# CHARACTERIZING DIOXIN AND FURAN EXPOSURE FROM PULP AND PAPER MILL EFFLUENTS USING CAGED MUSSELS IN THE KENNEBEC RIVER, MAINE

Michael H. Salazar and Sandra M. Salazar

Applied Biomonitoring, 11648 - 72nd PL NE, Kirkland, WA, USA 98034

## Introduction

There is an increasing trend toward using more *in-situ* methodologies, more integrated approaches, and weight-of-evidence criteria in environmental monitoring and assessment and subsequent decision-making by regulators. All these paradigms apply to organochlorines. Transplant studies with caged bivalves can provide more environmentally realistic, robust data for these decisions because this approach is based on natural routes of uptake. Ecological risk assessments are frequently used to assess pulp mill effluents because they provide a focused approach to environmental monitoring and they includes characterizations of both exposure and effects. Transplant studies with caged bivalves should be included in these assessments for organochlorines because they can be used to simultaneously assess exposure and effects and provide a combination of experimental control and environmental realism not possible with traditional field monitoring or laboratory toxicity testing[1].

In addition to characterizing exposure and effects over space and time, and under site-specific conditions, controlled field experiments can be used to identify and distinguish chemical sources. The strategic placement of caged bivalves along suspected chemical gradients in three dimensional space and resulting tissue chemistry data can be used to map chemicals in those effluent plumes as well as chemicals in associated sediment (Figure 1). The utility of these mapping data has been increased significantly with the development of chemical fingerprinting methods such as congener-specific analysis for PCBs, alkylated homolog analysis for PAHs, and pattern recognition analysis. The discriminating power of caged mussel monitoring at specific locations over well-defined exposure periods combined with sophisticated chemical fingerprinting analyses is a potentially powerful monitoring and assessment tool.

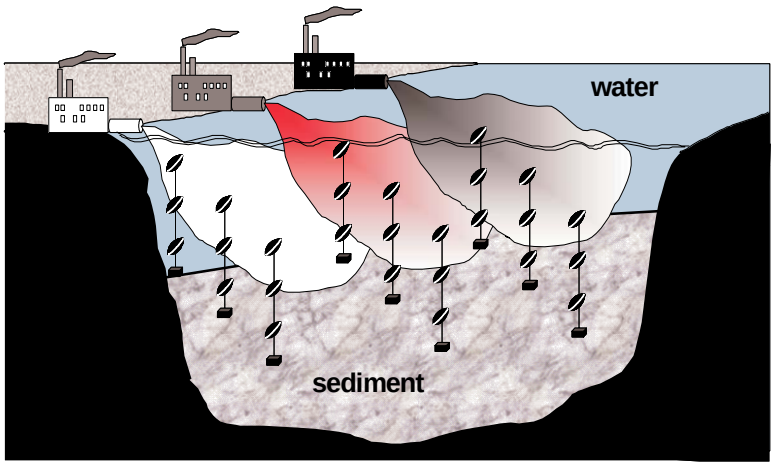


Figure 1. Identifying chemical sources and mapping effluent plumes using caged bivalves transplanted along suspected chemical gradients.

Using caged bivalves to monitor organochlorine exposure and effects has a long history. In 1960 and 1980, reduced growth and reproduction were measured in caged marine bivalves deployed in the vicinity of a Canadian pulp and paper mill outfall. These results were correlated with previous studies showing reduced densities of natural bivalve populations near the outfall. In Finland, caged bivalves have been routinely used since 1984 to monitor exposure from freshwater pulp and paper mill effluents. Unfortunately, most previous studies did not adequately integrate measurements of exposure and effects. Some only included effects measurements while others only included measurements of exposure. The methods for using field bioassays with caged bivalves have been refined to facilitate synoptic measurements of bioaccumulation and growth and codified into a standardized protocol[2].

This paper summarizes the caged bivalve methodology and a framework for incorporating this approach into organochlorine monitoring programs. A pilot study conducted on the Kennebec River, Maine with caged freshwater mussels will be used as a case study. The main purpose of this mussel study was to determine whether this approach would be a reasonable surrogate for resident fish used in above versus below comparisons of dioxins and furans associated with pulp and paper mill effluents.

**Materials and Methods**

The 53-day pilot study was conducted during the summer of 2000 in the Kennebec River, Maine. The State of Maine requires that dioxin exposure not be higher below the mill discharge as measured in fish or some acceptable surrogate. Caged bivalves were used to determine whether measurable and biologically available concentrations of these chemicals are leaving the pulp and paper mill by comparing exposure at locations above and below the mill.

The study design consisted of collecting freshwater mussels (*Elliptio complanata*) from Nequasset Lake, a relatively clean lake within the Kennebec watershed, selecting individuals with a 58 to 67 mm shell length, and transplanting the caged mussels to two locations on the Kennebec River, one above and one below the mill. Ten cages with 36 mussels each were deployed for 53 days at locations 13 miles above and 11 miles below a pulp and paper mill, in accordance with the above/below test paradigm. Mussels were only deployed at these two locations because they were the closest areas where fish could be collected. The primary limitations of fish sampling include 1) a series of dams on the river which preclude collecting fish close to the mill, 2) the mobility of fish and exposure at unknown locations or uncertain periods, and 3) the ability of fish to accumulate dioxins from sediment associated with previous discharges and from water associated with current discharges. Figure 2 shows the gradient design originally proposed and the 2-station design actually used. Semi-permeable membrane devices (SPMDs) were deployed at the same locations as part of another study. After retrieval, the whole soft tissues of mussels were analyzed for dioxins and furans, percent lipids, and percent moisture. Percent lipids were measured to normalize the chemical measurements on a lipid basis.

**Results and Discussion**

Mean concentrations of total dioxins and furans in mussels increased from below detection (DL for individual congeners ranged from 0.1 to 0.5 ng/kg-ww) at the beginning of the test to 4.33 ± 1.19

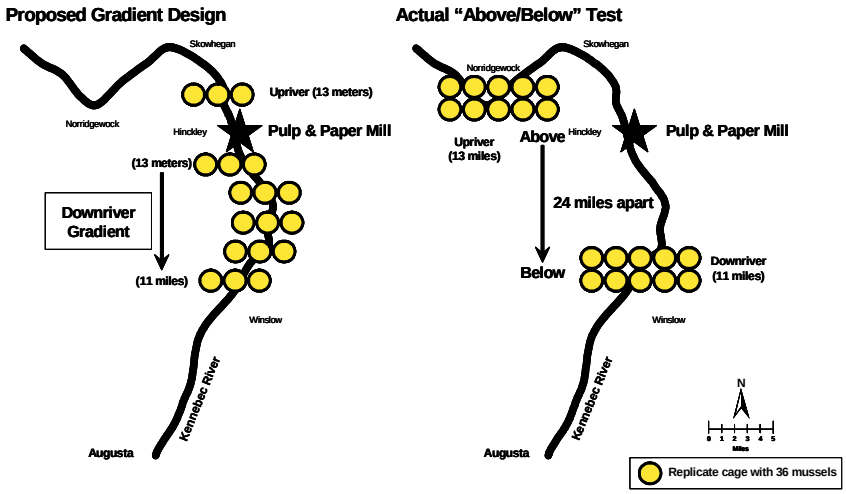


Figure 2. Gradient design originally proposed and actual "above/below" test.

(95% CI) and 4.67 ± 1.01 (95% CI) ng/kg-ww at the above and below stations, respectively, at the end of the test. Measured concentrations were higher below than above on a wet-weight and a lipid-normalized basis, but the difference was not statistically significant. More individual dioxin and furan congeners were measured in mussel tissues from both above (15 congeners) and below (13 congeners) locations than in SPMDs (11 and 12 congeners) or fish tissues (4 and 5 congeners). These results are encouraging with respect to using caged mussels as a surrogate for fish, particularly since the below station was located 11 miles from the mill and mussels still accumulated both dioxins and furans. The gradient design could have proven the existence of dioxins and furans closer to the mill if they were really being discharged by the mill. Exposure from current discharges remains an outstanding issue due to the generic limitations of fish sampling previously identified and uncertainty in the fish tissue chemistry data revealed in a recent review of the dioxin monitoring program by industry consultants.

Although the concentration of total dioxins and furans in fish tissues was significantly higher 11 miles downstream (4.19 ng/kg-ww) than 13 miles upstream (2.76 ng/kg-ww) of the mill, lipid-normalized concentrations of total dioxins and furans in fish collected at above and below stations were not significantly different. As with the data for SPMDs, the lipid-normalized concentrations for fish were higher above than below, but not significantly different. These data reinforce the significance of the important questions mentioned earlier regarding where the fish were exposed to dioxins and furans, whether they accumulated dioxins and furans from sediment or food that was contaminated from previous, rather than recent mill discharges, or how long ago exposure and accumulation occurred. Total dioxins and furans in SPMDs were higher upstream than downstream on both a lipid-normalized and a non-lipid-normalized basis although these differences were not statistically significant either. The SPMDs consistently demonstrated higher concentrations of dioxins and furans above than below. The weight of evidence from both mussel and SPMD data suggest higher concentrations above than below, but the fish did not detect this. A

statistically significant difference in accumulation of dioxins and furans in up- versus downstream fish was found because the concentrations in upstream fish were so low. Fish and mussels accumulated roughly equivalent concentrations of dioxins and furans at the downstream station.

There was much greater uncertainty in the SPMD data when compared to the mussel and fish tissue chemistry data. Nearly 40% of the congeners in mussel tissues were present at concentrations exceeding the detection limit, compared to approximately 20% for fish, and less than 10% for the SPMDs. This is based on results of the congener analyses that yielded 153 values for mussel tissues, 81 values for fish tissues, and 77 values for SPMDs. Some results for both the mussel tissues (<10%) and SPMDs (<40%) were reported at concentrations greater than zero, but less than the detection limit. For the SPMDs, these concentrations were generally at least one order of magnitude lower than the detection limit. The SPMD data in particular suggest that the extremely low measured concentrations and the large number of non-detects from samples collected 13 miles upstream and 11 miles downstream are not reliable indicators of dioxin and furan exposure, and that there may have been an analytical problems associated with these data.

Collectively, the results showed that more congeners were detected in mussels than in SPMDs or fish. The larger number of mussel samples above the detection limit also suggests that mussels were better indicators of dioxins and furans than SPMDs or fish. The most important question to be asked may be whether or not the fish data are believable, particularly given their ability to move and accumulate dioxins and furans through other exposure pathways. Just because the fish test satisfied the requirements of the above/below test and implicated the mill, does not mean that these data represent “real-world” conditions at the sampling locations located 13 miles upstream and 11 miles downstream.

While the experimental design in the caged mussel pilot study may have been appropriate for comparing dioxin and furan exposures with those in fish and SPMDs, it was not appropriate for addressing all the above/below issues concerning these potential fish surrogates and therefore was not a true test of the caged mussel methodology. Caged mussels and SPMDs should have been placed as close to the pulp mill discharge as possible for a more accurate evaluation of their ability to detect upstream/downstream differences. We believe that we have: 1) developed a method that is useful in dioxin monitoring, 2) provided data with as much or more promise as SPMDs or fish, and 3) shown that several anomalies in the 2000 data suggest the pilot study should be redone using the gradient design originally proposed.

**References**

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