Using Caged Mussels to Monitor Dioxins and Furans in the Kennebec River, Maine.

M.H. Salazar and S.M. Salazar, Applied Biomonitoring, Kirkland, WA.

Abstract

During the summer of 2000, a 53-day pilot study was conducted in the Kennebec River, Maine to determine whether caged freshwater mussels (*Elliptio complanata*) would be a reasonable surrogate for resident fish to assess upstream and downstream exposures of dioxins and furans associated with pulp and paper mill effluents. Caged mussels were deployed 13 miles upstream and 11 miles downstream from a pulp and paper mill. Mussels were deployed at these locations because they were the closest areas where fish could be collected due to the limitations of fish sampling and dams on the river. Mean total dioxin/furan concentrations in mussel tissues increased from below detection before deployment to 4.33 and 4.67 ng/kg-ww (parts-per-trillion) at the upstream and downstream stations after deployment. There was no statistically significant difference between upstream and downstream total dioxin/furan concentrations. More individual dioxin/furan congeners were measured in mussel tissues from both upstream and downstream locations than in either semi-permeable membrane devices (SPMDs) or fish tissues collected during the same time period. Advantages and disadvantages of caged mussels, natural fish populations, and SPMDs will be discussed along with the benefits of a gradient sampling design relative to using only upstream and downstream comparisons where the fish could be caught by angling.

Background

The State of Maine Department of Environmental Protection (DEP) has expressed concern regarding the ability to detect statistically significant differences in chemical exposure when comparing upstream and locations from pulp and paper mills due to declining tissue concentrations of dioxins and furans in fish. These comparisons are important because environmental regulations do not allow significant differences in upstream versus downstream exposures associated with those effluents. Academic and public environmental groups and mill representatives have all expressed concerns about using the fish test for this purpose and the fish test has limited support outside DEP. Many have identified problems with monitoring indigenous fish populations for upstream/downstream comparisons at mill sites, including uncertainty associated with mobility, accumulation from other sources, accumulation from previous mill discharges sequestered in sediments, and the inability to collect fish near the mill discharge. One environmental group supported and advocated the caged mussel pilot study anticipating that concerns regarding fish monitoring could be eliminated by using a surrogate, such as caged mussels, that could be deployed closer to the mill discharge where fish could not be collected.

DEP is responsible for developing a monitoring program to assess the nature and extent of dioxin and furan contamination in the waters and fisheries of the state but many have suggested that they have yet to develop an appropriate test. Maine has adopted the most stringent environmental regulations for dioxins in the US, and the primary objective of the dioxin/furan monitoring program is to assess potential ecological and human health effects by measuring chemical exposure in fish tissues. Interestingly, Environment Canada has adopted the opposite approach and focused on measuring effects in fish or suitable surrogates. Caged mussels and mesocosms have been accepted as alternatives to the adult fish survey in required Environmental Effects Monitoring (EEM) at pulp and paper mills in Canada. A secondary objective of dioxin monitoring in Maine is to document the status and trends of dioxin/furan exposures, evaluate progress in reducing environmental concentrations by compliance with existing regulations, and the need for even more stringent regulations. The third, and most specific objective is to determine if kraft pulp mills are discharging dioxins or furans into the

rivers of Maine. A state law enacted in 1997 prohibits such discharges and requires compliance by December 31, 2002. In practice, environmental exposures of dioxins and furans estimated by measuring concentrations in fish tissues or some surrogate, cannot be higher downstream of a pulp mill discharge than upstream. This is commonly referred to as the "above/below" test.

In 2000, DEP continued development of an appropriate "above/below" fish test, but as dioxin and furan concentrations decline, there were concerns that the existing monitoring approach may not be sufficiently sensitive to detect statistically or environmentally significant differences in exposure to properly evaluate compliance with the 1997 state law. Many believe that limitations of the fish test may preclude a scientifically or legally defensible use of the fish test in its current form. Instead of considering methods such as the caged mussels or SPMDs as surrogates for fish, it might be more appropriate to consider the use of fish in addition to surrogate tests in a weight-of-evidence approach. Although concentrations of dioxins and furans measured in fish tissues were higher below than above pulp mill discharges in 1999, questions remain about the suitability of fish as effective monitors. These questions are related to: 1) The mobility of fish and where exposure to dioxins and furans actually occurred, 2) Whether fish accumulated dioxins and furans from sediment or food that was contaminated from previous, rather than recent mill discharges and 3) When exposure and accumulation in collected fish occurred. In response to some of these questions, DEP modified the 2000 fish monitoring program to include measuring dioxins and furans in tissues of caged mussels and in lipids of SPMDs as potential surrogates for monitoring dioxins and furans in fish tissues.

Caged freshwater bivalves have been used to monitor dioxins and furans associated with pulp and paper mill effluents in Finland and for similar chemicals such as PCBs in Canada for approximately 20 years. Environment Canada has recently adopted caged bivalve monitoring as an alternative to the required adult fish survey in their EEM program for pulp and paper mills in Canada. Standardized protocols have been adopted by the American Society for Testing and Materials (ASTM) for conducting caged bivalve studies, and a standard guide appeared for the first time in the 2001 ASTM Annual Book of Standards. A revised version will also appear in the upcoming Standard Methods for the Examination of Water and Wastewater. Caged bivalves are a potentially powerful tool because of their ability to quantify exposure and effects over space and time. In situ studies with caged bivalves could complement and help establish links between various elements of the existing DEP monitoring program through the use of tissue chemistry and mussel growth measurements. This approach could also help reduce uncertainty in the current approach and answer questions within government, industry, and the public regarding chemical exposure and biological effects associated with pulp mill effluents. It is also consistent with the ecological risk assessment process of characterizing exposure through bioaccumulation and characterizing effects through mussel growth rates. As mentioned previously, Environment Canada has focused on characterizing effects in its EEM program while the State of Maine has focused on characterizing exposure. The ecological risk assessment paradigm suggests equal emphasis on exposure and effects in a more balanced approach.

Methods

Freshwater mussels (*Elliptio complanata*) were collected from Nequasset Lake, a relatively clean lake within the Kennebec watershed in Woolwich, Maine, caging individuals of a minimum size range, and transplanting them 13 miles upstream and 11 miles downstream from a pulp and paper mill in the Kennebec River (Figure 1). The mill is located in Hinckley, approximately 30 miles north of Augusta, Maine. DEP insisted on using only one upstream and one downstream station, i.e., locations closest to the mill where fish could be collected, so that mussel data could be directly compared with fish data. They did not allow us to place caged

mussels any closer to the mill than 13 miles upstream and 11 miles downstream, even though there were extra cages that could have been used. This precluded a thorough evaluation of the caged mussel methodology.

Ten cages with 36 mussels each were deployed for 53 days at these two locations in accordance with the upstream/downstream test paradigm. Figure 1 also shows our proposed experimental design with only three cages upstream and three cages placed at each of five downstream stations in a gradient design. This was our recommended approach and the one advocated in the ASTM Standard Guide for conducting in-situ bioassays with caged marine, estuarine, and freshwater mussels. After retrieval, the whole soft tissues of mussels were analyzed for dioxins and furans, percent lipids, and percent moisture. Percent lipids were measured as another indicator of animal health and to normalize the measurements on a lipid basis. Percent moisture was another indicator of animal health and used to normalize the data on a dry weight basis. Percent survival and multiple growth metrics were used as the primary indicators of animal health.

Results

Survival and growth of caged mussels indicated they were all in adequate health to accumulate dioxins and furans if present. Mean concentrations of total dioxins/furans in mussels increased from below detection at the beginning of the test to 4.33 and 4.67 ng/kg-ww at the upstream and downstream stations, respectively, at the end of the test (Figure 2). Concentrations were higher downstream than upstream, but the difference was not statistically significant between upstream and downstream total PCDD/PCDF concentrations at the end of the test. More individual dioxin/furan congeners were measured in mussel tissues from both upstream (15 congeners) and downstream (13 congeners) locations than in SPMDs (11 and 12 congeners) or fish tissues (4 and 5 congeners) (Figure 3A, B). We believe these results are encouraging with respect to using caged mussels as a surrogate for fish, particularly since the downstream station was located 11 miles from the mill and mussels still accumulated both dioxins/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.

The concentration of total dioxins/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 (Figure 4). These data suggest that fish are better able to detect differences in dioxin and furan exposure than mussels or SPMDs, and the existing fish monitoring approach is appropriate. However, on a lipid-normalized basis, concentrations of total dioxins/furans in fish collected at upstream and downstream stations are not significantly different. As with the data for SPMDs, the lipid-normalized concentrations for fish are higher upstream than downstream, 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.

Figure 4 also shows that total dioxin and furan concentrations in caged mussel tissues were higher downstream than upstream on both a lipid-normalized and a non-lipid normalized basis, although the differences were not statistically significant. Total dioxins and furans in SPMDs were higher upstream and downstream on both a lipid-normalized and a non-lipid-normalized basis although these differences were not statistically significant either. However, the SPMDs consistently demonstrated higher concentrations of dioxins/furans upstream than downstream. The fish demonstrated higher concentrations downstream when the data were not lipid normalized and higher concentrations upstream when the data were lipid normalized.

There was also 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 (Figure 5). This is based on results of congener-specific 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. Plots of the ratio of measured concentrations of the individual congeners divided by the method detection limit for each congener for mussels, SPMDs, and fish show the greater uncertainty in the SPMD data (Figure 5). Only 10 of the measured values (12%) for SPMDs are above the detection limit, only one value within 50% of the detection limit, and the rest of the values were between 0.4% and 29% of the detection limit. These reported concentrations were estimated from the calibration curve of the analytical instrument, but have the greatest uncertainty because they are so far away from the instrument detection limit. These data 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/furan exposure, and that there may have been analytical problems associated with these data. A recent quality assurance/quality control (QA/QC) review has suggested a methodological problem at the laboratory conducting the analyses, and when extra fish samples were analyzed, the concentrations were significantly higher. The original data were questioned when the reported concentrations were significantly lower than the previous year and there were no process changes at many of the mills that were being monitored.

Discussion

Collectively, the congener-specific data which showed detection of more congeners in mussels than SPMDs and fish, results that showed higher concentrations in mussels downstream than upstream (on both a lipid-normalized and non-lipid-normalized basis), and the larger number of samples above the detection limit suggests that mussels were better dioxin/furan indicators 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. This appeared to be one of the most important considerations for DEP in evaluating the suitability of caged mussels as a surrogate test. These questions, as well as concerns regarding upstream and downstream comparisons, can be addressed, at least in part, by using a weight of evidence approach. We carefully scrutinized the total concentrations of dioxins and furans measured in each test matrix (mussels, SPMDs, fish), the lipid normalized concentrations, and the concentrations of individual congeners.

Although the fish appeared to be the most suitable monitoring tool based on the ability to detect statistically significant differences between upstream and downstream concentrations of total dioxins and furans, the congener analysis and the lipid-normalized data suggest that they are not. On a congener basis the data suggest that mussels and SPMDs are more representative of all dioxin and furan exposures. Further, on a lipid-normalized basis there was no statistically significant difference between upstream and downstream locations in the fish data. More importantly, the concentrations were higher upstream than downstream. The caged mussel and SPMD data further suggest that the using the fish test at these upstream and downstream locations is inappropriate since the upstream station appears to be contaminated by another

source upstream of the mill. The downstream station was too far away to know whether fish are being exposed to current dioxin and furan dishcarges from the mill, other sources, or previous discharges from the mill. 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 the upstream/downstream issues concerning these potential fish surrogates. That would be a gradient design as used in most effluent monitoring studies. 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. A more direct approach would be to repeat the caged mussel pilot study with more stations closer to the mill in a gradient design as originally proposed.

With respect to comparing the results of the two surrogate tests evaluated as part of this study, the following conclusions reached by DEP in their 2000 Dioxin Monitoring Report are not scientifically defensible based on the available data.

"Since the development of the Above/Below test began in 1997, over 78 tests have been conducted for different dioxins, species, tissue types, and other surrogates in an attempt to develop a test powerful enough to accurately measure any differences above and below a mill. Bass and semi-permeable membrane devices show the most promise and will be tested again in the 2001 program."

"Freshwater mussels did not appear to be a useful monitoring device, perhaps because they are at a lower trophic level than fish."

There are no data or statements in the DEP report that support these conclusions. The concentrations of dioxins/furans in fish were significantly higher downstream than upstream, the difference was very small and on a lipid-normalized basis the dioxin/furan concentrations were higher in fish upstream than downstream. DEP chose to ignore the lipid-normalized data. Most SPMD samples were below the detection limit, the SPMD data were the most unreliable because reported concentrations were estimated at a fraction of the detection limit, and the response of the mussels was more like fish than the responses of the SPMDs. In addition, approximately 29% and 61% of the total dioxins/furans at upstream and downstream sites was attributable to a single congener (2,3,7,8-tcdf). In almost every study where SPMDs have been compared with mussels, SPMDs have been shown to "over-trap" the lower molecular weight organic compounds. Concentrations of this furan congener were about six times higher than fish or mussels and suggest that the majority of the dioxins/furans from SPMDs did not represent fish or any other living organism and that it was an artifact of the surrogate sampling procedures. In other words the SPMDs were good accumulators of the compound which was least environmentally relevant to fish or mussels. For comparative purposes, 2,3,7,8-tcdf is approximately 1/20 as toxic as 2,3,7,8-tcdd.

This integrated pilot study compared three approaches as alternative monitoring tools for assessing the fate and effects of dioxins and furans associated with a pulp mill effluent. While water samples have been used to characterize aqueous chemical exposures for over 50 years, new elements used here include the use of caged mussels to integrate chemical exposure and associated biological effects. Caged mussels have been used for approximately 30 years, but recent refinements have increased the sensitivity of this approach to a new level, and these methods have only recently been adopted by the ASTM. SPMDs represent the newest of these methodologies and applications of this approach are still being refined. This study is unique not only in terms of comparing these three monitoring methods, but applying them in areas where

they have not been commonly measured in Maine, using state-of-the-art chemical analyses with low detection limits, and using extensive experience and expertise to interpret the results of congener analysis (i.e., dioxins and furans) and mussel growth rates.

There are too many uncertainties in the results from accumulation of dioxins and furans in caged mussels, SPMDs, and fish tissues to unconditionally accept the results and make important programmatic decisions regarding the utility of these three methods. Another pilot study is suggested that directly tests the utility of the caged mussel methodology (and SPMDs) using a gradient design downstream from the mill and placing cages as close as possible to the effluent discharge. The weight of evidence from bivalve biomonitoring studies conducted on chlorinated hydrocarbons such as dioxins furans, and PCBs throughout the world suggest that caged bivalves can be an effective monitoring tool for pulp and paper mill effluents in the State of Maine. This is not to say that bivalves should be the only monitoring tool. Most experts have agreed that there is no perfect monitoring tool and that a weight of evidence approach should be used to make the most meaningful assessments. It seems reasonable to assume that a triad approach using caged mussels, SPMDs, and fish would provide DEP with the best possible data to make informed decisions with respect to potential exposure from dioxins and furans from pulp and paper mills on the Kennebec River. We have previously suggested that the best way to measure water quality is to not measure chemicals in water but measure them in mussel tissues because they provide a more integrated picture of exposure. As anomalous as it may seem, the best way to quantify exposure in fish may be to measure chemicals in caged mussels rather than fish. Mussels are potentially better indicators of dioxin/furan exposures because they do not move and because they can be placed closer to the mill.

Summary

- Mussels detected more congeners than either fish or lipid bags.
- The total concentration of dioxins/furans in mussel tissues were higher downstream than upstream whether or not the data were lipid normalized.
- More reported concentrations from congener-specific analyses were above the detection limit for mussel tissues than either fish tissues or SPMDs.

Conclusions

- Surrogate mussels may be a better indicator of exposure than fish or SPMDs.
- Surrogate mussels have a greater potential for the above/below test because they don't move and could be transplanted along suspected chemical gradients.
- DEP was biased in their interpretation of available data.

Recommendations

- Conduct another study using gradient design with stations close to mill.
- Have samples analyzed by another lab to avoid bias and poor methodology.
- Require documentation from DEP to support conclusions in 2000 report.

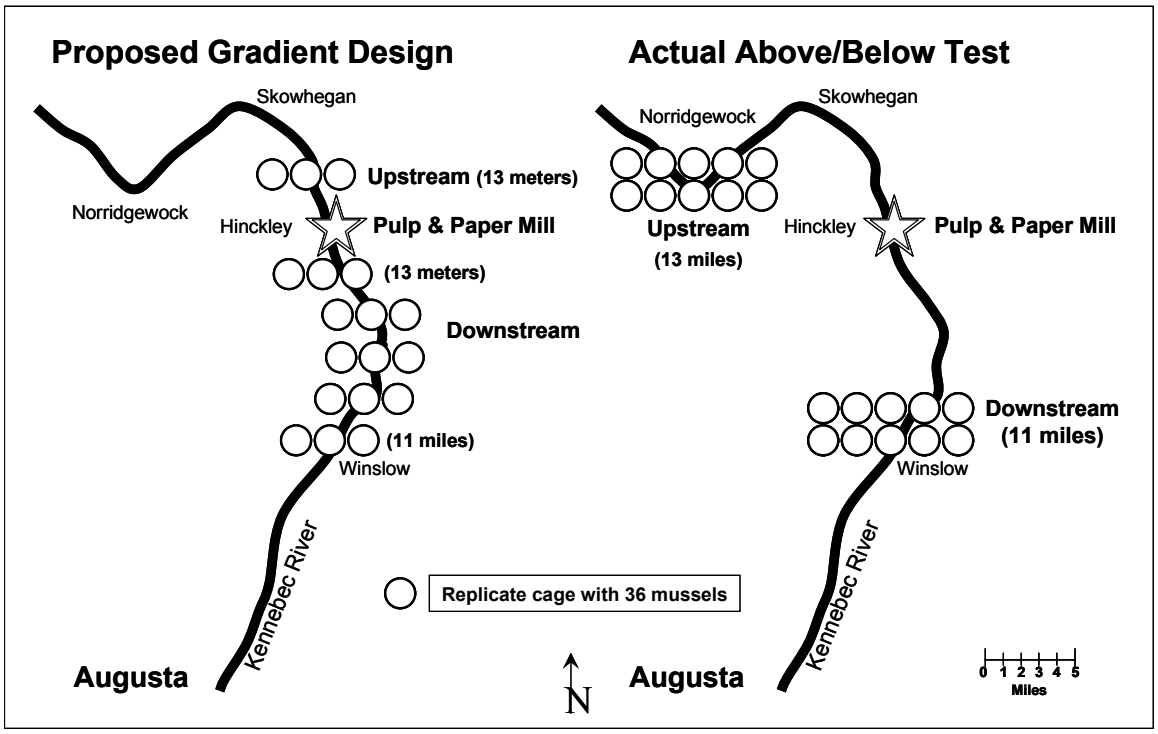

Figure 1. Site map showing proposed experimental design versus stations where caged mussels were deployed.

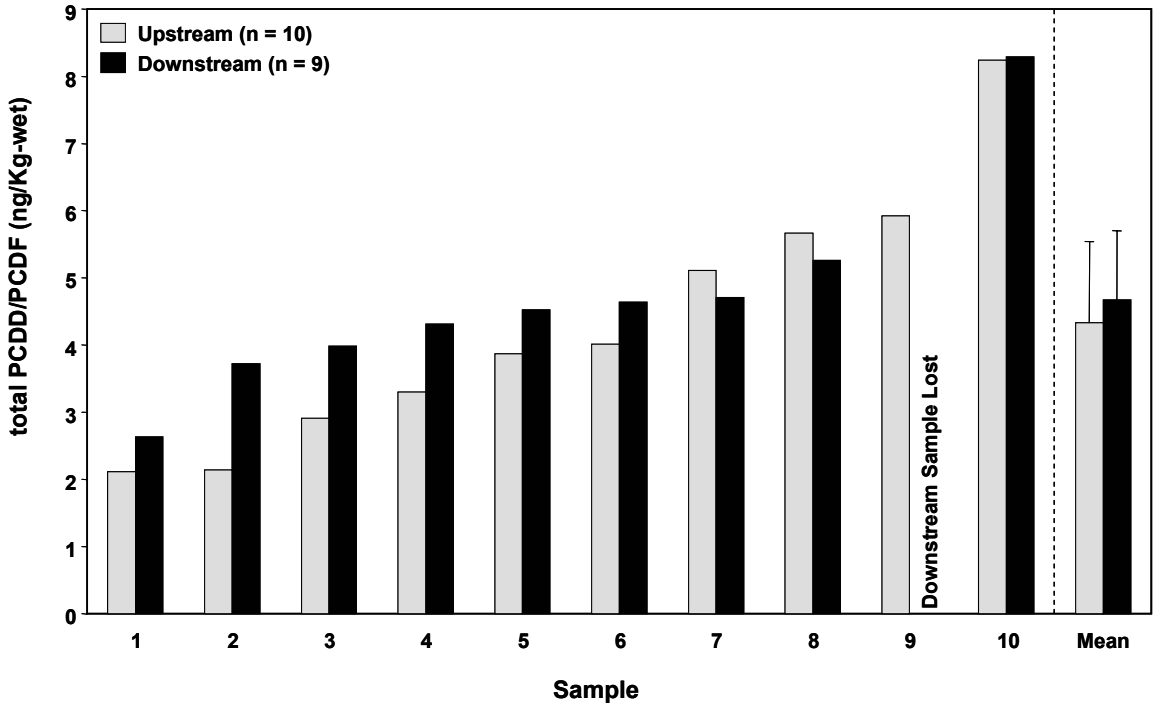

Figure 2. Total dioxins and furans in caged mussels from upstream and downstream stations.

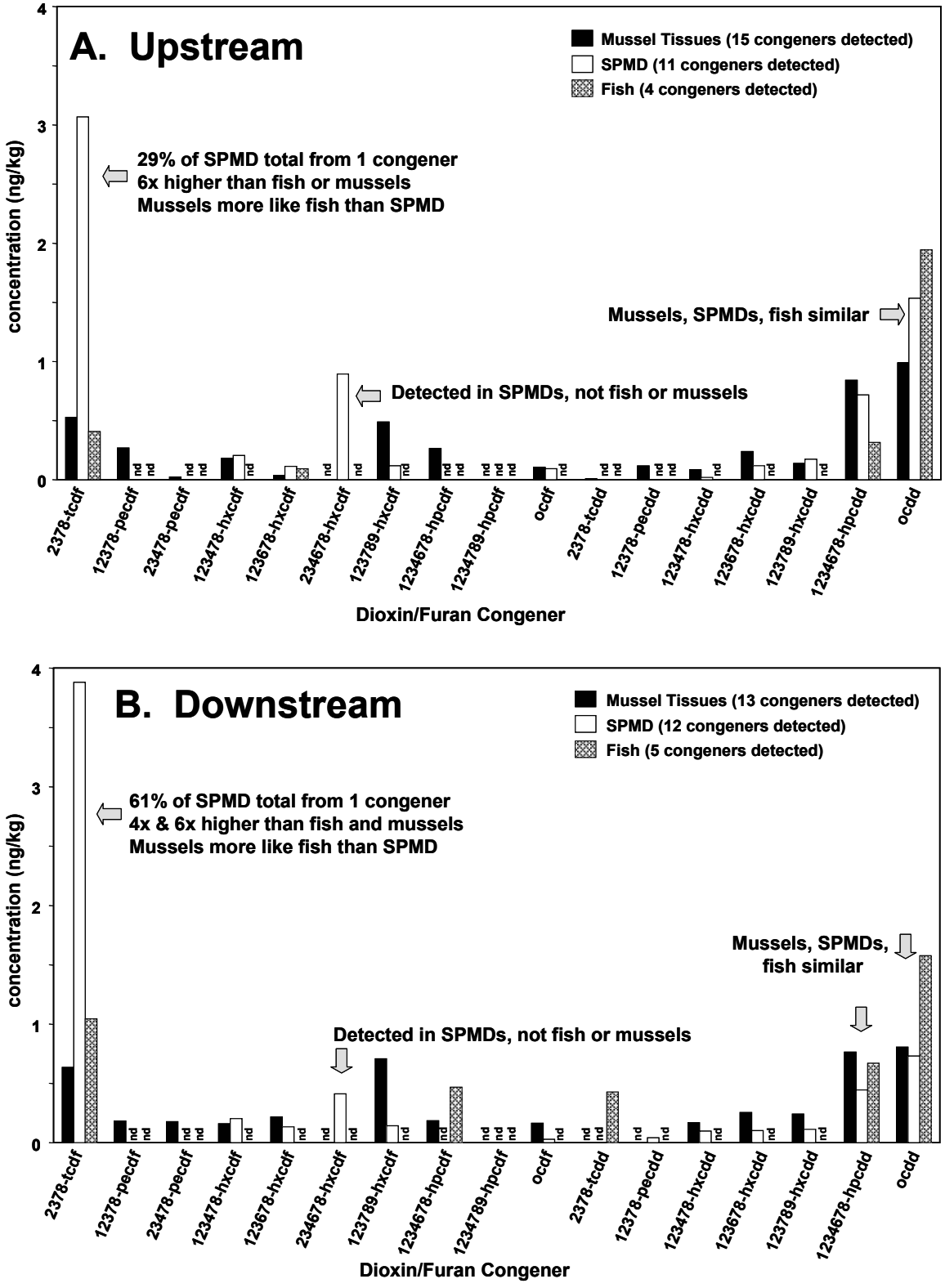

Figure 3. Mean concentration (ng/kg) of individual congeners measured in mussel tissues, SPMDs, and fish tissues. A = Upstream dioxin station; B = Downstream dioxin station. ND = not detected.

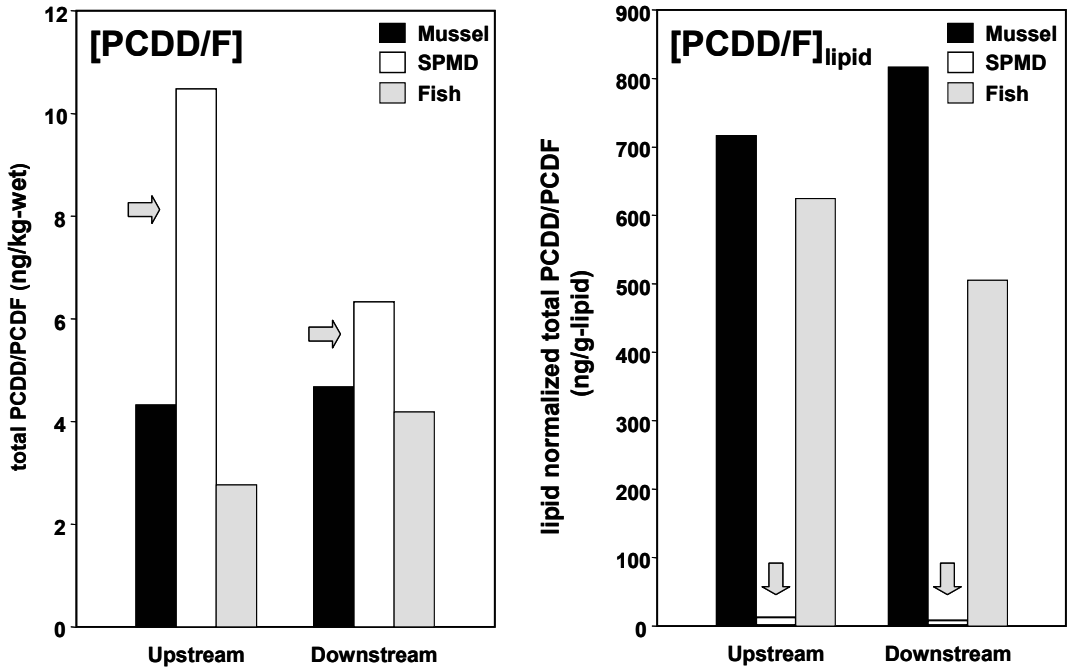

Figure 4. Total dioxins and furans in caged mussel tissues, SPMDs, and fish from upstream and downstream stations, and on a lipid-normalized basis.

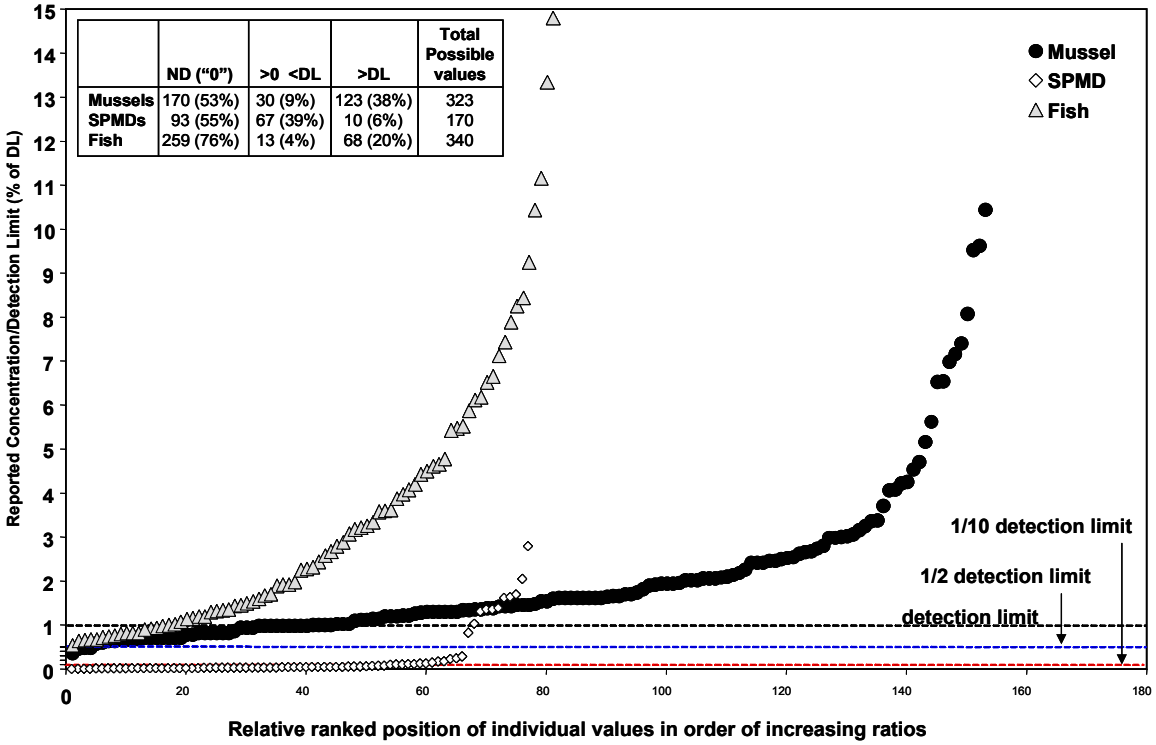

Figure 5. Percent of values >0 compared distribution detection limits (DL) for caged mussels, SPMDs, and fish. For non-detected (ND) values, a "0" was used to represent reported concentration. Total possible values = number of samples analyzed x 17 congeners. Graph does not show percent of values that were non-detects.