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USING AN INTEGRATED RISK ASSESSMENT STRATEGY TO CHARACTERIZE EXPOSURE AND EFFECTS IN THE FIELD WITH CAGED BIVALVES

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Abstract – As the concentration of potentially toxic chemicals decreases, it is becoming increasingly difficult to characterize the fate and effects of pulp and paper mill effluents and establish causal relationships between chemical exposure and associated biological effects. In response to these difficulties, many monitoring programs have emphasized either ***exposure-based*** or ***effects-based*** approaches using indigenous populations of fish. The ecological risk assessment (ERA) framework can be used to characterize exposure and effects to the extent necessary for understanding the subtle effects of mill effluents and establishing links with chemical exposure. Furthermore, the emphasis on wild fish in many monitoring programs has contributed to the uncertainty in these assessments and limited the ability to identify the chemicals associated with measured effects. In addition to an ***ERA-based*** approach, we suggest integrating field experiments with caged bivalves into regular monitoring programs to facilitate characterizations of exposure and effects and to help establish cause-and-effect relationships. Caging bivalves along suspected chemical gradients helps to identify sources of accumulated chemicals and facilitates the measurement of effects by controlling the location and duration of exposure as well as the exposure and genetic history of the test animals. The caged bivalve approach can be used to characterize exposure and effects over space and time under environmentally realistic conditions in the field and has a number of advantages over using wild fish. An integrated approach using fish and bivalves in laboratory and field experiments, with supporting tissue chemistry data, would enhance the assessments and help identify the chemicals associated with adverse effects. Two case studies are presented to highlight the difference between ***exposure-based*** and ***effects-based*** approaches and the need for an ***ERA-based*** approach. The first case study, based on work conducted on the Kennebec River, Maine, is an example of an exposure-based approach and compared the accumulation of dioxins and furans in caged bivalves, fish, and semipermeable membrane devices (SPMDs). The second case study, work conducted on the Moose River, Canada, is an example of an effects-based study that emphasized measuring effects on fish. The level of uncertainty in both studies could have been reduced by using an ERA-based approach. This paper focuses on the need for more integrated exposure and effects studies on pulp and paper mill effluents, and routine monitoring with equal emphasis on characterizing exposure and effects in the lab and in the field.

Keywords – ecological risk assessment, caged bivalves, exposure, effects

INTRODUCTION

There are several indications that the concentrations of potentially toxic chemicals in pulp and paper mill effluents are decreasing and that it is becoming increasingly difficult to characterize exposure and effects. One of the problems is that the concentrations of dioxins and furans have decreased to near detection limits in the tissues of biota and are below the practical detection limit in most receiving waters. Some of the difficulties associated with establishing cause-and-effect relationships come from uncertainties associated with making extrapolations across fish species used in field and laboratory assessments and variations in the response pattern associated with location and duration of exposure [1]. Many of these problems could be reduced by using the same species of bivalves in national monitoring and assessment programs. The fate and effects theme of this conference emphasizes the importance of characterizing exposure and effects. These are the major analysis elements of ERA. The United States Environmental Protection Agency (USEPA) [2] ERA paradigm provides a focus to environmental monitoring and assessment because it includes a characterization of exposure and effects. We have previously described how caged bivalves could be used to characterize exposure and effects associated with pulp and paper mill effluents [3] and provided an exposure-dose-response triad framework to show how caged bivalves could be used to support an integrated ecological risk assessment-based monitoring strategy [4]. Others have outlined an ERA-based field and laboratory approach to assess endocrine disruption and suggested that the ERA framework is sufficiently robust to accommodate the specific characteristics of endocrine-disrupting chemicals [5]. The ERA paradigm may be most appropriate for assessing ecological risks associated with pulp and paper mill effluents and various applications of this approach have been outlined [6,7].

Some routine compliance monitoring programs for pulp and paper mill effluents are exposure-based and others are effects-based. The narrow focus of each approach is inconsistent with ERA that includes characterizations of exposure and effects. Minimizing costs in compliance-based monitoring often results in utilizing only exposure-based or effects-based approaches. More progress might be made in establishing cause-and-effect relationships and identifying the chemicals responsible for observed effects if equal emphasis was placed on characterizing exposure and effects in both laboratory and field studies . The critical issue is not measuring toxicity. It is about adequately characterizing and relating exposure associated with the measured toxic effects. “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,” [8]. Furthermore, experimental field approaches are needed to reduce uncertainties associated with fish surveys such as location and duration of exposure, comparisons with different species, and exposure to historical rather than current discharges. Establishing a relationship between exposure, dose, and response in the field would help predict effects and establish causality.

Environment Canada’s Environmental Effects Monitoring (EEM) for pulp and paper mill effluents uses a tiered approach with emphasis on laboratory toxicity testing to establish causality. We suggest that cause-and-effect relationships might be established more quickly and easily by placing equal emphasis on exposure and effects in both laboratory and field studies and integrating the caged bivalve methodology with fish surveys for a more complete characterization of exposure and effects. Canada’s Aquatic Effects Technology Evaluation Program for mining [9], recommends the following important questions serve as a framework for monitoring and assessment: 1) Are contaminants entering system? 2) Are contaminants bioavailable? 3) Is there a measurable response? 4) Are contaminants causing the response? Exposure-based monitoring successfully addresses questions 1 and 2. Effects-based monitoring only addresses question 3. Neither approach alone completely addresses question 4. In addition to identifying key questions that need answering in the context of characterizing exposure and characterizing effects, these questions highlight the need to integrate the correct elements. In other words, just because a monitoring and assessment program is integrated does not mean that it includes all the elements necessary to answer the most important questions [10].

Although this chapter is not about conducting ERAs, we suggest that the major analysis elements of the ERA paradigm can be used to reduce uncertainties in monitoring the exposure and effects associated with pulp and paper mill effluents. This chapter demonstrates how the existing ERA framework can be applied to pulp and paper mill monitoring for a more integrated investigation of cause by characterizing exposure and effects in the field. The potential utility of this framework is shown by reviewing two pulp and paper mill case studies, neither of which adequately characterized both exposure and effects. One study was primarily exposure-based and the other was primarily effects-based, and both monitoring studies were based on collecting wild fish. The advantages of ERA-based monitoring and field studies with caged bivalves will be discussed.

STATE OF MAINE, USA: THE KENNEBEC RIVER CASE STUDY

The state of Maine has adopted the most stringent environmental regulations for dioxins in the US, with the primary objective of their dioxin-furan monitoring program to assess potential ecological and human health effects by measuring chemical exposure in fish tissues [11, 12]. These regulations were established to regulate the discharge of dioxins from pulp and paper mills. 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 currently discharging dioxins or furans into the rivers of Maine. 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. Their monitoring program is based on resident fish, and they rely completely on the ability to detect concentrations of dioxins and furans in fish tissues at 1 part per trillion or less. Since their program only measures tissue chemistry, it could be referred to as exposure-based monitoring.

Over recent years, the concentrations of dioxins and furans in fish tissues have declined [11, 12], and the Department of Environmental Protection (DEP) has expressed concern regarding the ability to detect statistically significant differences in dioxins and furans in fish collected from locations above and below the pulp mill discharge. Many consultant, academic and public environmental groups have expressed concerns whether the observed differences in above-below comparisons are real or associated with the many uncertainties attributable to monitoring mobile fish, including uncertainty associated with mobility, accumulation from other sources, accumulation from previous mill discharges sequestered in sediments, and the reluctance by DEP to collect fish near the mill discharge. In this pilot study, mussel tissues and lipids from the SPMDs were assessed as potential surrogates for dioxin monitoring in fish. The intent was to eliminate concerns with monitoring fish by using a surrogate, such as caged mussels, that could be deployed closer to the mill discharge, i.e., areas where fish are not currently collected.

Ten cages of freshwater mussels (*Elliptio complanata*) were transplanted to each of two stations in the Kennebec River where fish are collected as part of the dioxin monitoring program: an upstream station 13 miles from the mill and a downstream station 11 miles from the mill (24 miles apart). The presence of fish was the primary criterion for station selection, rather than testing the ability of the caged mussels to identify a gradient in chemical concentration in the vicinity of the mill, because DEP wanted a direct comparison of dioxin accumulation between fish and caged mussels. It is virtually impossible to adequately characterize exposure with an above-below comparison. Furthermore, effects in fish were not measured.

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, after the 53-day deployment. 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; these differences were not statistically significant. The concentration of total dioxins-furans in fish tissues was significantly higher downstream (4.19 ng/kg-ww) than upstream (2.76 ng/kg-ww) of the mill. However, the lipid-normalized concentrations of total dioxins-furans in fish tissues were higher upstream than downstream, and there was no significant difference between upstream and downstream. These data reinforce the significance of the important questions mentioned earlier regarding where fish exposure to dioxins and furans occurred, whether fish 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. There was less uncertainty in the mussel data when compared to the SPMD and fish data. For mussels, 38% of the values exceeded the detection limit, compared to approximately 20% for fish and 6% for the SPMDs [13, 14, 15]. Mussels also had fewer non-detects and fewer values that were between non-detect and the detection limit.

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 decisions regarding the utility of these three methods. Important questions regarding the fish data remain unanswered. Given the large percentage of non-detects, their ability to move and either avoid exposure or accumulate dioxins and furans through other exposure pathways, and the inability to distinguish between current and previous discharges, using the fish data may be problematic since the regulations are based on current and not previous mill discharges. 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 question is "why did this difference exist?" Why was the concentration of total dioxins and furans so low in the upstream fish? A statistically significant difference could not be found between the up- and downstream mussels because the mussels accumulated more dioxins and furans from the upstream station relative to the fish. Furthermore, no attempt has been made to collect fish in the impoundment where the mill discharge is located or to measure effects in fish. The regulations and the compliance monitoring is completely exposure-based.

The caged mussel and SPMD data further suggest that using these upstream and downstream locations is inappropriate because the upstream station appears to be contaminated by a source upstream of the mill. The downstream station was too far away to know whether the fish accumulated dioxins and furans associated with current mill discharges, from previous discharges from the mill (i.e., sediment-bound dioxins and furans), or via the food chain. While the experimental design in the pilot study may have been appropriate for comparing dioxin and furan exposures in fish with those in caged mussels and SPMDs, it was not appropriate for addressing the upstream-downstream issues concerning these potential surrogates. Caged mussels and SPMDs should have been placed as close to the mill discharge as possible for a more accurate evaluation of their ability to detect upstream-downstream differences and in a gradient design to determine if dioxins are furans are currently being discharged by the mill.

CANADA: THE MOOSE RIVER CASE STUDY

Environment Canada has established an Environmental Effects Monitoring (EEM) Program to evaluate potential effects of effluents on fish, fish habitat, and the use of fisheries resources. The EEM Program includes: a fish population survey to assess the health of fish; a benthic invertebrate community survey to assess fish habitat; a study of dioxins and furans in edible fish tissue and a tainting study as assessments of the usability of fisheries resources; sublethal toxicity testing in the laboratory to assess effluent quality; and supporting water and sediment quality variables to aid in the interpretation of biological data. Since the program primarily measures effects, it could be referred to as effects-based monitoring.

Although the EEM program is clearly an integrated approach, the emphasis on the adult fish survey and effects-based monitoring may not be the most efficient approach for establishing causality. The approach might be improved with the addition of caged bivalves for routine compliance monitoring. Without analyzing tissues for the most likely chemicals of concern, it is difficult to use the effects data to make management decisions with respect to mill process changes. Furthermore, there are too many uncertainties associated with measuring effects in mobile fish and making comparisons between exposure and reference areas to provide definitive answers to questions of causality. Causality might be established more easily by characterizing exposure and effects in a more integrated ERA-based approach with equal emphasis on field and laboratory testing with the most appropriate monitoring tools. In this approach, selected screening of suspected chemicals in wild fish and caged mussel tissues would be conducted to examine potential correlations between exposure and effects.

The Moose River study [16] could be considered as one of the best examples of effects-based monitoring, and even included measuring dioxins and furans in fish tissues. The Moose River study examined fish performance at 20 sites through a series of 50 fish collections over several years. The focus of the study was to develop an effects-driven cumulative effects assessment of fish responses to industrial development within the system. Industrial development not only includes structural changes to the environment but changes in water quality through the addition of chemicals and changes in temperature. The intended use of the data was to refine the effects-based assessment approach, provide guidance on how to conduct an effects-driven assessment, develop a philosophical framework to assist in decision-making related to cumulative effects, and provide baseline data for future comparisons. Even with the comprehensive collections and multi-year effects studies, the causes for the measured effects could not be identified. Paired exposure and effects measurements would have increased the utility and the robustness of the data, and facilitated better management decisions. Another reason for measuring tissue chemistry is to eliminate or explain effects that have been attributed to non-chemical stressors. While it has been argued that it is not efficient to measure the entire suite of chemicals when the specific chemical causing effects in fish remains unidentified, there has been sufficient progress to evaluate groups of compounds suspected of causing the observed effects.

Munkittrick et al. [16] suggest that stressor-based assessments have only been successful where effects were associated with gross inputs, and that many stressor-based assessments fail to adequately incorporate temporal and spatial aspects of responses, interactions between potential stressors, and thresholds for effects. While this may be true for some stressor-based assessments, it is not necessarily true for all. A sound, well planned study design based on an ecological risk assessment paradigm can provide data to address these important issues, and such a study design should contain both exposure and effects measurements. Although the Moose River study did measure dioxins and furans in fish tissues and included some experimental approaches, the basic elements of fish surveys in the Moose River study and in EEM approaches are observational, not experimental. In the context of reducing the uncertainties associated with observational data from fish surveys, characterizing exposure and effects and attempting to establish causality could be improved by using an integrated monitoring approach. Given the multiple chemical sources and multiple environments throughout the Moose River Basin, an integrated approach that included characterizing exposure and effects would have reduced uncertainty and provided corroborative data for measured effects in fish. For example, caged bivalve studies could be used to help identify the causes of potential effects, identify potential contaminant sources, and map the influence of discharges from these sources. Even though pulp and paper mill effluents contain hundreds of compounds, this approach is viable because a growing body of evidence suggests that the chemical causing many effects in fish is a polycyclic aromatic hydrocarbon (PAH) compound [6]. Furthermore, compounds such as retene and phenanthrene and their metabolites have been associated with adverse effects [17]. In addition to the advantage of experimental control mentioned previously, bivalves have a very limited ability to metabolize PAHs and could make it easier to detect and map compounds that are affecting fish, but are not as easily detected in fish tissues. This means that this class of compounds could be screened in routine monitoring with fish, caged mussels, and mesocosms to help establish causality or lack thereof.

ADVANTAGES OF ERA-BASED MONITORING

In the context of understanding the fate and effects of pulp and paper mill effluents, one major advantage of ERA is that it provides a focus for environmental monitoring and assessment. It serves as a reminder that there should be equal emphasis on charactering exposure and effects and that emphasizing or eliminating one or the other may limit the ability to correctly interpret the data.

An ERA-Based Framework

A conceptual model is the first step to establishing ecotoxicological links necessary to establish causality and predict effects. This is normally done in the problem formulation phase of ERA. The conceptual model proposed here (Figure 1) was developed from guidelines for ERA [2] and an ecotoxicological framework [18]. This model is the same as that outlined by the USEPA, with slight clarification. The exposure characterization is divided into two distinct parts: (1) external exposures associated with chemicals in water and sediment, and (2) internal exposure measured by the concentration of chemicals in the tissues of biota (i.e., dose). The exposure characterization has been divided to emphasize the need for simultaneous measurement of each element. The modified model also characterizes effects by measuring biological responses. This model can also be used to predict effects and establish causality [18]. We have referred to this conceptual model as the exposure-dose-response triad to emphasize the importance of characterizing exposure with measurements of chemicals in water and sediments as well as bivalve tissues [3, 4]. Each of these components of the ERA-based approach contribute to answering different questions and increasing resolution towards stressor identification, characterizing and understanding processes, predicting effects, and establishing causality.

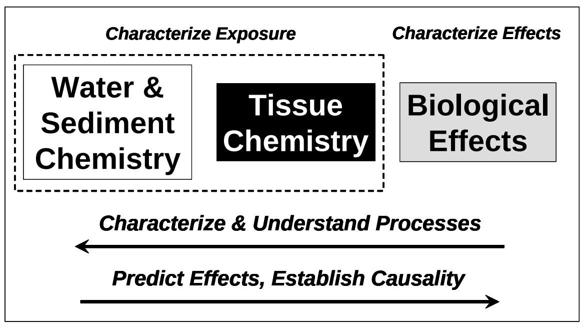

Figure 1. Modified ERA model.

Monitoring and Assessment Framework - Collecting and Using the Data

This ERA-based approach can be applied by using tissue chemistry (i.e., dose) as the central link in any monitoring and assessment program (Figure 2). Tissue chemistry is the link between the environment and the organism and can serve as a common thread to evaluate and interpret field and laboratory bioassays and benthic community assemblages. It is important to characterize the chemicals accumulated and retained within tissues because of the uncertainties associated with bioavailability of chemicals in water and sediment. Without fully characterizing the dose in addition to the external exposure, one can never be sure that the response measured in laboratory toxicity tests or in wild fish is related to, or at least associated with, exposure to specific chemicals. However, this approach is not new. GESAMP [19] stated it succinctly as follows: "... Without observations linking levels (of pollutants) in the water or sediment with tissue concentrations and then with effects on organisms and populations and, ultimately, with the well being of the ecosystem as a whole, an adequate assessment of pollution is impossible."

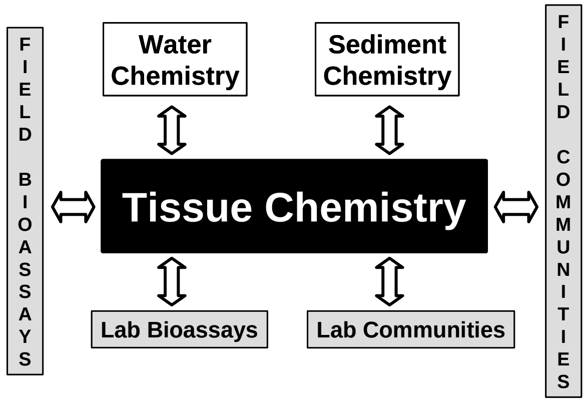

Figure 2. Establishing Links.

Foundation of the Tissue Residue Effects Theory

The tissue residue effects theory links critical body residues (CBRs) with measured effects to help predict threshold concentrations of chemicals where specific effects begin to occur. A CBR is defined as the concentration of a chemical accumulated in tissues of an aquatic organism that is linked to a specific toxicity endpoint such as mortality, reduced growth, or reduced reproduction. It is important to note in the context of pulp and paper mill effluents where the specific chemicals associated with effects are uncertain, that a CBR can be defined for either individual chemicals or for classes of chemicals that have the same mode of action. These have been shown to be relatively constant across a wide range of aquatic species and taxonomic groups [20]. According to this theory, CBRs within a defined mode of action category should be relatively constant across different chemicals, species, and exposure conditions. This approach has been supported by both theoretical and experimental evidence [21]. Although the relationships derived by McCarty [22] were theoretical, they were based on the use of quantitative structure-activity relationships to estimate the toxic internal concentrations of organic chemicals in fish. Whereas McCarty estimated critical body residues, Donkin et al. [23] actually measured critical body residues in mussel tissues and associated them with effects on filtration. The relationships between hydrophobicity, sublethal toxicity and critical tissue residues established by Donkin et al. [23] are virtually identical to those demonstrated by McCarty [22]. This study also shows that in addition to using bivalves in field experiments, they can also be used in the laboratory and in mesocosms to help characterize exposure and effects.

The Concept and Importance of the Dose

The dose can provide valuable confirmatory evidence that exposure has occurred and could be linked to possible effects. McCarty [24] makes an important distinction between concentration-response and dose-response and the need to combine both endpoints, “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’s example can also be applied to field monitoring and assessment to include an element of potency (tissue residue) and an element of effects (toxicity). In the context of ecotoxicology, the ERA paradigm serves as a reminder that it is not sufficient to measure only exposure or effects [5]. The approach is based on characterizing exposure and effects.

Measuring body residues may be a better representation of actual exposure at receptors of concern than measuring chemicals in water or sediment because the chemicals are closer to the internal receptors of concern. It is generally agreed that effects are elicited when potentially toxic chemicals interact with internal receptors. While it may not be possible to measure the precise concentration of chemicals at each internal receptor, McCarty and Mackay [20] suggested that whole body tissue residues are a reasonable surrogate for the critical concentrations at receptors of concern. Tissue chemistry is the most direct and reliable method of estimating bioavailable chemicals [10]. Direct measurement of chemicals in water or sediment cannot be used to quantify chemical bioavailable or potential effects after short- or long-term exposure because chemicals in water or sediment are further removed from the receptors of concern. In addition, tissue chemistry represents an integration of exposure rather than a "snapshot" of chemical conditions as commonly occurs with analysis of discrete water or sediment samples that only represent an instant in time.

ADVANTAGES OF CAGED BIVALVES

Bivalves have a number of advantages over other species such as fish for characterizing exposure and effects in routine monitoring programs throughout the world [18, 25, 26]. 1) They are dominant members of many benthic communities in marine and freshwater environments, which minimizes problems associated with comparing results from different species. 2) They are sedentary, and therefore more appropriate than mobile species as indicators of both exposure and effects. 3) They are relatively tolerant but not insensitive to a wide variety of environmental conditions and chemicals. 4) Most are suspension feeders that pump large volumes of water as they feed, concentrating and integrating chemicals found in water into their tissues. These elevated concentrations in tissues make it easier to measure the chemicals of concern. 5) The measurement of chemicals in tissues also has a greater toxicological significance than measuring chemicals in water or sediment. 6) Compared to fish, bivalves have a limited capacity to metabolize most organic chemicals and therefore more accurately reflect environmental exposure. This limited metabolic capacity is particularly important for using caged bivalves to characterize chemical exposure from pulp and paper mill effluents where the chemicals causing effects in fish appear to be organic. It may also help explain why early efforts to identify chemical tracers of mill effluents were largely unsuccessful. 7) Bivalve populations are relatively stable and can be sufficiently large for repetitive sampling. 8) They can be easily transplanted and maintained in cages for extended periods of time even in areas where they might not be naturally found. Furthermore, caging facilitates measuring both exposure and effects endpoints and almost any clinical measurement such as biomarkers. It is more difficult to conduct field studies with caged fish because caging could affect pathways of chemical exposure and the health of the test animals. 9) Because many marine bivalves are commercially and recreationally important and many freshwater bivalves are imperiled, they are both ecologically relevant with respect to natural resources. Interestingly, bivalves are widely used as sentinel organisms for chemical exposure in marine environments throughout the world, but this approach has not been as well developed for freshwater environments. The use of bivalves as sentinel organisms for biological effects is relatively recent, with more emphasis on marine than freshwater bivalves.

Characterizing exposure and effects over space and time

In the context of understanding the fate and effects of pulp and paper mill effluents, one major advantage of the caged bivalve methodology is that it provides a characterization of exposure and effects over space and time and under environmentally realistic conditions. Deploying caged bivalves along suspected chemical gradients helps to identify the source of accumulated chemicals and facilitates the measurement of effects. The location and duration of exposure are controlled as well as the exposure and genetic history of the test animals. This approach has a number of advantages over using wild fish, and can also be used to establish cause-and-effect relationships.

It is important to note that collecting wild fish is not an experiment and does not facilitate hypothesis testing through experimentation. Without an ERA-based approach many will remain skeptical about the fish results. To understand the context of effects studies with fish, experiments must be conducted under environmentally realistic test conditions that simulate the real world. While the State of Maine has emphasized monitoring exposure and Canada has emphasized monitoring effects, the ERA-based approach suggests that it is necessary to include both in any meaningful monitoring program.

Source identification, predicting effects, and establishing causality

Concentrations of potentially toxic chemicals in pulp and paper mill effluents are decreasing due to better management practices, and therefore, it is extremely difficult to measure these chemicals in the receiving waters. It is also difficult to characterize exposure and effects in some resident organisms, such as fish, because of their mobility. These difficulties hinder the establishment of cause-effect, field-based relationships. Caged bivalves are a potentially powerful monitoring tool for pulp mill effluents because they are sedentary, can be placed in areas of concern, and are able to concentrate and integrate chemicals from water and sediment in their tissues. Bivalves can be used to quantify exposure and effects over space and time [3, 18, 25, 26, 27]. Chemicals in bivalve tissues provide a direct link between chemical exposure and associated biological effects. This relationship provides a way to compare the results of bioassays and population or community responses in the field. The caged bivalve approach is accepted world-wide as demonstrated by its long-term use in Finland and Canada, and more recent use in Argentina, Australia, Brazil, France, Germany, Hong Kong, Japan, New Zealand, Russia, and Sweden [13, 28, 29, 30]. 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 developed through a consensus-based process in an international standards organization [30], and the approach is consistent with the ERA process of characterizing exposure through bioaccumulation and characterizing effects through growth and other endpoints. Although caging freshwater mussels may be restrict their ability to bury in sediment, bioaccumulation and growth are generally not affected because these are filter feeding organisms that generally utilize overlying water for food. Furthermore, we have developed caging methods where freshwater mussels are not placed in compartmentalized cages and are free to bury themselves in contaminated sediment [31, 32]. Field bioassays with caged bivalves have several advantages over assessments with wild fish, with the most important probably being a defined and controlled exposure period. In addition, studies with caged bivalves offer more experimental control, although not as much as in standard laboratory bioassays.

Important links can be established between exposure and effects by using cultured or wild mussels, transplanting them along suspected chemical gradients, and analyzing their tissues for groups of chemicals suspected of inducing effects in fish. For example, in the caged mussel study at the Port Alice Pulp and Paper Mill we established a significant relationship between campesterol in mussel tissues and mussel growth rates [33, 34]. While this does not establish causality, it is a working hypothesis that could be used for other chemicals as well. Conversely, in a series of caged mussel studies in Canada as part of the EEM program, no chemical measurements were made and no information was gained regarding possible relationships between exposure, dose, and response [35]. There is no question that controlled laboratory tests within the internal waste streams of the mill could be used to establish links between exposure, dose, and response as suggested recently [36]. The conceptual approach of first confirming that effects in the field have occurred and then moving into the lab to confirm causality could be viewed as an ERA-based approach. However, characterizing exposure an effects as part of an iterative process in the laboratory and the field would provide additional insights into causal relationships. Furthermore, using the caged bivalve methodology provides a practical approach for gathering the information necessary to establish those relationships under environmentally realistic conditions with field experimentation.

Field studies with caged bivalves are appropriate for both marine and freshwater ecosystems. Pulp and paper mill effluents are discharged to both freshwater and marine environments and can be assessed with both freshwater and marine bivalve species. As a whole, freshwater mussels may be as important a resource as fish because many have an imperiled status. There are, however, many freshwater bivalve species that are not of imperiled or endangered status, and these are the species used in the work cited herein and recommended for future studies. Marine bivalves, which include mussels, oysters, and clams, are also an economically important resource, but few marine species have the imperiled or endangered status that many freshwater species have. Both freshwater and marine bivalves are recommended for environmental monitoring to better understand the consequences of chemicals in the environment and protect those species that are imperiled. All studies should be conducted with abundant species, and collections should limit harvesting effects on native populations.

Establishing links between effects in bivalves and fish

We have been working with Environment Canada scientists at the St. Lawrence Center in Montreal over the last five years to develop a suite of biomarkers for marine and freshwater bivalves that have been tested upstream and downstream of a municipal effluent and other sites that could be applied to pulp and paper mill effluents. These include an assay for immunocompetence [37, 38], several biomarkers including cytochromeP450, DNA damage [39], a vitellin assay that was linked to possible endocrine disruption and concentrations of coprostanol in caged mussel tissues [40, 41, 42] and experimentally-induced sex reversal in mussels caged downstream of a municipal effluent for a period of 1 year [43]. During this process we have also measured mussel growth to help calibrate the sensitivity of the various biochemical responses. Furthermore, we have developed a benthic cage that facilitates holding mussels in bottom sediment for a period of 1 year to measure all of those responses [43]. Collectively, these studies demonstrate that most effects endpoints commonly measured in fish can also be measured in caged bivalves. Perhaps more importantly, similar effects on hepatic vitellin and reproductive function were demonstrated in spottail shiners at sites downstream of the same municipal effluent [44]. Caged freshwater mussels also showed significant endocrine and reproductive effects downstream of a pulp and paper mill in Florida that were similar to those reported for largemouth bass [45].

PROSPECTUS: INTEGRATED MONITORING STRATEGY

An integrated ERA-based monitoring strategy is suggested to reduce uncertainties in current environmental assessment approaches and to establish causality. There are three basic components to ecological risk assessment: problem formulation, analysis, and risk characterization. Most assessments of pulp and paper mill effluents have not fully utilized the ERA approach in all aspects of their monitoring, but have used only selected elements in the lab and the field. In the examples provided earlier, the State of Maine emphasizes exposure characterization and Environment Canada emphasizes effects characterization. These are their basic conceptual models. However, if the conceptual model developed during the problem formulation is flawed or deficient, the resulting components that follow will also have increased uncertainty. Therefore, results of the analysis phase, which is supposed to include an integration of characterizing exposure and characterizing effects, may be biased because the analysis is either exposure-based on effects-based. Similarly, there can be no reliable characterization of risks in the third phase, because the appropriate elements have not been included previously. It may be misleading or inappropriate to suggest that either exposure-based, stressor-based, or effects-based monitoring will successfully reduce uncertainty and answer critical questions that remain with respect to effects on wild fish. An integrated ERA-based approach is the best way to accomplish those tasks.

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