Investigation of Cause in Pulp and Paper Environmental Effects Monitoring

L. Mark Hewitt,1* Monique G. Dubé,2 Sandra C. Ribey,3 Joseph M. Culp,4,5 Richard Lowell,6 Kathleen Hedley,6 Bruce Kilgour,7 Cameron Portt,8 Deborah L. MacLatchy9 and Kelly R. Munkittrick9

1Aquatic Ecosystem Protection Research Branch, National Water Research Institute, Environment Canada, Burlington, Ontario
2Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan
3International Affairs Division, Natural Resources Canada, Ottawa, Ontario
4Aquatic Ecosystem Impacts Research Branch, National Water Research Institute, Environment Canada, Saskatoon, Saskatchewan
5National Water Research Institute and Canadian Rivers Institute at the Department of Biology, University of New Brunswick, Fredericton, New Brunswick
6Water Quality Monitoring Branch, National Water Research Institute, Environment Canada, Ottawa, Ontario
7Stantec, Ottawa, Ontario
8C. Portt and Associates, Guelph, Ontario
9Canadian Rivers Institute and Department of Biology, University of New Brunswick, Saint John, New Brunswick

Environmental Effects Monitoring (EEM) Programs in Canada have been developed for the pulp and paper and metal mining industries. The EEM Program conducts cyclical evaluations of receiving environments to determine whether effects exist when facilities comply with existing regulations. Investigation of cause (IOC) is a specific stage in the EEM Program that is used after environmental effects in fish and/or benthos have been detected, confirmed and their extent and magnitude have been documented. This paper presents an overview of the processes associated with this phase of monitoring.

The objective of an IOC is to obtain sufficient information so that the source of the effect can be identified and removed, or its effects reduced to an acceptable level. The initial direction of an IOC is dependent upon the type of response patterns observed for fish and/or benthos during EEM cycles and extent/magnitude studies. The framework presented in this paper is based on an amalgamation of research projects conducted at Canadian pulp mills over the last decade and selected studies are summarized as examples. It also represents an integration of several research philosophies and scientific disciplines. The framework is constructed with a progression of investigative levels designed to provide more information on the causative factors. Each of these phases also represents a decision point for stakeholders to determine if sufficient information has been attained about the causal factor(s) and whether the IOC should be concluded. It is expected that the framework will evolve with a growing knowledge base of causal factors, as facilities enter into this phase of the EEM Program.

Key words: environmental effects monitoring, investigation of cause, pulp and paper, effluent, toxicity, fish, benthos

Introduction

The Environmental Effects Monitoring (EEM) Program is a regulated monitoring program under the Fisheries Act designed to determine if effluent from the pulp and paper and metal mining industries are causing environmental effects. The objective of the EEM Program is to evaluate the effects of effluents on fish, fish habitat and the use of fisheries resources by humans, so that the adequacy of the regulations can be assessed. Studies are carried out in multi-year cycles which provide stakeholders with an opportunity to review results between cycles and allow the program to evolve as new information on effects and monitoring technologies evolve. The EEM Program was designed to span about six cycles of data (18 to 24 years) in order to understand the potential impacts of effluents. Effects on fish are assessed through comparison of adult fish exposed to effluent with unexposed adult fish. Effects on fish habitat are assessed through comparison of benthic invertebrate communities from areas exposed and unexposed to effluent. The first monitoring cycle of EEM for the pulp and paper sector was completed in 1996, the second cycle in 2000 and the third cycle in 2004. Monitoring for metal mining started in 2004. Within EEM, an effect on the fish population and on the benthic invertebrate community is defined as
a statistically significant difference in a measured parameter, between an area exposed to effluent and a reference area or a statistically significant gradient within the exposure area (Environment Canada 2003). The EEM Program functions in a series of iterative steps (Fig. 1). If an effect has been identified, the effect is first confirmed, and then the magnitude, geographical extent and possible cause(s) of the effect are determined.

The objective of any investigation of cause (IOC) within EEM is to collect sufficient information so that the effect can be characterized and information is available to consider possible follow-up actions if deemed appropriate by stakeholders. The present paper outlines the IOC phase of the EEM Program and focuses on investigations of pulp and paper mill effluent. The tools, approaches and case studies presented are based on investigations of pulp and paper mill effluent. The design of the IOC phase must account for uncertainties regarding the source of the effect and additional contributions from other stressors in the receiving environment (e.g., municipal sewage, agricultural runoff, etc.). It is critical at the outset of any IOC to link cause to the effluent in question, e.g., discriminating between historical and present-day contamination.

The current understanding of biotic responses to pulp and paper effluent is largely based on the extensive literature available on the subject (Culp et al. 2003; reviewed in McMaster et al. 2003), as well as on the results of the National Assessment of the second cycle of EEM data. After analysis of the second cycle of pulp and paper EEM, it was found that the benthic community structure was altered at the majority of mills in Canada (Lowell et al. 2003). Eutrophication was the most common response pattern in benthos and was characterized by increased abundance and taxa richness (Fig. 2). The average national pattern in fish responses was a decrease in relative gonad weight with increases in liver weight, condition factor and weight at age. This response is believed to be indicative of some form of metabolic disruption or impairment of endocrine functioning in combination with an eutrophication effect (Fig. 2; Lowell et al. 2003). The response patterns described above are examples of interpretable patterns which will direct studies into the causes of the effects.

When Should an IOC be Conducted?

Progressing from Extent and Magnitude to IOC Studies

Questions asked during the IOC phase are based upon information collected from previous EEM cycles, including evaluations of the extent and magnitude of effects. The design of the IOC phase must account for uncertainties regarding the source of the effect and additional contributions from other stressors in the receiving environment (e.g., municipal sewage, agricultural runoff, etc.). It is critical at the outset of any IOC to link cause to the

Using Response Patterns to Investigate Cause

Based upon analysis of the first and second cycles of data, and knowledge about the response patterns observed, biotic response patterns have been categorized into two classifications for IOC—an eutrophication response and a contaminant response (including metabolic disruption) (Fig. 3). It is acknowledged that the biotic response patterns are likely more complicated than this classification scheme and it is anticipated that as the understanding of patterns evolves so too will the approaches for investigating their underlying causes. More specific criteria will be developed once the patterns of effect become evident after successive cycles of monitoring have been completed.

In attempting to define which pattern(s) are present in fish and benthos for a given site, mills would categorize the responses according to Fig. 2. First, the benthic results should be examined to determine if they fall into a known stressor category, either eutrophication or contaminant-based. In the absence of fish data, the mill may proceed to either an eutrophication or a contaminant investigation of cause based on the results of the benthic invertebrate survey (Fig. 3). If either fish or benthos exhibit a contaminant-based response (or metabolic disruption for fish), then those responses can be investigated using the investigation of cause framework outlined in Fig. 3. In cases where there is benthic eutrophication and fish metabolic disruption (which commonly occur together), each is treated separately.

In some cases a consistent but uninterpretable response pattern may exist at a site (Fig. 2). While every effort has been made to categorize fish and benthic

![Decision tree to assist with determination of when a mill should progress through the different phases of the Pulp and Paper EEM Program.](image-url)
responses into patterns that are interpretable, it is possible that a pattern at a given site may fall outside that which is currently interpretable. Colby (1984) initially proposed response patterns in fish to particular stressors can be measured. Munkittrick and Dixon (1989a,b) later proposed that response patterns could be used to define the status of a fish population. Gibbons and Munkittrick (1994) further modified the response patterns and proposed follow-up studies for fish. There may be situations where the effects found in fish are completely inconsistent with the effects found in benthic invertebrates, such as food limitation in fish and moderate eutrophication in benthic invertebrates. In such cases, mills will continue monitoring this pattern, perhaps including other biotic components of the ecosystem. However, if the magnitude or extent or effect size were to worsen over time it may be necessary to conduct an IOC on that specific pattern, employing the framework outlined below.

**Investigation of Cause Framework**

Defining the type of stressor(s) based on response patterns will greatly benefit any IOC at the outset by narrowing the focus to the possible causes and sources of the effect. Once the response pattern has been defined, then the types of questions to be asked to identify the stressor for that pattern type are shown in the Investigation of Cause section of Fig. 3. The approaches to address these questions have evolved through review of the published literature, the ongoing results of the EEM Program, and as the result of an integration of research projects and their philosophies. This methodology has been developed and applied over the past decade at pulp mills in Canada, spanning several geographical regions and scientific disciplines (Hewitt et al. 2003a). The questions follow a tiered approach, defined by a continuum of investigative phases, each providing more information regarding the cause of the effect with concomitant investments in time and resources. A review of relevant information concerning mill history, process type, process or operational changes, extent and magnitude information and response patterns observed in EEM cycles is critical before decisions can be made regarding the initial phases and direction of the IOC. The IOC framework has largely been constructed around case studies that have been individually conducted at pulp and paper mills and it is recommended that those studies pertaining to a particular response pattern be reviewed as part of the planning process of an IOC. Case study outcomes can be recorded and stored in a database to aid in future studies.

After defining the response pattern (Fig. 2), there may be sufficient information for an IOC, without going into a detailed study (Fig. 3). For example, in cases where the response pattern demonstrated by the fish and benthic surveys are difficult to interpret, and the spatial magnitude and extent of the response has been determined, a facility may simply continue to monitor in three-year cycles to determine if the temporal extent and magnitude improves or deteriorates.
Contaminant Investigations
Including Metabolic Disruption

In-mill source identification. The purpose of source identification is to attempt to specify or isolate specific waste streams within the manufacturing or treatment process that are responsible for the observed effects measured in the receiving environment. There are a variety of approaches that have been tried, ranging from simple, on-site static exposures involving containers of waste from different sources within the mill (Parrott et al. 2000a), to flow-through, on-site mesocosm exposures to investigate waste streams selected by acute toxicity tests (Dubé and MacLatchy 2001). Determining the source(s) of the effect has several potentially important outcomes including: (1) focus further investigations to a particular area of the mill for a more detailed inventory of process stream sources, quantities and waste stream qualities and toxicities; (2) identify an area of the mill where operations can be reviewed to ensure “normal operations” are occurring (e.g., spill control is being implemented as defined) and unknown anomalies in operations are not resulting in the effect; (3) evaluate the potential for source treatment and the consequences to final effluent quality; and (4) focus subsequent, detailed investigations of the waste stream sources(s) including identification of chemical class characteristics and compound identification. This level of approach has been applied and refined in several case studies that are described later (see Case Studies Concerning Metabolic Disruption).

The first step in attempting to isolate the mill stream that is causing effects is to tour the mill and acquire detailed data on mill operations. It is essential to speak with mill engineers in order to understand the process at that particular mill and the waste streams that contribute to final effluent. It is necessary to obtain or construct a sewer map (Fig. 4), in order to identify where the waste streams go, which streams contribute to the final effluent, which streams are recycled in the mill, and where the waste streams are mixed. As well, it is important to get information on the quality (e.g., pH, conductivity, toxicity) and quantity of the different waste streams over time and to calculate a mill sewer flow balance. There may be sensitivities in sharing this level of mill-specific information from a business or “competitive-edge” standpoint. IOC investigators should be aware of

Fig. 3. Iterative questions addressed in defining biotic response patterns and proceeding into causal investigations for eutrophication and investigations concerning contaminants.
this and ensure appropriate discussions and agreements are considered to facilitate sharing of information in a manner agreeable to all parties involved.

Once the dominant (e.g., based on flow proportion) streams are identified, the next step is to conduct acute toxicity tests and general chemical analyses (e.g., pH, conductivity, BOD) to determine their toxicity to fish and benthos. Conductivity, as well as sodium can often be used to assist with flow balance estimates. Acute toxicity information is extremely valuable to compare the quality of the different waste streams. It is also a relatively cost-effective and rapid mechanism to assess process stream quality. Conducting both invertebrate (e.g., *Daphnia magna* 48-h LC50) and fish (e.g., rainbow trout 96-h LC50) acute tests helps build a collection of knowledge on the waste stream toxicity. Effluent quality tests should be conducted at the same time as the acute toxicity tests and under normal operating conditions.

Using the acute toxicity data, mill sewer balance and effluent quality information on the waste streams and final effluent, it is then possible to narrow down the waste streams that will be used for longer-term sublethal testing. The number of waste streams selected for longer-term testing is usually affected by logistics, i.e., there are limits to how many streams can be tested and the number of concentrations each stream can be tested at. The number of streams to be tested depends upon the method selected and how many treatments the method can accommodate for the desired level of statistical replication. Figure 4 is an example of a waste stream summary for a mill including sewer flows, quality and toxicity information. The goal is to have the fewest streams possible to gain maximum information, in order to be cost effective. In this example, a subset of streams was to be tested using a fathead minnow partial lifecycle test (Rickwood et al. In press). The fathead minnow test method could accommodate 7 treatments in total (3 replicate aquaria per treatment and 3 spawning pairs per aquaria in a flow-through mesocosm-type trailer). Based on this information, one control was tested as well as Stream #1 (2 concentrations) and Streams #2, 3, 4 and 5 (one concentration each). Stream #6 was not selected because there was little interest in monitoring a stream that contained fibres that would settle out of the effluent with primary treatment and not be discharged with the final effluent. The focus was on those effluent constituents that would be carried through primary treatment and would be affecting final effluent quality.

Once the streams were selected then the next major decision was to set the test concentrations. This decision is very site-specific and must balance environmental relevance against toxicity and flow proportion. Studies con-

![Figure 4](https://example.com/figure4.png)

**Fig. 4.** Example of a waste stream summary for a mill including sewer flows, quality and toxicity information. (USGPM = U.S. gallons/min; LCC = low contaminated condensates; GPM = gallons/min; Cond = conductivity; RBT = rainbow trout; DM = *Daphnia magna*).
ducted by Martel et al. (1997) used a flow proportion approach to set test concentrations. Using the example illustrated in Fig. 4, the final treated effluent (Stream #1) was tested at both an environmentally relevant concentration (1%) as well as at 100% (concentration of maximum toxicity). Stream #2 was tested at 17.0% which was 40% of the LC50 for rainbow trout (projected concentration of maximum toxicity). Stream #2 was a mixture of Streams #3, 4 and 5. Thus, Streams #3, 4 and 5 were tested at concentrations that reflected their flow proportion contribution to Stream #2. For example, Stream #3 was 9% of the flow of Stream #2. Thus, Stream #3 was tested at 9% of 17% (40% of Stream #2 LC50 for trout). Stream #4 was 68% of the flow of Stream #2 and was therefore tested at 11.5%. Stream #5 represented 23% of the flow of Stream #2 and was thus tested at 4%.

Once the streams are selected and the concentrations to test established, longer-term testing can occur using a variety of approaches including mesocosms, laboratory bioassays, partial-to-full lifecycle studies, etc. The objective is to use a method and test species that are best suited to establish the cause of the responses measured in the field.

**Chemical isolation and characterization.** While the ultimate objective of an IOC would be to establish definitively the specific chemicals causing the effect in order to eliminate the effect, this represents an undertaking that can be time consuming, expensive and has no guarantee of success. It may not be necessary to proceed to ultimate chemical identification and confirmation, and in many cases, the identification of the specific waste stream or process responsible for the toxicity can be sufficient to allow the identification of remedial measures and options, if any are available. If identification of waste streams has not provided sufficient information to characterize or eliminate the effect, then this section provides information on procedures to characterize, isolate and identify the responsible substances, which may lead to remedial measures.

The approaches described here are designed to identify specific characteristics of the chemical(s) that are responsible for effects of concern. This is accomplished with a modification of an original version of an IOC framework (Hewitt et al. 2003a) incorporated in Fig. 4. The objective is to reduce what may seem to be an overwhelming task of absolute identification of individual chemicals responsible for an effect into achievable goals at which the IOC may be halted if sufficient information is gained such that a process modification or treatment solution can be found (Fig. 4). For example, the first question addresses the characteristics of the chemical class involved in the effect. In this first level of chemical identification it may be determined that specific manipulations of effluents or source wastes by aeration or pH adjustment removes the effect. In this case, a simple mitigative solution might therefore be found without further investigation. Asking progressively more detailed questions such as the identity of the chemical class itself leads to more levels of information but requires dedicated time and resources.

**Gather information that indicates the properties of the chemicals involved.** If mitigation of the effect is not possible based on previous studies, determining the properties of causative agents and the chemical classes involved may be required. All information available from field studies, source identification and laboratory studies needs to be integrated to determine what properties and broad chemical classes can be inferred. The exposure profile of the effect can indicate several chemical properties. For example, if the response in aquatic biota occurs rapidly upon exposure it indicates the responsible compounds are readily bioavailable. If the effect only occurs within a short distance of the outfall and in areas with little dilution, it may indicate that the causative agents are biodegraded rapidly under certain environmental conditions or that they are hydrophobic substances bound to sediments and therefore exposure is restricted to an immediate depositional zone.

**Toxicity identification evaluation (TIE) procedures.** The next steps are based on the comprehensive toxicity-based approaches outlined by the U.S. EPA’s Toxicity Identification Evaluation (TIE) procedures (U.S. EPA 1991, 1993a,b, 1997). The TIE approach uses the responses of organisms or appropriate bioassay to detect the presence of active agents. This approach characterizes the active substances of interest in a complex matrix in three phases (U.S. EPA 1991, 1993a,b, 1997). Each phase relates to the continuum of questions asked in Fig. 4; Can characteristics of a chemical class be identified? (Phase I), Can the chemical class be identified? (Phase II), Can the specific causative agents be identified? (Phase III). The three phases will be briefly summarized here, but for specific details that may pertain to individual types of toxicity and effluent matrices, consultation of the U.S. EPA manuals is recommended.

**Can characteristics of a chemical class be identified?** This phase of a TIE involves: (i) determining the characteristics of the active agents and (ii) establishing whether or not the effect is caused by the same substances. Failure to establish effect variability related to the active substances could lead to erroneous conclusions and control measures that do not eliminate the effect. The physical/chemical properties of the active substances can be described using effluent manipulations coupled to a bioassay that either duplicates the field effects or is mechanistically linked to them. Each test is designed to alter the substances themselves or change their bioavailability so that information on the nature of the sub-
stances can be obtained. Repeating these tests over time on the same sample will provide information on the consistence of the substances to cause the effect. Examples of effluent manipulations include filtration, pH adjustments, addition of oxidizing agents and chelating agents, temperature adjustments, aeration and solid phase extraction. If relatively simple modifications of this stage remove the effect during testing it may be possible that the investigation can be halted at this juncture and these manipulations employed on an industrial scale.

**Can the chemical class be identified?** The first phase (above) involves specific methods to isolate active chemicals and propose structures for their identification. In this step, active components are further separated from inactive substances for their identification and confirmation. These methods are specific to the classes of chemicals outlined above and utilize bioassay responses to evaluate the success or failure of extraction, separation and concentration of bioactive substances. The question of whether one or more bioactive substances are involved complicates this process and the solution is to focus on the active component that is easiest to identify. Examples of isolation techniques include solid phase extraction, HPLC and solvent extraction. Chemical isolation steps proceed in an iterative fashion, directed by bioassay responses until either further isolations are not possible or candidate chemicals are identified. Once there is strong evidence that one or more candidate chemicals are associated with the response, the last phase can be initiated.

**Can the specific causative chemicals be identified?** This step involves techniques that confirm the proposed substances are in fact responsible for the observed toxicity. This is usually accomplished through a weight of evidence assemblage of information that collectively establishes the identity of the active compounds. It is also equally important to establish that the cause of the effect is consistent over time so that amelioration efforts can adequately address the effect. Some judgment can be exercised in terms of the extent to which confirmatory tests are carried out, which reflects the authenticity of the results. For example, if a suspected substance can be removed by inexpensive pretreatment or process modification, a higher level of uncertainty may be acceptable than if an expensive treatment plant is required. Confirmatory approaches include:

i) **Correlation Approach.** A strong consistent relationship between the concentrations of the suspected agents and the bioassay response can be established.

ii) **Symptom Approach.** Different active substances often produce different symptoms in response. By comparing exposures of the effluent sample to those of pure suspected active substances, one can obtain further evidence that the suspected agents are responsible or not. Examples of symptoms include: species sensitivities, shapes of dose-response curves and time for the effect to occur.

iii) **Spiking Approach.** Suspected agents are added to the effluent to determine if a proportional response in the bioassay is obtained.

With this information and tentative chemical structures it may be sufficient to tentatively assign cause and proceed on a course of action which stakeholders agree upon. Posing the chemical identification IOC objective as a question allows stakeholders to decide to either: (i) proceed forward with confirmation studies as to the exact cause, or (ii) decide that a pilot-scale treatment may be appropriate to target a specific class of chemicals that has been identified.

**Case Studies Concerning Metabolic Disruption**

**Source identification studies.** It is important to note that several source identification studies described below are based on examination of mixed function oxygenase (MFO) detoxification enzyme activity in process streams (Martel et al. 1997), steroid depressions (Parrott et al. 2000a; Dubé and MacLatchy 2000a, 2001) or acute toxicity (Dubé and MacLatchy 2000b). These are not effects endpoints examined in the current EEM Program; however, the methodologies employed for treatment selection and testing have relevance for design of IOC studies under the EEM Program.

In addition to the Martel et al. (1997) study described previously, systematic waste stream approaches were applied to investigate sources of chemicals with the ability to affect sex steroid levels in fish at three other pulp mills. Extensive investigations on waste streams within two mills were conducted to determine their potential to elicit effects on circulating steroids in fish (Parrott et al. 2000a). Effluents before and after treatment were evaluated at a bleached kraft mill (18 streams) and a bleached sulfite mill (14 streams). In both cases, individual process wastes within the mill did not affect steroid levels or steroid production in goldfish (Carassius auratus) but final effluent from both mills after secondary treatment did cause significant steroid depressions.

An extensive investigation was conducted at a bleached kraft mill in Saint John, New Brunswick, which is one of a select few of pulp mills in Canada that does not employ secondary treatment. The study involved systematic characterization of process stream quality and toxicity (Dubé and MacLatchy 2000b) as well as exposures of mummichog (Fundulus heteroclitus) to in-mill process wastes to determine the waste stream source(s) contributing to depressed sex steroids associated with exposure to final effluent. These exposures were first conducted in a field-based, mobile, artificial stream system (Dubé and MacLatchy 2000a) and later confirmed...
with laboratory studies (Dubé and MacLatchy 2001). This work resulted in the identification of chemical recovery condensates as a primary source of substances depressing circulating testosterone in fish. Reverse osmosis (RO) treatment of condensates was also conclusively proven with off-on trials to remove the active substances prior to their reuse in brownstock washing and dilution before bleaching (Dubé et al. 2000; Dubé and MacLatchy 2001). RO treatment resulted in a non- acutely lethal final effluent and the sublethal toxicity of the final effluent was reduced in three different marine species (Dubé and MacLatchy 2000b). At this mill, IOC could have stopped at this stage of investigation as improvement in effluent quality was documented. However, further investigations of the condensates were undertaken by Hewitt et al. (2002) for characterization and compound identification.

In the Miramichi River in 1999, studies examining the effects of primary and secondary bleached kraft pulp mill effluent (1% v/v) on Fundulus heteroclitus after 23 d of exposure were evaluated using a redesigned large artificial stream system (Dubé et al. 2002b). Results showed that although there were no significant differences in length, weight, condition, LSI or GSI between the treatments after 23 d of effluent exposure, both sexes of mummichog exposed to secondary treated effluent showed significant, 5-fold depression in plasma testosterone concentrations relative to control. These concentrations were also significantly depressed relative to levels measured in fish exposed to 1% primary treated effluent.

**Chemical identification studies.** There have been several research projects that have investigated the identities of chemicals associated with induction of MFO activity and metabolic disruption in fish. TIEs, or bioassay-directed effluent fractionations, have been employed to isolate and characterize compounds associated with MFO induction in final effluents since suitable bioassays have existed to drive chemical separations. While it is recognized that these are not EEM endpoints, the approaches and methodologies employed would be applicable to any biological endpoint chosen to drive chemical separations.

In one of the first studies to address the role of secondary treatment in affecting MFO activity, Hewitt et al. (1996) fractionated effluents before and after treatment and after a maintenance shutdown at a bleached kraft mill. Laboratory rainbow trout were exposed to treated and untreated effluent, whole and filtered (<1 µm) effluent, resuspended solids and two fractions of effluent that had been generated by nanofiltration. Comparisons of relative MFO activity levels in the different effluents with chemical levels in the samples provided insight into correlations of chemicals with the biological responses. These analyses eliminated resin acids, fatty acids, bacterial fatty acids, terpenes, chlorophenolics, aliphatic alkanes, plant sterols and chlorinated dimethyl-sulphones as candidates.

A classical TIE approach on final effluent from two bleached kraft mills located in Ontario was conducted using centrifugation, tangential flow filtration and C18 solid phase extraction (SPE; Burnison et al. 1996). HPLC isolations determined that the active substances were present in a relatively nonpolar region of the chromatographic separation, with a logarithmic octanol/water partition coefficient ($K_{ow}$) of 4.6 to 5.1 (Burnison et al. 1996). As a result of follow-up studies using rainbow trout exposures and incubations with a rat hepatic carcinoma cell line (H4 interleukin) which directed HPLC fractionations of the methanol extract of the high molecular weight material, a chlorinated pterostilbene structure was postulated for an unknown compound strongly associated with induction (Burnison et al. 1999). This was significant in that it showed a natural product, modified in the bleach plant, was eliciting the biological response.

Martel et al. (1997) determined the source and identities of two substances associated with induction present in the primary treated effluent of a newsprint thermomechanical pulp (TMP) mill. Various process effluents were sampled throughout the mill. Exposure concentrations were based on the flow of these process streams in relation to final effluent. Contaminated TMP stream condensates were identified as the major process source of MFO-inducing substances. The major constituents were identified by gas chromatography/mass spectrometry (GC-MS) as juvabione, dehydrojuvabione and manool, all naturally occurring extractives in balsam fir (Abies balsamea).

A bioaccumulation model was developed in the late 1990s to circumvent problems associated with the high level of effluent complexity. The bioavailability model circumvents effluent complexity by investigating tissue burdens of bioactive substances. These investigations have utilized controlled exposures of fish to final effluents that are associated with reproductive problems in wild fish. Studies of accumulated compounds from final effluents consider chemical reactions and modification processes that could occur through mixing of process effluents, effluent treatment, environmental processes and metabolic activation. Such processes may be involved in toxicological interactions or the formation of the putative substances involved in the effects that would not be detected in evaluations of individual process streams or final combined effluent.

These investigations have shown that caging studies of wild fish can be used to examine compounds associated with MFO activity (Parrott et al. 2000b) as well as those compounds with the potential to affect sex hormone signaling and transport in fish (Hewitt et al. 2000, 2003b, 2004). These studies also have shown that multiple bioactive compounds, with a range of hydrophobicities, are accumulated in hepatic tissues during short-term caging in
effluents from mills utilizing kraft and sulfate pulping processes. Some chemicals appear to be metabolized rapidly upon removal of the exposure but the body burdens of others were maintained, indicating different chemicals were involved in the responses (Hewitt et al. 2003b). Finally, this model can detect accumulations of bioactive substances in wild fish exposed in the receiving environment (Hewitt et al. 2004, 2005). Ongoing studies are aimed to pursue this line of investigation to structurally characterize the active components accumulated by fish.

IOC studies are currently being conducted on chemical recovery condensates in the Saint John, New Brunswick, bleached kraft mill that are associated with steroid depressions. Using steroid depressions in mummichogs, a two-step solid phase extraction (SPE) method was developed which completely recovered the active chemicals from the condensates (Hewitt et al. 2002). As a first approximation of the content of the biologically active SPE extract, GC-MS analyses of both fractions revealed relatively simple mixtures of <20 chemicals and the mass spectra of several unknowns appeared to be consistent with lignin degradation products (Hewitt et al. 2002; Belknap et al. 2004).

Investigations Concerning Eutrophication

Two questions are associated with delineation of the cause for an eutrophication response pattern:

1. Can the causative nutrients be identified?
2. Can the source of the nutrients within the mill be identified? (Fig. 3).

Causative nutrients are those that limit growth in the receiving environment. Identifying causative nutrients requires an understanding of the concentration and forms of nutrients discharged by the mill. It also requires an understanding of what levels might be required at the end-of-pipe to reduce algal accrual and enrichment in the receiving environment both temporally and spatially. This begins with examination of both receiving water chemistry as well as effluent chemistry. Concentrations of nutrients (e.g., dissolved phosphorus [DP], orthophosphorus [OP], soluble reactive phosphorus [SRP], dissolved inorganic nitrogen [DIN]) in both the reference and exposure areas should be examined. Thresholds for nutrient saturation are approximately 100 µg/L DIN, 1 to 10 µg/L SRP for saturation of cellular growth rates in algae, and 30 to 40 µg/L SRP for saturation of areal algal biomass. Although these numbers are based on site-specific work of Bothwell and Daley (1981), Bothwell (1989), Bothwell et al. (1992), Bothwell and Culp (1993) and Chambers et al. (2000b, 2001), they serve as a starting point for assessment.

Examination of water and effluent chemistry, as well as algal accumulation, in the receiving environment may be sufficient to identify the nutrient that is limiting growth in the receiving environment. Systems can be nitrogen-limited, phosphorus-limited or co-limited by these nutrients. Further investigation could involve in situ techniques including the use of nutrient diffusing substrates (NDS) (Dubé et al. 1997; Chambers 1996; Scrimgeour and Chambers 1997; Chambers et al. 2000a; Tank and Dodds 2003) or mesocosm studies. In the NDS studies, containers that release nitrogen, phosphorus or both nutrients to saturation levels are placed in the reference and exposure areas. After a period of 15 to 30 d, algal biomass is measured. As an example, if a river system is phosphorus-limited and pulp mill discharge relaxes this limitation, then increased algal growth would occur in the phosphorus (P) treatments relative to the control upstream of the mill, but algal biomass would be similar on the P and control treatments downstream of the mill. Nutrient limitation is a dynamic process and may be expected to vary seasonally (Chambers 1996; Scrimgeour and Chambers 1997).

Identifying limiting nutrients and separating confounded effects. Before a mill embarks upon nutrient limitation studies, a review of the existing case studies and supporting literature should be conducted. Growth curves for periphyton exposed to different nutrient levels have been generated for several sites by Bothwell (1989), Chambers et al. (2000b) and Cash et al. (2004). Although nutrient limitation is site-specific, examination of these existing growth curves provides a benchmark for other studies to build upon. The limiting nutrient, generally either nitrogen (N) or P, can be defined as the nutrient that restricts plant growth when it is not available in sufficient quantities. A first cut at determining the limiting nutrient can be accomplished by comparing the levels of nutrients in the receiver to plant stoichiometry (Hecky et al. 1993). The ratio of N to P in plant biomass can be used to gauge nutrient limitation. Specifically, the classic study of Healey and Hendzel (1980) indicates that a plant tissue molar N:P >20 indicates P limitation, moderate P deficiency when N:P is between 10 to 20, and nitrogen limitation if N:P <10.

Wapiti and Athabasca River Studies

The Northern Rivers Basin Study (NRBS) and Northern Rivers Ecosystem Initiative provide good examples of the progression from identification that nutrient levels may be causing eutrophication, to specific studies that determine the limiting nutrient. These ecosystem studies were conducted on the nutrient-poor Athabasca, Peace and Slave rivers in northern Alberta. NRBS studies characterized nutrient loading from point and non-point sources (Chambers 1996; Chambers et al. 2000b). It was determined that between 4 and 20% of the annual TN load, and 6 and 22% of the annual TP load on the Athabasca and Wapiti rivers could be attributed to municipal and pulp mill effluent (Chambers et al. 2001).
Once the limiting nutrient(s) have been identified then the next step is to examine mill processes (Fig. 4) and process control to determine where in the process nutrients are being added and if approaches are available for reduction of concentrations at the end-of-pipe. In addition to field surveys, mesocosm studies can also be used to determine the limiting nutrient. During the NREI program Cash et al. (2004) used field-deployed mesocosms to determine that biomass in the Wapiti River, Alberta, was co-limited by both N and P.

Culp et al. (2004) also used mesocosms to separate the effects of municipal sewage effluent (MSE) and pulp mill effluent (PME) on the Wapiti River. They found that MSE was a major source of N and the PME was a major source of P and carbon (C). Algal biomass increased with exposure to the effluents, and was related more strongly to N than P or C, suggesting greater importance of N limitation downstream of the effluent discharges. The combined total emergence from both 1% MSE and 3% PME was only 60% of that of the 1% MSE + 3% PME stream. Thus, the two effluents produced a synergistic effect on insect emergence. As shown by Cash et al. (2004), this mesocosm experiment provided evidence that algal biomass in the Wapiti River is sensitive to both N and P.

One of the first applications of mesocosms to assess the effects of PME on benthic invertebrate and periphytic algae communities occurred in the Athabasca River (Culp and Podemski 1996; Culp et al. 1996, 2000b; Podemski and Culp 1996; Podemski 1999). The objective of the Athabasca River studies for NRBS was to use artificial streams to separate the effects of nutrients in whole mill effluent from contaminants. Nutrient effects were separated from contaminant effects on the basis of directional differences. Specifically, moderate eutrophication would increase primary and secondary productivity whereas contaminant effects would decrease growth, reproduction and eventually result in mortality (Culp and Podemski 1996; Podemski and Culp 1996; Culp and Lowell 1998; Culp et al. 2000b).

Thompson River Studies

Additional studies on the Thompson River illustrate how using field and multiple mesocosm studies with different species can be integrated to understand cause at a preliminary level. The Thompson River is enriched largely because of PME discharge, a problem that has been investigated since the early 1970s, when excessive accumulations of periphytic algae occurred in the river downstream of the pulp mill discharge (Dubé et al. 2002a; Federal-Provincial Thompson River Task Force 1976). A series of studies by Bothwell and Daley (1981), Bothwell (1985), Bothwell et al. (1992) and Bothwell and Culp (1993) illustrated how bioavailable P discharged in PME enriched periphytic algae growth. However, further research was required to determine the role of contaminants (Bothwell et al. 1992). Increased algal biomass was possible through two mechanisms: nutrient enrichment due to the PME, and/or reduced grazing pressure caused by pulp mill contaminants reducing benthic invertebrate abundance.

In the Thompson River studies, artificial streams were used to tease apart the interacting effects of nutrients and contaminants in PME on algae and benthic invertebrates. The approach differed from the NRBS studies in that a dose-response design was employed with the expectation to observe nutrient effects at low effluent concentrations, and contaminant effects at higher concentrations. In 1993 and 1994, periphytic algae and chironomids were exposed to a dilution series of PME (0.25 to 10% v/v) (Dubé and Culp 1996; Lowell and Culp 1999). Smaller artificial streams were used for testing the effects of the PME on single insect species (Lowell et al. 1995, 1996) and simplified benthic food webs (Dubé and Culp 1996). The single species approach focused the assessment of effects on key sentinel taxa to improve the understanding of species-specific responses (Culp et al. 2000c). Algae and chironomid larvae (Diptera: Orthocladiinae) from a reference area were placed into the streams and changes in algae and chironomid biomass were measured after 2 to 3 weeks of effluent exposure (Dubé and Culp 1996). It was reported that algal biomass (chlorophyll a) increased in all effluent concentrations due to nutrient enrichment. Total chironomid biomass and individual weight were also enriched at low effluent concentrations (<5%). At higher concentrations (5 and 10%) chironomid biomass decreased possibly due to contaminant effects.

In 1993, Lowell et al. (1995, 1996) conducted small-scale artificial stream experiments on the Thompson River in concert with those of Dubé and Culp (1996). Using the mayfly, Baetis tricaudatus, the effects of PME (1 and 10% v/v) on survival, growth, molting and morphological development were investigated under two feeding regimes (low and high). Effluent exposure significantly stimulated growth and development with 20 to 50% increases in dry body weight relative to controls. Although molting frequency increased with moderate effluent exposure (1%), higher exposure (10%) reduced molting frequency suggesting a contaminant-mediated mechanism (Lowell et al. 1996). These artificial stream results using mayflies as the sentinel species were consistent with the chironomid exposures conducted by Dubé and Culp (1996) showing an enrichment response at low PME concentrations and the appearance of inhibitory effects at higher concentrations.

In addition to consistency amongst artificial stream experiments, these results were consistent with field survey results (Culp and Lowell 1998). Long-term trends in historical data sets on the Thompson River were analyzed (Lowell et al. 1996, 2000). In addition, from 1991 to 1994 Dubé et al. (1997) collected algae and benthic invertebrate samples once every two weeks at sites near-
field (50 km) and far-field (120 km) from the pulp mill. Long-term trend analysis showed that several families of stoneflies (Plecoptera), caddisflies (Trichoptera) and mayflies (Ephemeroptera) were more abundant in the years when the mill output of suspended solids and phosphorus were higher (Lowell et al. 1996). Field monitoring by Dubé et al. (1997) also showed that temporal and spatial patterns in water column P, periphyton biomass and chironomid biomass (Diptera: Orthocladiinae) were consistent under normal mill operating conditions. The effects of the mill on the Thompson River benthic food web were restricted to nutrient enrichment. However, Dubé (1995) also observed that toxic effects of mill-related contaminants decreased chironomid densities in the Thompson River at far-field sites in 1992 when the mill’s secondary effluent treatment system shut down.

The small-scale artificial stream studies conducted on the Thompson River were instrumental in providing information to substantiate field observations on the effects of PME on the benthic food web (Culp and Lowell 1998; Lowell et al. 2000). They illustrated the nutrient enhancement effects of PME on endemic primary and secondary producers under conditions of effluent exposure typically found in the river (1%). These studies also showed the toxicity potential of the effluent under scenarios of increased effluent discharge (5 and 10%) or decreased effluent quality. The results of these studies were used as a basis to amend provincial operating permits for the mill’s final effluent discharge (Dubé 1995).

Investigating Cause when the Response Pattern is “Uninterpretable”

If the response pattern found is not interpretable based on current scientific knowledge, the mill will conduct an IOC focused on continued monitoring. For example, monitoring could track whether or not the effects that are found are changing (are the magnitude of the effects increasing or decreasing?). Other options may include changing species or to monitor other biota in the receiving environment. Information could also be gathered to better quantify the physical and chemical characteristics of the receiving environment, in order to determine what is causing the changes in the fish or benthic invertebrate community.

References


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