISTORICAL PERSPECTIVE**

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### **ABSTRACT**

There is general agreement between regulatory agencies and industry that new, and integrated approaches are necessary to monitor and assess environmental conditions in the vicinity of pulp and paper mill effluents. Traditional monitoring approaches have considerable uncertainty, and any single method is clearly not adequate. Current monitoring approaches utilize laboratory bioassays, benthic community structure and adult fish to surveys to estimate exposure and effects. There are several problems even with this integrated approach. Sources of uncertainty include extrapolation from laboratory bioassays to site-specific conditions, availability of appropriate fish species, and difficulties in interpreting effects and exposure data from benthic communities. Perhaps most frustrating is the use of fish to characterize chemical bioavailability. Numerous fish samples have been analyzed and detectable concentrations of the chemicals of concern have only been found in a small percentage of the samples. Often, fish are not even found in the areas of concern. Measurements of biochemical indicators have been inconsistent, and there is no clear relationship between the induction of these biomarkers and fish population effects. Adult fish surveys have not been able to answer the questions most commonly asked by government, industry and the public "Are conditions getting better, worse, or staying the same?" This paper will provide the rationale, historical perspective, and an example of using caged bivalves as a new approach to reduce uncertainty in monitoring and assessing pulp and paper mill effluents. The historical use of caged bivalves in aquatic monitoring programs will be reviewed. In addition, results will be presented from a recent caged mussel study conducted in the Pacific Northwest to evaluate conditions in the vicinity of effluents discharged from a pulp and paper mill.

### **INTRODUCTION**

While the stated object of the Environmental Effects Monitoring (EEM) Program in Canada is to test the effectiveness of current regulations on fish health and fish habitats, measuring fish may not be the most appropriate method for making that assessment. As contradictory as that might seem, measuring water concentrations of chemicals is not the best way to assess water quality. Biological indicators were originally developed as a measurement tool for chemicals in water and sediment when the concentrations were below detection limits of currently available analytical techniques (Phillips, 1980). Biological indicators integrate and concentrate bioavailable chemicals and add a biological component to the chemical analysis. Field bioassays with caged bivalves maximize the utility of a bioindicator approach and minimize the uncertainty associated with traditional laboratory and field methods. Caging facilitates synoptic measurements of exposure and effects over space and time. This approach combines the experimental control of laboratory bioassays and the environmental realism of field monitoring. Over the past 20 years the design and methodology has been refined to include mussels, clams, and oysters; marine, estuarine, and freshwater habitats; and depths to 80 m. Transplanting caged bivalves in the immediate vicinity of outfalls will guarantee an exposure and almost certainly produce measurable tissue burdens if the chemicals are biologically available. Placing test animals along chemical gradients will also help identify potential sources. Field studies with caged bivalves facilitate measuring bioaccumulation and bioeffects because (1) bivalves are easy to collect, cage, and measure, (2) bivalves will accumulate bioavailable chemicals, and (3) bivalves can be measured for adverse effects (Salazar and Salazar, 1995). Recently, we have described the application of caged bivalves to

support ecological risk assessments (Salazar and Salazar, in review) and to characterize exposure and effects associated with pulp and paper mill effluents (Salazar and Salazar, in press).

It is timely to consider the use of new and innovative approaches for monitoring effluents because the results of Cycle I monitoring are currently being reviewed. Results of the review will be used to improve the approach for Cycle II of the EEM Program. Further, it is always helpful to return to the questions that are being asked as part of any monitoring program to ensure the best methods are being used. Quantifiable, scientifically defensible, and easily understood answers are needed that reduce the uncertainty in the risk assessment process. The questions should also be asked within the context of an ecological risk assessment: (1) What is the characterization of exposure in the major environmental compartments—water, sediment, and tissue? (2) What is the characterization of effects in ecologically significant receptors? (3) Are conditions getting better, worse, or staying the same?

Government, industry, and the public are frustrated with the high degree of uncertainty in traditional approaches. Many expensive fish surveys have been conducted only to result in non-detectable concentrations of chemicals in the tissues or biomarker measurements of chemical exposure that are ambiguous, difficult to interpret, and of limited value. Some tagged fish studies have been conducted where none of the tagged fish were recovered. Cost per unit effort is very high. Mixing zones are often poorly defined and chemical analysis of discrete water samples do not address the integrated exposure and effects associated with chemicals of concern. Every extrapolation adds to the uncertainty. Numerous extrapolations must be made on data from laboratory bioassays, analyses of benthic community structure and from monitoring various ecological receptors in natural fish populations. These types of monitoring tools characterize chemical exposure and associated effects with relatively high levels of uncertainty.

## RATIONALE

One way to reduce the uncertainty associated with traditional monitoring methods is the use of field bioassays with caged bivalves. Field bioassays bridge the gap between traditional laboratory bioassays and field studies; they combine the experimental control from laboratory bioassays and the environmental realism from field monitoring into a cost-effective monitoring tool (Figure 1). The main advantages of field bioassays with caged bivalves are: (1) the ability to obtain site-specific measurements and establish site-specific links under environmentally realistic conditions; (2) strategic placement of test animals along suspected chemical gradients where they do not move; (3) testable hypotheses; and (4) test species are always available and the same species can be used in many different environments. In the field bioassay approach, exposure is characterized by measuring chemicals in tissues, and effects are characterized by measuring sublethal endpoints like growth. There is a greater likelihood of accurately evaluating the extent of bioavailable chemicals because bivalves, such as mussels, efficiently concentrate and integrate chemicals. Strategic placement of caged bivalves integrates exposure and effects over space and time (Figure 2). There is relatively no additional expense associated with extending exposure times, so field bioassays can be conducted until chemical equilibrium has been reached. Most laboratory bioassays have been designed to minimize cost and exposure time. However, it is uncertain whether chemical equilibrium has been reached in test animals and whether the measured response reflects realistic exposure conditions.

Bioaccumulation provides the most direct estimate of biologically available chemicals. It provides a direct link between the external environment (i.e., water and sediment) and the receptor sites (i.e., the internal environment of the organism). Bivalves integrate chemical exposure over time as they filter the water for food and sequester the chemicals along with the food. Both natural and transplanted populations of bivalves have been used for bioaccumulation studies around the world (Phillips, 1980). Growth represents an integration of all internal biological processes and can be associated with water, sediment, and tissue concentrations of chemicals.

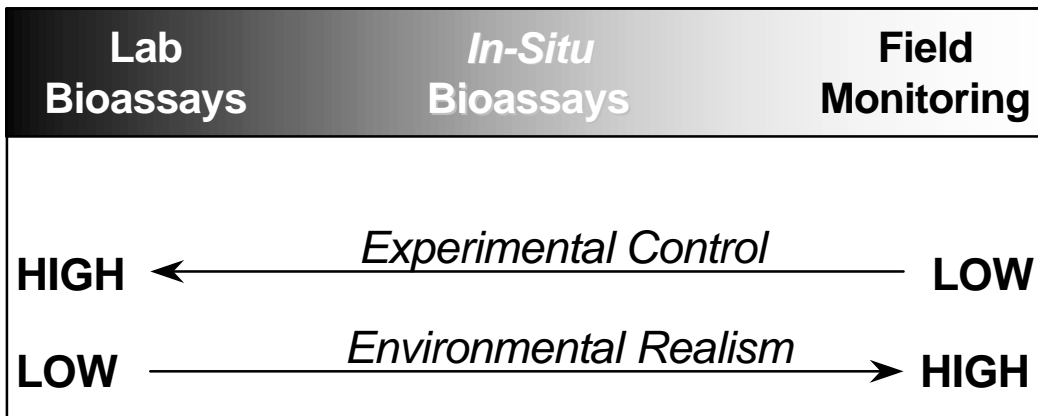


Fig. 1. Bridging the gap between traditional laboratory bioassays and field monitoring with *in situ* field bioassays.

There is a perception that because bivalves are routinely used in bioaccumulation studies, they are insensitive to chemicals. A number of recent studies have shown that bivalves are equally or more sensitive than other commonly used bioassay organisms, and growth is an integrated effects endpoint that can be readily quantified. For example, Burgess and Morrison (1994) have shown that for marine sediments, although clam (*Mulinia lateralis*) and amphipod (*Ampelisca abdita*) acute endpoints are relatively similar, the sensitivity is increased in clams when growth is used as a sublethal endpoint. In their tests on six freshwater pulp mill effluents, McKinney and Wade (1996) showed that 9-day mussel mortality (*Anodonta imbecilis*) demonstrated greater sensitivity than 7-day daphnia mortality (*Ceriodaphnia dubia*). On a tissue residue basis, Salazar and Salazar (in review) have shown that an 84-day growth endpoint in caged mussels (*Mytilus galloprovincialis*) was an order of magnitude more sensitive to tributyltin (TBT) than the mortality endpoint in four marine amphipod species reported by Meador et al. (1996).

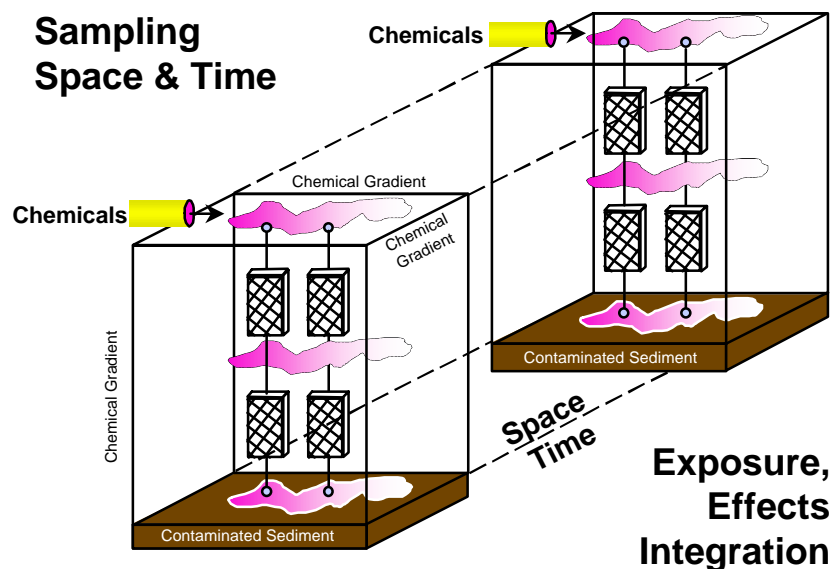


Fig. 2. Using caged bivalves to monitor chemical exposure and biological effects over space and time through integrated measurements.

Bivalves are sedentary and have hard shells. This makes them easy to collect, cage, and measure. Using farms or culture facilities as the source of test animals has the added benefit of a population with a known genetic and environmental history. There is also an extremely large database for exposure and effects data from both laboratory and field studies. Caging does not affect chemical exposure to bivalves as it does with fish; caged fish do not have the freedom to feed in their usual way because of confinement. Further, most bivalves have a limited capacity to metabolize petroleum hydrocarbons (PAHs) and other chemicals in mill effluents that have been associated with MFO induction in fish. The main problem in using biomarkers of chemical exposure like MFO is that they are too ephemeral and persist for only a short period of time after exposure. Recent work has also suggested that there is no direct link between MFO induction and population effects (Munkittrick et al., submitted).

It is important that the chemicals in the effluent plume be accurately mapped. Likewise, it is necessary to accurately evaluate the effects from that chemical exposure. Caged mussels provide a direct method of mapping the extent of chemical contamination, defining mixing zones, and assessing the subsequent biological effects. Caged bivalves accumulate and respond to only biologically available chemicals. Routine analysis of thousands of water samples cannot reveal which chemicals are bioavailable or the effects of exposure to multiple chemicals. It is very difficult to define the extent of chemical contamination or map the effluent plume using natural fish populations because they are so mobile. This mobility results in high uncertainty associated with the exposure period and position.

#### Historical Perspective

Since the initiation of environmental monitoring programs for pulp and paper mills, fish have been the emphasis for monitoring receiving waters. Techniques for collecting and analyzing tissues of resident fish populations are well established and used routinely. However, it is becoming increasingly apparent that using mobile species may not be the best approach. Scandinavian scientists were among the first to associate effects on growth and reproduction in fish with chemicals in effluents. They also associated specific biomarkers with those organismal effects. This approach was adopted by Canadian regulatory agencies early in the EEM Program. Unfortunately, even though significant progress has been made in quantifying the endpoints, and even the compounds responsible for these effects and MFO induction, there is still no clear relationship between induction and population effects (Hodson, 1996; Munkittrick, submitted). This could have been predicted several years ago on the basis of the mobility of the fish populations and the short duration of MFO induction after exposure ceases. The Scandinavians have shown additional foresight by developing an integrated monitoring program that includes bivalves and fish (Rantio et al, 1996). Integrated programs at several levels of organization have been suggested previously (Chapman, 1996; Hall, 1996).

Historically, caged bivalves have been used to monitor pulp and paper mills in freshwater and marine environments, but the emphasis has been on bioaccumulation of chemicals in tissues. The largest data set for bioavailable chemicals comes from Finland, where they have been using this approach since 1984 (Herve et al., 1988; Herve, 1991; Herve et al., 1996). They primarily use the freshwater mussel *Anodonta piscinalis* and the method referred to as "mussel incubation." Much work has been done in Canada with the freshwater mussel *Elliptio complanata*, but again much of the emphasis has been on bioaccumulation (Metcalf and Hayton, 1989). Another complicating factor is that much of the information is either unpublished or found in the grey literature. Other examples of using freshwater bivalves to monitor similar effluents include the use of *Corbicula fluminea* to evaluate effluents from a wood treatment facility discharged to the San Joaquin River, California (Hayward et al., 1996), *Anodonta cygnea* to evaluate extractable organic halogens discharged into the Ton River in France (Hayer and Pihan, 1996) and *Hydriddella menziesi* to assess accumulation and depuration of resin acids in a freshwater pond in New Zealand (Burggraaf et al., 1996).

The proceedings of the 17<sup>th</sup> Aquatic Toxicity Workshop (Parker et al., 1991) contained a draft proposal for the Canadian EEM Program that included field bioassays with caged bivalves to estimate chemical exposure. This approach was excluded from the final EEM regulations; the reasons for exclusion were

unclear. Field bioassays with caged bivalves are currently required by several countries, but usually to document exposure by measuring chemicals in their tissues. This approach has also been required at one U.S. pulp and paper mill as part of their NPDES monitoring program. Caged mussel studies were included in the permit because no natural populations could be found to demonstrate whether chemicals were biologically available and having adverse effects. In their effort to understand the fate of chemicals once the effluent is discharged, the Canadian EEM Program requires both the effluent and receiving waters to be monitored. Except for the one particular case mentioned above, the major effort in the U.S. is directed toward characterizing only the effluent. Field bioassays with caged bivalves could enhance monitoring programs in both Canada and the U.S. In Canada, this shift in focus would reduce the uncertainty by decreasing the reliance on fish to characterize exposure and effects in the receiving waters. In the U.S., this shift in focus would reduce the uncertainty by decreasing the reliance on laboratory bioassays to characterize the receiving waters.

### Example

In the U.S., one pulp and paper mill has been required to use field bioassays with caged mussels. Mussels (*Mytilus trossulus*) were collected from an uncontaminated reference site, measured, placed in cages, and transplanted to seven sites. These included two reference sites and five sites in the vicinity of the effluent discharge at distances of 100, 300, 600, 800, and 1000 meters from the outfall. A 60-day exposure period was used. Three replicates of 100 animals each were deployed at each site. The following mussel metrics were used to determine the effect of chemical exposure on growth: whole-animal wet-weight, shell length, shell weight, and tissue weight. All mussels were measured at both the beginning and the end of the test. Measurements included whole-animal wet-weights and shell lengths and were recorded and tracked on an individual basis. An additional 300 mussels were measured at the beginning of the test for whole-animal wet-weight, length, tissue weight, and shell weight. The tissue weights from these animals were used to estimate tissue weights of the caged mussels at the beginning of the test. Tissues from these 300 mussels were analyzed for the chemicals of concern to determine background concentrations at the reference site. Individual tissue and shell weight measurements were also made at the end of the test for all deployed mussels. After the 60-day deployment, mussel tissues were chemically analyzed to determine concentration of selected chemicals, including dioxins (PCDDs) and furans (PCDFs). This facilitated estimates of bioavailability and comparisons between beginning and end-of-test tissue burdens.

Results can be summarized as follows: (1) compared to the initial tissue concentrations, total PCDD/PCDF concentrations after 60 days of exposure were significantly elevated in mussels within 1000 meters of the effluent discharge; (2) in all mussel tissues, a large percentage of total PCDD/PCDF was octachlorodibenzo-p-dioxin (OCDD); (3) based on the Toxic Equivalence Concentration (TEQ), the concentrations of dioxin and furan compounds in mussel tissues were about an order of magnitude below the predicted low risk TEQ of 50 pg/g; and (4) based on changes in whole-animal wet-weight and shell length, mussels at the test sites had significantly lower growth than the reference sites.

The concentrations of PCDD/PCDFs in mussel tissues increased by about an order of magnitude over the 60 day exposure period (Figure 3). The maximum concentration measured in mussel tissues was about 400 ng/kg. Interestingly, the highest concentrations were not found at the site closest to the effluent pipe. OCDD constituted approximately 80 percent of the total PCDD/PCDF at each test site. Although mussels at the mill sites showed statistically significant elevations in dioxins/furans, it does not appear that these tissue burdens are environmentally significant. The predicted low risk Toxic Equivalency Concentration (TEQ) is 50 pg/g (US EPA, 1993). The TEQs determined for mussels in the vicinity of the mill were about an order of magnitude below this predicted concentration (Figure 4).

Although the lower growth rates measured in the vicinity of the mill effluent were statistically significant, these differences may not have been environmentally significant because the concentration of PCDD/PCDFs in tissues were not environmentally significant and the absolute differences in mussel metrics were very small. In this study, changes in whole-animal wet-weight were the most discriminating

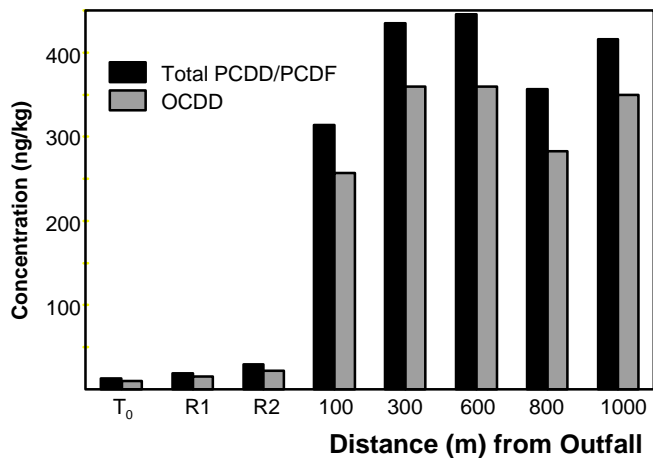


Figure 3. Concentrations of Total PCDD/PCDF versus OCDD in mussel tissues at the start ( $T_0$ ) of the test, deployed at two reference sites, and at various distances from the mill outfall.

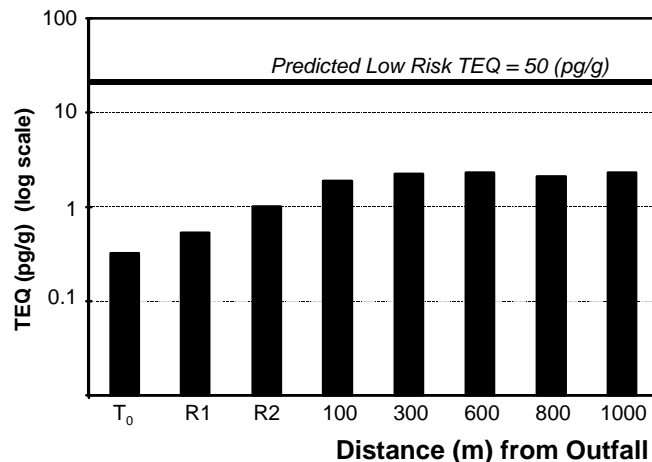


Figure 4. Toxic equivalence concentration (TEQ) for dioxins and furans in mussel tissues compared to the low risk TEQ of 50 pg/g.

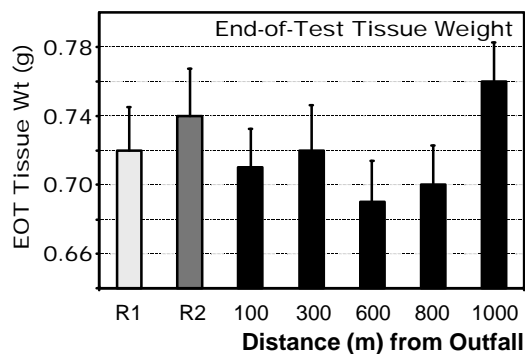
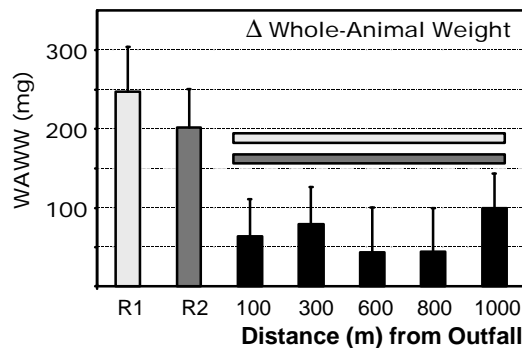
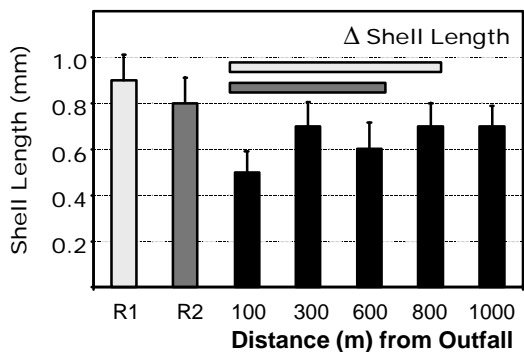


Figure 5. Differences in mussel growth at two reference sites and at various distances from the mill outfall using three different metrics: change in shell length, change in whole-animal weight, and end-of-test tissue weight. Horizontal bars indicate statistically different from the reference sites.

Statistically different from R1
  Statistically different from R2

metric and end-of-test tissue weights were the least discriminating (Figure 5). Based on our most recent work in other areas with slow-growing mussels, we expected tissue weights to be the most discriminating. Two factors may have precluded the ability of this metric to be more discriminating. First, the test was conducted from January through March when mussels approached their primary spawning season. Early spawners could have added to the variability in end-of-test tissue weights, and thus prevented a more discriminating statistical analysis. Second, the overall size range was about 8 mm and this could have added to the variability in tissue weights. To reduce these potential confounding effects, fall deployments with a size range of 5 mm or less has been recommended (Salazar and Salazar, 1995).

## SUMMARY AND CONCLUSIONS

We have shown the utility of caged bivalve monitoring for pulp and paper mill effluents by providing the rationale, historical perspective, and an example. The results of the example provided here demonstrate the discriminating power and the significance of initiating these tests with mussels of uniform size and health. In addition, it is important to consider the time of year and reproductive status of test animals during the interpretation of test results. Since tissue burdens provide a direct estimate of bioavailable chemicals in water and sediment they are being used more often in environmental risk assessments. Some regulations are also shifting toward a tissue residue basis. Field bioassays with caged bivalves are a potentially powerful tool in the risk assessment process, but data interpretation depends on understanding the physical and biological processes that affect chemical exposure and associated biological effects.

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