



Environmental Effects Monitoring Science Symposium 2008

Proceedings



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Hotel Château Cartier, Gatineau, Quebec April 29th - 30th, 2008

Environmental Effects Monitoring 2008 Science Symposium Hôtel Chateau Cartier, 1170 Chemin Aylmer, Gatineau (QC) April 29 and 30, 2008

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Executive Summary

More than150 participants interested in the environmental effects of the pulp and paper and metal mining effluents met to share and discuss current science in this field. Representatives from industry, academia, government and consulting agencies presented research results relating to four themes - Fish and Benthos studies, Investigation of Cause research and Sublethal Toxicity testing. This was the first EEM Science Symposium to bring pulp and paper and metal mining environmental effects stakeholders together.

These proceedings list the program as it unfolded over the two days and include summaries of discussion sessions that occurred at the end of each theme. Abstracts follow program list and discussion summaries. The document ends with a list of participants.

Fish Studies

Keeping in mind that the Environmental Effects Monitoring Program (EEM) for pulp and paper was designed to determine, over six three-year cycles, if the applicable regulations were adequately protecting the environment, effects on the national scale have been detected after 3 cycles. The primary effects are eutrophication and reduction in fish gonad size. It is now possible to move forward towards first finding the causes of these effects and then determining solutions. Many agreed that the EEM program is at a crossroad where more funds are needed for further research, common research initiatives would be applicable and all players have a role to play in leveraging funds and initiatives.

Benthos Studies

A wealth of information on Reference Condition Approach science, analysis and results was shared and several questions were raised concerning bench mark variability, re-sampling and climate change.

Investigation of Cause Research (IOC)

The national IOC project on fish gonads is progressing and researchers are expected to work at new mills and with new effluents in summer 2008. IOC fish gonad studies may be influenced by changes in effluent characteristics as effects on gonads may disappear and re-appear. It was shown that there is value in testing the effluent with increased frequency and that working with fresh effluent is important.

The Forest Products Association of Canada's (FPAC) Best Management Practices document is intended to help mills with eutrophication problems in designing studies to investigate causes and finding solutions. The EEM program is re-assessing alternative methods to fish surveys and it is hoped that these methods can also help mills to determine effects contributions in confounded situations.

Sublethal Toxicity Testing (SLT)

Although SLT responses still need to be linked to standard field endpoints it was stated that temporal variability in effluent quality is the most important characteristic SLT is capable of capturing and therefore it is important to maintain a chosen methodology in order to compare results over longer periods. Research on toxicity of metals is being conducted in many research centers around the world and this should help to advance knowledge. Historical contamination and re-opening of mines was seen as a problem and solutions were suggested including good baseline studies and treating historical contamination as a confounding factor. Innovative methods for IOC techniques will need to be developed for use by the Metal Mining industry.

Symposium Program

Note: Contact presenter for full presentation. See abstracts for presentation details, presenter affiliation and contact information.

Fish Studies: Chairs - Kelly Munkittrick and Robert Prairie

Robert Praine	Introduction to the fish studies session
Greg Kaminski	National overview of Pulp and Paper Environmental Effects Monitoring (EEM) Cycle 4 results and alternative to fish surveys studies in Cycles 2 to 4
Carrie Rickwood	Using artificial streams for fish survey alternatives; questions which can be answered
Sylvie St-Jean	Responses to environmental stressors of wild versus caged mussels
Mark McMaster	How to develop baseline information quickly for species that you don't know much about!
Rick Lowell	Solving the fish significant interactions puzzle
Kelly Munkittrick	Considerations for site-specifically assessing the health of fish populations exposed to industrial stressors

The fish session was co-chaired by Robert Prairie (Xstrata Zinc) and Kelly Munkittrick (UNB) and featured 5 presentations that summarized progress on several issues related to the fish survey and on reporting from several working groups. Greg Kaminski, EC Gatineau gave an overview of Cycle 4 results for the pulp and paper EEM program and summarized the outputs from a working group that has been discussing options for alternatives when fish surveys are not possible. He discussed some of the background related to increased perception of need for alternatives, some of the data gaps and the research needs associated with brining on new tests. Sylvie St-Jean (Jacques Whitford Consultants) updated progress since the 1997 workshop on alternatives. and described some of the advances and limitation of using caged bivalves, specifically related to species selection and timing of sampling. She highlighted some research needs and issues related to further developing the caged bivalve tools, and the need for additional guidance. There were two presentations by the EEM Fish Working Group related to new research advances and new guidance available for regional implementation of EEM fish surveys. The first presentation by Mark McMaster (EC Burlington) featured new information on data analyses that can provide insight into the reproductive strategies of small-bodied fish species, especially for species that baseline biology is not known. There are analyses that can help identify spawning strategies and potential sampling times. Rick Lowell (EC Saskatoon) presented the output from another working

group that has been looking at data interpretation issues in interpreting fish data when there are interactions in the data analysis. Historically these data have not been synthesized into the national averages because of challenges in interpreting an effect size when the data shows an interaction. He presented an option for calculating effect sizes when interactions were present in data analyses. The final presentation was also from the Fish Working Group from the National EEM Science Team and was presented by Kelly Munkittrick (University of New Brunswick). It discussed options for selecting reference sites and interpreting data, and highlighted the success that the EEM program has achieved in fulfilling its original design objectives.

The session effectively highlighted new research that can provide guidance, as well as data gaps and science needs for further improving the program. It is clear that there are a number of changes that should be incorporated into the next revision of the technical guidance documents to improve the study designs and options. It is also clear that the program has worked effectively for what it was designed to do, and that improvements to guidance and study design options will provide further refinement.

Question and Answer period/Discussion Points

The audience was reminded during the discussion that Pulp &Paper (P&P) Environmental Effects Monitoring (EEM) program was initially designed to be evaluated after 6 cycles. EEM was designed to answer the following question:

• are the regulations, with their current discharge limits, adequately protecting the environment (i.e. under conditions of compliance)

Effects on the national scale were detected after only 3 cycles: eutrophication and reduction in gonad size being two major ones. It is important that the program balances adequately national objectives versus site-specific needs. The program may still need 6 cycles to adequately describe effects on particular mill basis. It will be interesting to see how this will be balanced to inform the Metal Mining (MM) EEM program.

EEM practitioners felt that the concept of Critical Effect Sizes (CES) is better suited for the program than only a statistically significant difference between exposed and reference sites.

Questions were asked about the fit of investigation of cause (IOC) and investigation of solutions (IOS) studies – do they belong in the EEM program, which is a regulatory program, or should they be pursued outside of it, as a purely research initiatives? Some feared that IOC studies could last too long without any tangible results; hence no decisions on solutions could be made. It was felt that effluent effects should be verified periodically, even during the IOC studies – if there are no more effects due to improved effluent quality, no more studies should be conducted.

Industry was asking if its scarce funds should still be spent on continuous monitoring or should they be better used financing IOC and IOS studies? Industry representatives have expressed opinion that some mills would like to join the IOC/IOS studies but perceive that they are being prevented from doing so by Environment Canada (EC). The pulp and paper industry expects EC to support mills' participation in IOC studies on both national and regional levels. The industry would like to see the IOC and IOS work done mostly inside the mills – to fix their processes and effluent treatment systems (where warranted) rather than monitoring in the receiving environment. A clear decision-making process is necessary to allow mills to move to finding solutions and fixing problems.

Many agreed that the EEM program has reached a crossroad – more funds are needed for further research and requests for grants to finance common research initiatives should be made. In leveraging funds all players – EC, industry and industry associations have a role to play. It was noted that research and research funds were initially to be supplied by the government sector, but this has not come about fully. For the industry, the reasons for spending money need to be practical – just because money is available (and in present economic situation, it is not) is no longer good enough.

Costs of conducting studies are important to the industry – mesocosm/in-situ fish exposure studies are expensive, time-consuming, labour-intensive. It was asked if, alternatively, lab testing could be used (especially if artificial stream studies are moving now towards onsite bioassays) Researchers performing mesocosm studies noted that costs of artificial stream/mesocosm studies has recently come down. The units used to perform these studies are now more mobile and practitioners are achieving efficiencies, especially in the exposure period.

Even though effluent quality and composition has changed and continues to change over the short and long term, effluents still have measurable effects. Some noted that technical solutions are becoming available for dealing with eutrophication problem (for nitrogen and phosphorous).

Fate of other than P&P and MM sectors was discussed. Petroleum and Municipal Waste Water have received funding for research through Canadian Environmental Protection Act. Environment Canada is drafting Regulations for Municipal Waste Water that may include some EEM-like components. However, agriculture run-off would not be good candidate for regulations. Voluntary approaches are being used. Information about the status of Diamond Mining Regulations was requested – status was unknown to the panel, but process is looking at MMER for lesson's learned. Representatives of the Mining Association of Canada informed that it is being consulted by EC regarding Diamond Mining Regulations.

Some consultants observed that performing MM EEM studies in the North is difficult due to lack of baseline information; requested more guidance. It was said that work is being done on guidance for sampling species with little known biology and how to choose species that would work best in such conditions; Traditional Ecological Knowledge could also be of used in northern areas.

Questions were asked about the potential decadal effects of climate change on multi-cycle EEM data. Such analyses have not been done. Currently researchers working with the data are trying to take out noise between cycles. Considering effluent quality changes and variable study designs, there is probably too much noise to see climate change effects. However, the Experimental Lakes Area program is doing some Climate Change effects research. St Lawrence Centre in Montreal is conducting some mussels and Climate Change research.

It was noted that this was the first EEM symposium to bring together P&P and MM industries and practitioners – and a great venue to disseminate knowledge and exchange information.

Rick Lowell	Introduction to the benthos session
Lee Grapentine	Accommodating changing reference conditions in assessments of nearshore benthic conditions of the Great Lakes
Michelle Bowman	Developing reference benchmarks: A comparison of ecoregion, clustering – discriminant analysis, and nearest-neighbour classifications
Bruce Kilgour	Using Regression in Reference Condition Approach (RCA) Designs
Keith Somers	Teasing apart the cumulative effects of multiple stressors
Tim Arciszewski	The potential of population-level indicators in invertebrate species for monitoring pulp mill impacts
Michelle Bowman	Reference benchmarks for benthic algae, macroinvertebrates, and fish and their sensitivities to mining developments in a World Heritage Site, the South Nahanni River Watershed, NWT, Canada
Joseph Culp	Approaches for setting sediment and nutrient standards for agricultural streams

Benthos Studies: Chairs - Rick Lowell and Keith Somers

A series of seven talks were presented in the Benthos Studies session, chaired by Richard Lowell (Environment Canada, Saskatoon) and Keith Somers (Ontario Ministry of the Environment). These talks covered a variety of issues relevant to benthic surveys in the EEM Program, including study designs, cumulative effects, alternative methods, benthic endpoints, and related monitoring in other sectors.

The first three presentations dealt with recent developments in benthic study designs. Lee Grapentine (Environment Canada, Burlington) suggested a number of ways of accommodating changing reference conditions when using the Reference Condition Approach (RCA) to assess nearshore benthic communities in the Great Lakes. Reference conditions can change over the years due to a number of factors such as climate change, invasive species, and land-use changes. Suggested analytical solutions included deleting old data or, preferably, incorporating time and nuisance stressors as predictor variables. Michelle Bowman (University of Toronto) continued the RCA theme, focusing on selection of appropriate RCA designs for mining areas in northern Ontario. The three major models considered were based on 1) ecoregions, 2) clustering-

discriminant function analysis, and 3) nearest neighbour analysis. With sufficient availability of reference sites, the three models yielded comparable site assessments, although the nearest neighbour model was particularly useful for sharing reference site data among mining areas. In a third talk on RCA designs, Bruce Kilgour (Kilgour & Associates Ltd.) highlighted the use of regression analysis when data are collected along a natural ecological gradient, using examples from data collected in Ontario rivers and streams. The regression approach had a number of advantages, including a more straightforward model, increased statistical power, accommodation of lower sample sizes, and reduced chance of falsely declaring an impact.

The issue of teasing apart the cumulative effects of multiple stressors was addressed in a presentation by Keith Somers (Ontario Ministry of the Environment). He pointed out that, traditionally, cumulative effects assessments often rely on descriptive approaches that can yield subjective conclusions. He went on to propose a quantitative approach that utilized multivariate multiple regression to partition effects due to two or more stressors, using northern Ontario RCA data to illustrate the analyses. In the next presentation, Tim Arciszewski (University of New Brunswick) proposed an alternative EEM survey method to measure sewage and pulp mill effluent effects, by utilizing freshwater invertebrates (stoneflies) to evaluate condition, development, growth, and reproduction endpoints. Thus, the approach evaluated the same kinds of individual/population-level endpoints measured in EEM fish surveys, and the results were similar to a concurrent slimy sculpin study in New Brunswick, but with the added advantage of ready availability and high site fidelity of sampled organisms. Michelle Bowman (University of Saskatchewan) next addressed the issue of the relative sensitivity of different benthic endpoints and study designs. In a comparison of control/impact versus RCA designs used to monitor mining effects in the South Nahanni River, she found that the control/impact designs had less inherent variability, but also less power, than RCA designs, so that the sensitivities of the two designs were similar. In the final presentation, Joseph Culp (Environment Canada, Fredericton) described monitoring approaches used in the agricultural sector by the National Agri-Environmental Standards Initiative (NAESI). In addition, he described approaches for setting up sediment and nutrient standards for agricultural streams. He highlighted a number of similarities (e.g., effects based approach, evaluating multiple stressors) and differences (e.g., non-point impacts, non-regulatory) of the NAESI program in comparison to the National EEM Program.

It was apparent from the presentations in this session that the benthic monitoring component of the National EEM Program has progressed a long way since the program was initiated in the early 1990's. The approaches used in the program have proved quite valuable in evaluating effluent effects in a wide variety of scenarios. The presentations in this session illustrated how recent developments in monitoring science are being (or can be) applied to further improve the program.

Question and Answer period/Discussion Points

Following the presentations, questions were asked on how often reference sites should be re-sampled in a Reference Condition Approach (RCA). It is hard to generalize since it will depend on the variability observed. However, it is important to go back and check to ensure benchmark sites are not changing due to effects from climate changes or other sources – the U.S. EPA recommends revisiting 20 to 30 percent of the sampling sites per year, however 10 to 20% should be able to give a solid baseline.

The possibility of EEM programs collaborating with the CABIN (**C**anadian **A**quatic **B**Iomonitoring **N**etwork) program in the development of reference conditions across the country should be explored. NAESI program (National Agri-Environmental Standards Initiative) established benchmarks for environmental protection in the non-point source effluent environment. There is a possibility of continued monitoring by the Regional Aquatics Monitoring Program (RAMP) which assesses the health of rivers and lakes in the Oil Sands Region of north-eastern Alberta. Oil and gas sector monitoring has some similarities with P&P and MM EEM, and a collective study approach would allow for monitoring to go farther and improve study designs.

Investigation of Cause Research: Chairs - Pierre Martel and Mark Hewitt

Pierre Martel Mark Hewitt	Overview of IOC in EEM
Bernard Vigneault	Tools for determining permissible metal loadings to the aquatic environment: A study of rivers in northwestern Quebec that receive mine effluents
Roger Cook	Best Management Practices (BMP) Guide for Nutrient Management in Biological Treatment of Pulp and Paper Mill Effluents
Raymond Chabot	Eutrophication effect of pulp mill effluents: a possible pathway for investigation of solution studies
Mark Hewitt	Update on the national investigation of cause project on reduced gonad sizes in fish
Pierre Martel	Investigation of solution (IOS) studies at FPInnovations - Paprican
Craig Milestone	Determining biologically active components in kraft mill chemical recovery condensates
Thijs Bosker	The effect of thermomechanical pulp mill effluent on reproduction in mummichog (<i>Fundulus heteroclitus</i>): an investigation of cause.

The Investigation of Cause Session was chaired by Mark Hewitt (EC) and Pierre Martel (FPInnovations) and topics covered the metal mining and pulp and paper sectors. In all, seven papers were presented highlighting the recent developments in chemical analysis, establishing linkages between the field and the laboratory and the development of diagnostic tools.

The session started with a presentation given by Bernard Vigneault (NRC) summarizing a series of studies conducted on metal mining effluents. An ecotoxicological approach using biochemical tools to detect metal-induced effects in aquatic organisms supported the development of estimates of metal exposure. These estimates were compared to results of laboratory toxicity tests with ambient water and sediments.

The focus of the session then shifted over to the pulp and paper sector, starting with the issue of nutrient management in the biological treatment of pulp and paper mill effluents. Roger Cook (FPAC) presented the outline of a Best

Management Practice Guide to Nutriment Management to help mills that reported nutrient enrichment down-stream to develop an action plan as part of their EEM Investigation of Cause and Solutions. Raymond Chabot (EC) presented a case study of the actions taken by the staff of a thermomechanical pulp mill in Quebec to reduce residual phosphorus release in their effluents. Optimized control of phosphoric acid addition to the treatment has reduced residual phosphorus levels in the effluents while maintaining treatment efficiency.

An update on the national investigation of cause project was then presented by Mark Hewitt (EC). A team of researchers from industry, government and academia studied a mill effluent in the field and in the laboratory to select appropriate diagnostic tools that could be used to evaluate in-mill and end-ofpipe treatment options for removing substances affecting fish reproduction. Results obtained from the first mill effluent studied shows wild fish results can be reproduced in the laboratory lifecycle experiments and that short-term egg production tests show promise for investigating causes and solutions. While the selection of diagnostic tools is ongoing, preliminary work was conducted to characterize the potential of effluents to cause reproductive effects in a laboratory test with fathead minnows.

The presentation by P. Martel (FPInnovations) suggests that when effects are noted in the laboratory with short term reproduction tests they are not related to any particular pulping or bleaching technologies and that conventional effluent treatment can be beneficial. However, effluent-related effects persist as some mills. In trials conducted to refine conventional activated sludge and investigate ozone treatments neither options eliminated effects on fish reproduction illustrating the refractory nature of the causative agents.

Efforts to identify agents present in condensates streams from the Kraft recovery process and causing a depression of sex steroids in mummichog were summarized by Craig Milestone (UNB). Using solid phase extraction (SPE) techniques one eluted fraction was identified as containing the majority of biological activity. Attempts to further separate the elutriate by HPLC was not successful in retaining the biological activity. The solid phase extraction procedure is being refined to produce chemically distinct fractions for in-vivo an in-vitro bioassays.

An approach to identify the source of effects on gonad size in mummichog at a thermomechanical pulp mill was presented by Thijs Bosker (UNB). Following earlier findings that the final effluents from this mill caused a significant reduction in gonad size in mummichog an investigation of source in mill effluent streams was conducted. Three experiments conducted over the last 12 months have shown no clear pattern of response from the final effluent and in-mill streams on gonad size, plasma steroid hormones nor egg production suggesting low potential for these effluents to cause effects on fish reproduction.

Question and Answer period/Discussion Points

IOC fish gonad studies may be influenced by changes in effluent characteristics; effects on gonads may disappear and re-appear. Changes may be linked to process changes, raw material variability, etc.:

- Conventional effluent parameters may not change (TSS, BOD, turbidity etc.) while other, less conventional do.
- This is true for both P&P and MM, although the sources of changes may be different.
- Notable P&P effluent improvements have occurred over the last 15 years, so that less and more subtle effects are now seen using fish bioassays.
- Possibility of using the fish bioassays as additional tests in designing alternative to standard fish surveys tests.

It was pointed out that the Technical Guidance Document (TGD) allows the regulatees to suggest studies other than mesocosm and caged mussels studies, as long as scientific justification is presented. Also, the EEM program is in the process of re-assessing the alternative methods – the alternative methods working group recently submitted its questions/recommendations to the EEM Science Committee and National Office.

Fish bioassay responses have to be linked to the standard endpoints and there is some concern that bioassays can give a false negative. Before a modified fathead minnow test is approved as a standard test further method standardisation is necessary (test is being standardized in the U.S.); work is being done on mummichog test standardization. Ankley test is now being standardized (round robin) in the U.S.

Questions were asked if there are process changes that can be correlated to improvements in fish surveys results. It is difficult to attribute improvements to a single change, since mills often close while process changes are being made and several changes are often employed at the same time. General process efficiencies did seem to improve effects.

It is uncertain if short-term changes in effluent quality may have long-term effects on residual wild fish populations. It would be of value to test effluent while studies are on-going.

While asked about response patterns that have been observed in other effluents, researchers said that they were different from these observed for P&P. For example, increased condition and gonad size with decreased egg production have been observed in municipal effluents (rather than fatter fish with smaller gonads as observed for P&P). Pharmaceuticals such as antidepressants, birth control medications and heart medications play a role in mimicking certain hormones and influencing fish physiology in municipal effluents while in the case of P&P effluents dominant effects maybe linked to eutrophication.

Research work on indentifying the chemicals responsible for effects seen in fathead minnow tests is continuing – it's very important to work with fresh effluent since effluent characteristics change very quickly.

The EC EEM office in Quebec Region collaborates with the provincial ministry on a nutrient abatement program for certain Quebec mills. This allows these mills to meet the regulatory requirements of both jurisdictions with one action plan.

Best Management Practices (BMP) document sponsored by FPAC is intended to help mills designing IOC and IOS studies when eutrophication (excess of N and P in the effluent) is a problem. It is expected that once these improvements to methods are implemented, the solutions will be validated by consecutive field studies.

Mills with confounding factors in the receiving environment would also like to be allowed to conduct IOC studies – what are the suggested methods in order to show how much of a problem can be attributed to mill's effect? It was suggested that consultants working together with mills on pursuing IOC studies determine the best alternative methods logically suitable for a particular situation. These alternative methods should be included in the Study Design Reports submitted to the regional EC offices. Environment Canada offices will help mills in choosing the best methods and may suggest improvements to the study designs.

Sublethal Toxicity Testing: Chairs - Rick Scroggins and Tibor Kovacs

Tibor Kovacs	Introduction to SLT Session
Rick Scroggins	Uses of Sublethal Toxicity in Pulp and Paper and Metal Mining EEM
Wes Plant	Comparisons of the Ontario Metal Mining EEM benthic invertebrate community data with sublethal toxicity data using the Lab-To-Field rating system
Lisa Taylor	National, Regional and Mine-Type Trends of Sublethal Toxicity Testing in the Metal Mining EEM Program
Leana Van der Vliet	New Developments and Improvements in SLT Methods
Bernard Vigneault	Relating sublethal toxicity to effluent chemistry using characterization data from the metal mining EEM program
Keith Holtze	Utility of toxicity tests with <i>Hyalella azteca</i> in differentiating between current effluent quality and historical sediment contamination

The aim of the session was to provide a state-of-the-art overview of the:

- Uses of sublethal toxicity testing within environmental effects monitoring
- Application of the test results
- New developments in methodology
- New applications of SLT

Following is a brief synopsis of the presentations:

<u>Uses of SLT within the EEM program:</u> The key uses of sublethal toxicity tests include the monitoring of effluent quality with time, help with interpretation of field data from complex situations that as may occur at multiple discharge sites and Investigation of Cause/Investigation of Solution studies. (Presentation by Rick Scroggins)

<u>Current application of test results:</u> Sublethal toxicity testing can provide regional and national trends of effluent quality and can also provide a comparison of effluent quality from different types of industrial complexes. (Presentations by Wes Plant and Lisa Taylor)

<u>New developments in test methodology:</u> Improvements in methodology have been made or are planned for all the sublethal toxicity tests in the EEM program.

These should provide greater consistency and applicability of the tests. In addition, much work has been done to strengthen the statistical analyses of the test data. Of particular note is regression analysis to estimate sublethal toxicity thresholds. (Presentation by Leana van der Vliet)

<u>New applications of SLT:</u> With metal mining effluents, relating the sublethal toxicity test results to effluent chemistry it was possible to identify some metals and increased water hardness as causes of sublethal effects (Presentation by Bernard Vigneault). Also in the case of metal mining effluents, the freshwater amphipod *Hyalella azteca* test was helpful in distinguishing the impact of current and historical discharges through comparison of water-only and water-sediment toxicity results (Presentation by Keith Holtze).

Overall, the session provided an update of the latest developments in SLT related to the EEM program and could serve as the basis for future discussions about the application of sublethal toxicity testing within the EEM.

Question and Answer period/Discussion Points

It was noted that there may be differences (for certain metals) in test results depending if receiving or conditioned lab water is used in SLT testing. The panel responded that although mines use conditioned lab water for the SLT testing, this procedure has been standardized by previous research.

Research on toxicity of metals is being conducted in many research centres around the world – this should advance the knowledge.

Sometimes unexpected results are obtained in tests using *Hyalella azteca*, different metals may be responsible for toxic results in these tests than metals responsible for effects on trout and *Daphnia*.

The panellists observed that temporal variability in effluent quality is the most important characteristic SLT testing is capable of capturing; therefore it is also very important to maintain a chosen methodology in order to be capable of comparing results.

More data is needed to evaluate MM SLT tests, one cycle of data is simply not enough. More research funds are necessary to advance the MM SLT (and other issues).

Trace metals may not be a big issue in MM SLT testing, there are existing capabilities to rule these out; however, other non-standard parameters that are not being measured may be causing effects, for example presence of flotation reagents. This issue needs to consider in IOC.

Historical contamination of re-opening mines was mentioned as a problem; suggested solutions – good baseline studies and the proponents should treat historical contamination as confounding factor.

Representatives of the Canadian Nuclear Safety Commission mentioned that Under CEAA it is important to ask it the current deposition would add to the historical deposits creating unacceptable conditions – cumulative effects are considered under that legislation.

It was suggested that in future innovative methods for IOC techniques have to be developed to be used by MM industry.

Possibility of marine/estuarine mills using freshwater SLT tests in order to reduce costs was suggested by the industry.

Symposium wrap-up: main points recorded

Discussion points – Fish Studies Session

- P&P EEM program initially designed for 6 cycles
 - Effects on aquatic environment: yes or no?
 - Are the Regulations, with their current discharge limits, adequately protecting the environment?
 - Effects on the national scale detected after 3 cycles
 - May still need 6 cycles to adequately describe effects on particular mill basis
 - National objectives versus site-specific needs must be balanced in the MM EEM program
- The concept of Critical Effect Sizes better suited for the program than only statistical significance
- Questions about the placement of the IOC and IOS studies do they belong to the program or should be conducted outside if it?
- Should (scarce) P&P industry funds be spent on continuous monitoring or should they be better used financing IOC and IOS studies?
- P&P industry is interested in finding the causes and fixing them; thinks that in certain cases continuous monitoring is a "waste of money"
- The industry would like to see the IOC and IOS work done mostly inside the mills – to fix their processes and effluent treatment systems (where warranted)
- Costs of conducting studies are important to the industry mesocosm/in situ fish exposure studies expensive, time-consuming, labour-intensive; alternatively, lab testing could be used
- The EEM program has reached a crossroad:
- More funds for further research needed
- Leverage EC, industry and industry associations have a role to play
- Clear decision-making process necessary to allow mills to move to finding solutions and fixing problems
- P&P industry expects EC to support mills' participation in IOC studies on both national and regional levels
- Effluent effects should be verified periodically, even during the IOC studies - no more effects, no more studies
- Performing MM EEM studies in the North difficult lack of baseline information, more guidance needed
- Effluent regulations for diamond mining industry and EC in the process of consultations, possibility of including some of the existing EEM Programs components
- This is a first EEM symposium that brings together P&P and MM industries and practitioners – great venue to disseminate knowledge and exchange information

Discussion points – Benthos Studies Session

- In benthos studies (RCA and other techniques) it is important to resample a part of (~10% to 20%) reference sites in order to maintain a steady baseline information
- Possibility of EEM programs collaborating with the CABIN (Canadian Aquatic Blomonitoring Network) program in the development of reference conditions across the country
- NAESI program (National Agri-Environmental Standards Initiative) established benchmarks for environmental protection in the non-point source effluent environment, possibility of continued monitoring by the RAMP program

Discussion points – IOC Studies Session

- IOC fish gonad studies may be influenced by changes in effluent characteristics
 - Changes linked to process changes, raw material variability, etc.
 - Conventional effluent parameters may not change (TSS, BOD, turbidity etc.) while other, less conventional do
 - This is true for both P&P and MM, although the sources of changes may be different
- Notable P&P effluent improvements seen in the last 15 years, less and more subtle effects seen using fish bioassays
- Possibility of using the fish bioassays as additional tests in designing alternative to fish standard surveys tests
- TGD allows the regulatees to suggest other than mesocosm and caged mussels studies, as long as scientific justification presented
- EEM programs are in the process of re-assessing the alternative methods, working group submitted its questions/recommendations to the Science Committee and the National EEM Office
- Fish bioassay responses have to be linked to the standard endpoints
- It is uncertain if short-term changes in effluent quality may have long-term effects on residual wild populations
- Before a modified fathead minnow test is approved as a standard test further method standardisation is necessary (test is being standardized in the U.S.); work is being done on mummichug test standardization
- Different response patterns have been observed in other effluents (such as municipal), but not fatter fish with smaller gonads
- Pharmaceuticals such as antidepressants, birth control medications and heart medications play role in mimicking certain hormones and influencing fish physiology in municipal effluents while in the case of P&P effluents dominant effects maybe linked to eutrophication
- Research work on indentifying the chemicals responsible for effects seen in fathead minnow tests is continuing it's very important to work with fresh effluent since effluent characteristic do change very quickly

- Regional EC EEM office in Quebec region collaborates with the provincial ministry (MDDEP) on nutrient abatement program for certain Quebec mills, allows them to meet the regulatory requirements of both jurisdictions with one action plan
- Best Management Practices (BMP) document sponsored by FPAC is intended to help the mills designing the IOC and IOS studies when eutrophication (excess of N and P in the effluent) is a problem
- It is expected that once these methods implemented improvements will be achieved and confirmed by consecutive field studies
- It is suggested that consultants working together with mills willing to pursue the IOC studies (also mills with confounding factors) determine the best alternative methods logically suitable for a particular situation
- These methods should be included in the Study Design Reports submitted to the regional EC offices
- EC offices will help mills in choosing the best methods and may suggest improvements to the study designs

Discussion points – SLT Studies Session

- There may be differences (for certain metals) in test results depending if receiving or conditioned lab water is used in SLT testing
- Research on toxicity of metals is being conducted in many research centres around the word this should advance the knowledge
- Sometimes unexpected results in tests using *Hyalella azteca*, different metals may be responsible for toxic results from these responsible for effects on trout and Daphnia
- Temporal variability most important to capture using SLT testing; maintain the chosen methodology
- More data needed to evaluate MM SLT tests
- Trace metals may not be a big issue in MM SLT testing, there are capabilities to rule these out
- Research funds necessary to advance the MM SLT (and other issues)
- Historical contamination of re-opening mines is a problem; suggested solutions – good baseline studies
- Under CEAA it is important to ask it the current deposition would add to the historical deposits creating unacceptable conditions
- Innovative methods for IOC techniques to be used by MM industry are needed
- Possibility of marine/estuarine mills using freshwater SLT tests suggested by the industry
- Mines use conditioned lab water for the SLT testing, but this has been standarized by previous research

Symposium Abstracts: Fish Studies

National overview of Pulp and Paper Environmental Effects Monitoring (EEM) Cycle 4 results and alternative to fish surveys studies in Cycles 2- 4 *Greg Kaminski, Environment Canada, Gatineau,* <u>Greg.Kaminski*ec.gc.ca</u>

The major trends in responses of core endpoints and magnitudes of effects on adult fish and benthic invertebrate community are assessed. Effects were similar to earlier cycles for both fish and benthos, but generally less pronounced. Main observed effects were nutrient enrichment/eutrophication and metabolic disruption (smaller gonads). Part of the reduction in effects may be attributable to the mills that have shown most pronounced effects in previous cycles pursuing Investigation of Cause (IOC) studies.

Alternative to fish survey studies have been historically used in such situations as hazardous conditions, unsuitable habitat for sampling, presence of confounding factors and repetitive failure to collect appropriate samples. Renewed interest in the alternative to fish survey studies comes from the Smart Regulations Process for pulp and paper that recommended flexibility in allowing industry to conduct IOC studies more effectively. Scientific guidance is sought to: increase the number of recommended methods, correlate the responses (standard surveys vs. alternative tests), use tests that are focused on reproductive responses and use tests able to measure reproductive capacity by means other than gonad size.

Keywords: Pulp and paper, environmental effects monitoring, fish surveys, fish reproductive tests

Using artificial streams for fish survey alternatives; questions which can be answered

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Since the early 1990s artificial stream systems have been used as an alternative monitoring tool for field surveys assessing environmental effects of both pulp and paper and metal mining effluents. Initial studies using these systems focused on pulp mill effluent effects on benthic invertebrates, however, the systems soon evolved to incorporate small-bodied and juvenile fish species. The predominant endpoints in fish assessments were individual-level effects, for example growth, gonad and liver size, fecundity and sex steroids. Whilst these studies offered an opportunity to measure EEM-relevant endpoints on wild-fish in confounded environments, it was difficult to extrapolate these individual-level effects to population level changes. In addition, these studies incorporated water-only exposures which were not reflective of the receiving environment, where sediments could play an important role in the bioavailability of certain contaminants e.g. metals. Over the last six years artificial stream systems have developed to incorporate 1) bioassays to assess population level endpoints, and 2) sediments and benthos to assess trophic-transfer of contaminants. Results of

these studies have answered a suite of hypothesis that would not have been possible before. Validation and continued development of artificial streams is ongoing for both the pulp mill and metal mining sectors.

Responses to environmental stressors of wild versus caged mussels

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The first cycle (1992-1996) of adult fish surveys carried out at most pulp and paper mills discharging into marine and estuarine environments failed to produce useful data. A workshop held in 1997 to discuss potential solutions concluded that the most promising alternatives were on-site bioassays (mesocosms) and caged bivalve applications. Following a recommendation that the applicability of these approaches to the PPER-EEM program should be demonstrated as quickly as possible, pilot projects with caged mussels were carried out at Port Alice, British Columbia in 1997 and at Pictou, Nova Scotia in 1998. These studies successfully measured survival and growth over 68 and 90 day periods respectively but did not provide measures of energy storage or reproduction. Caged mussel applications continued through the ensuing EEM cycles at certain pulp and paper mills in Atlantic Canada and an active research program was pursued in Pictou, Halifax NS, and in Vancouver, BC. Products of this research include the development of measures of reproductive output and energy storage, some of which has been incorporated into EEM Technical Advice already and some of which remains to be applied. The techniques were also field tested in monitoring programs in both Vancouver (municipal waste water) and Voisey's Bay (mine effluent). This work has also shown that different bivalve species may be more suitable to different monitoring environments, and therefore site specific conditions/circumstances and species-specific advice is required. Certain applications are better suited to juveniles than adults such as growth. As well, certain kinds of information require either caging over shorter periods (metal accumulation), many months (GSI, LSI) or supplementing the caging program with sampling of wild bivalves (when longer caging is inappropriate). Overall though, a well designed program sampling caged and/or wild bivalves can often provide useful information on effluent effects in environments not amenable to carrying out a standard fish survey. One area requiring further research is direct side-by-side comparisons of the responses of caged bivalves, wild bivalves and wild finfish exposed to the same effluent. One such comparison of mummichogs and caged mussels in Pictou yielded somewhat different responses between species, though results were confounded by the two species having been exposed to greatly different concentrations of pulp mill effluent and for very different periods of time.

How to develop baseline information quickly for species that you don't know much about!

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The recent expansion of large pulp mills in Chile, Brazil and Argentina has increased attention on monitoring programs focused on environmental health. There are serious challenges to developing environmental monitoring programs in South America because of the lack of baseline information on the fish populations and communities of many regions. While study designs in Canada have progressed to using fish population-based data, the absence of basic lifehistory information in South America affects species selection, study design and data interpretation. The challenges can be very large. In Chile, all native species are IUCN-red-listed which prohibits or severely limits lethal sampling and affects sampling designs. In Uruguay, there may be more than 200 fish species present in rivers, and for most very little is known about life history. Furthermore, some sampling designs are using migratory fish species as sentinels, which increases the complexity of interpretation. However, work with North American species and preliminary work with some South American species suggests that there are a limited number of life history patterns which can be readily identified through appropriate preliminary sampling. Critical issues for evaluating species include such factors as mobility, longevity, reproductive strategies, growth rates and feeding preferences, which are largely unknown for most species. Some simple collections can provide a wealth of information, and this presentation will discuss data examination techniques that can provide insight into critical life history characteristics and provide advice on the development of evaluation criteria for fish species to be used for environmental monitoring. These aspects are relevant for study designs for EEM, specially with the expansion of study designs into species we don't know much about and the challenges with designing marine monitoring programs.

Keywords: absence of baseline data, new fish species, interpretation, study design

Solving the fish significant interactions puzzle.

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For EEM data collected over the last decade, effluent effects are most often fairly constant within a population for fish of different sizes or ages (this usually occurs for well over half of the comparisons being made). Put another way, within a given adult population, big fish and small fish most often respond similarly. In statistical terms, the exposure and reference area slopes in an Analysis of Covariance (ANCOVA) can be treated as being parallel for these comparisons. ANCOVA is used to factor out the influence of size (weight or length) or age on

the response variable being measured. For a sizeable proportion of the data, however, the exposure and reference area slopes are significantly different (termed a significant interaction). That is, the magnitude of effect changes with increasing size or age of the fish. This can be a quite important effect of effluent exposure, but interpretation can be difficult, particularly when making decisions on whether the effect is great enough to require further study or corrective action. We present a new methodology for analyzing and interpreting such effects based on maximum observed deviations in regression lines. We have now tested this new methodology using data from both the metal mining and pulp and paper EEM programs.

Keywords: fish size; fish age; statistical interactions; effect size

Considerations for site-specifically assessing the health of fish populations exposed to industrial stressors

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After 20 years of controversy related to interpretation of field studies in EEM, most criticisms can be related to five major issues: the adequacy of reference sites, presence of confounding factors, movement of species between sites, level of variability and statistical design, and ecologically relevant effect sizes. There are a number of adjustments that can be made during study design that can reduce the friction associated with these concerns. We have been working on approaches to identify reference sites, characterize variability and fish movement, increase sensitivity on monitoring, and address issues of ecological relevance. *A priori* definition of the purpose of the study, monitoring targets, critical effect sizes, and unacceptable responses are important steps in the design of an assessment program, and studies have to be optimized to reduce the noise and to provide adequate warning. The relevance of some of these issues for EEM study design and interpretation will be discussed. *Key words*: Study design, interpretation, effect sizes, fish impacts

Symposium Abstracts: Benthos Studies

Accommodating changing reference conditions in assessments of nearshore benthic conditions of the Great Lakes

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Benthic macroinvertebrate communities have been periodically sampled from sediments in over 200 undisturbed sites in nearshore areas of the Great Lakes since the early 1990s. The data obtained are used to describe reference conditions for impact assessments of benthic communities in contaminated sediment sites. Through the years, reference sites in the Great Lakes have been affected by invasive species, climate change, and other regional stressors, potentially resulting in unstable or moving benchmarks for reference conditions. The simplest approach for accommodating variable reference conditions in environmental assessments is to exclude older data determined to be nonrepresentative of current conditions. Alternatively, reference condition descriptions can be statistically adjusted using bioassessment models that include time or the nuisance regional stressor variable as predictors. Examples of these procedures will be shown for Environment Canada's program of benthic assessments for Areas of Concern in the Great Lakes. *Keywords*: benthos, impact assessment, variable reference conditions

Developing reference benchmarks: A comparison of ecoregion, clustering – discriminant analysis, and nearest-neighbour classifications

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The northern Ontario benthic invertebrate biomonitoring network was designed to assist the metal mining industry in using the reference condition approach (RCA) to meet Canadian Environmental Effects Monitoring requirements. The premise underlying the RCA is that homogenous biological communities are expected to occur in areas with distinct sets of habitat characteristics. Traditionally, groups of reference sites with similar biological communities are identified either theoretically (a priori) or empirically (a posteriori). In the a priori ecoregion approach, homogeneous groups of sites are delineated by considering causal and integrative factors (e.g., soils, vegetation). In the alternative a posteriori approach, reference sites with similar biological communities are clustered, and habitat characteristics that separate community types are identified using discriminant analysis. Recently, another a posteriori approach using nearest neighbours (based on habitat similarity) was proposed for situations where distinct groups do not exist. We investigated the consequences of using these three classification approaches in the evaluation of simulated and real stream invertebrate communities from four mining regions in northern Ontario. Our

results highlighted the pros and cons of the various methods for matching test sites with appropriate reference sites and showed that the three approaches resulted in comparable bioassessments of mining sites.

Keywords: metal mining, reference condition approach, reference site classification

Using Regression in Reference Condition Approach (RCA) Designs

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The "Reference Condition Approach" or RCA has been recommended as an alternate design for studies of benthic macroinvertebrates in environmental effects monitoring (EEM) programs (1) when there are no "local" reference locations that are good matches for the exposure area in terms of habitat, and (2) to better characterize the background range of variability of indices of composition. Typical RCA designs involve the collection of data from lots of locations, often 100s. Analysis of the reference data can take many forms, the most common of which involves the use of multivariate clustering of benthic community composition and discriminant models based on habitat characteristics of the reference community groups. The use of discriminant models, when there is an underlying "gradient" in conditions, can lead to erroneous conclusions of impact. Regression analysis is computationally easier than the discriminantmodel approach, and will not produce the same rate of erroneous conclusions of impact when the reference data are from a natural ecological gradient (often the case). Two case studies will be provided demonstrating the use of regression models used to test for effects in (1) a large river, and (2) a small headwater stream.

Keywords: RCA, EEM, benthos, study design

Teasing apart the cumulative effects of multiple stressors

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Cumulative effects assessment has a long, but imperfect history in environmental impact assessment. Why imperfect? Most cumulative effects assessments are descriptive rather than quantitative. Despite thousands of studies, statistical tools to tease apart cumulative effects are generally lacking. Recent interest in multiple stressors has underscored the need to quantitatively evaluate the cumulative effects of two-or-more stressors. We propose that appropriate statistical tools exist and are widely used in aquatic community ecology. With data collected in a collaborative study of mines and reference sites in northern Ontario, we use multivariate multiple regression, or redundancy analysis, to partition variation from three distinct sources. That is, variation in four benthic community metrics is partitioned among natural reference-site habitat features, mining effluent and tailings impacts, and uncontrolled urban land-use effects. This approach quantifies the individual and combined effects of different factors

that potentially affect the benthic community, providing a significant quantitative tool for cumulative effects assessment, and permitting an objective evaluation of the separate impacts of multiple stressors.

Keywords: multiple regression, redundancy analysis, mining impacts, urban effects

The potential of population-level indicators in invertebrate species for monitoring pulp mill impacts.

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Typical monitoring programs often focus on fish populations and benthic invertebrate communities and may use alternative methods (i.e. mesocosms) when either are impossible or impractical. We examined the potential use of stonefly (Perlidae; Plecoptera) populations as an alternative method for examining nutrient enrichment downstream of sewage and pulp mill outfalls. We used life history characteristics including developmental stage (wing pad length: head capsule width), growth, fecundity, condition, thorax length, and dry weight of several stonefly genera (Acroneuria, Paragnetina, and Agnetina) and compared responses from upstream to downstream of both sewage inputs and a pulp mill outfall. We found that the stoneflies condition, developmental stage, and size responded at both family and genus levels downstream of the sewage. but responses to the pulp mill effluent at this site were absent. Exposure to sewage effluents caused increased growth, energy storage, and relative abundance of slimy sculpin, indicating that fish and invertebrates respond similarly to these discharges. Our findings suggest that invertebrate populations can be used as an alternative method in field studies to either replace or augment existing monitoring techniques.

Keywords: stonefly, population-level, alternative, absence of fish

Reference benchmarks for benthic algae, macroinvertebrates, and fish and their sensitivities to mining developments in a World Heritage Site, the South Nahanni River Watershed, NWT, Canada.

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Canada's north is changing at an unprecedented rate as a result of increased resource development in sectors such as oil and gas and mining as well as changes due to global warming. The goal of this study was to contribute to the understanding of variability in aquatic reference conditions of the north and identify species or communities that are feasible and sensitive indicators of human disturbance. Sediment type and chemistry, water chemistry and periphyton, benthic macroinvertebrate, and fish communities were characterized

at 20 reference sites and at near-field and far-field sites downstream two metal mines. Comparisons between the upstream, near-field, and far-field study design versus a reference condition approach showed that for detecting biological effects, setting an appropriate ecological threshold was of great consequence than the type of study design used. Biological indicators most sensitive to environmental change included benthic chlorophyll and periphyton community structure and were consistent with mild eutrophication effects. Using a regional, multi-trophic level approach to monitoring the effects of human disturbance improves inferential strength and broadens applicability. *Keywords:* metal mining, benthic communities, study design, effect size

Approaches for setting sediment and nutrient standards for agricultural streams.

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We report on results from the National Agri-Environmental Standards Initiative (NAESI) for "Ideal Performance Standards" (IPS) of sediments and nutrients. These IPS values for Canada's agricultural regions Canada specify thresholds predicted to be protective of environmental quality. To produce these standards, we applied 5 approaches, used for the development of water quality standards in Australia and New Zealand, Canada and the US, to historical and contemporary data sets from BC, AB, MB, ON, QC, NB and PEI. Agricultural land disturbance was linked to increased sediments and nutrients in streams across Canada, with these parameters exhibiting a positive increase with percent total agriculture within most catchments. The primary gaps in the science of sediment and nutrient standards development are related to methods development, monitoring capacity and Beneficial Management Practice (BMP) scenario modeling. We strongly recommend: the use of biological endpoints to establish IPS values; a comparison and contrast of relationships among sediment, nutrient and other NAESI standards; and the further development of approaches that integrate modeling with field assessments.

Keywords: agriculture, nutrient, sediment, water quality guideline.

Symposium Abstracts: Investigation of Cause Research

Tools for determining permissible metal loadings to the aquatic environment – a study of rivers in northwestern Quebec that receive mine effluents

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The main objective of this project was to test an ecotoxicological approach for assessing the effects of metals on the aquatic environment. The geographical focus of the project was the Canadian Shield, site of most of the past and current mining activities in Canada. The approach comprised several types of measurements along metal exposure gradients in rivers, up- and down-stream from mine effluent discharges: geochemical and limnological analyses; laboratory toxicity tests; and metal bioaccumulation, metallothionein induction and toxicity responses in indigenous biosentinel animals (mayfly larvae, Hexagenia limbata; floater molluscs, Pyganodon grandis; northern pike, Esox lucius; walleye, Stizostedion vitreum). The specific objectives of this project were: (a) to develop biochemical tools capable of detecting metal-induced deleterious effects in representative indigenous aguatic animals; (b) to link metal-induced effects in indigenous aquatic animals to estimates of metal exposure via the ambient water and / or sediment: (c) to link metal-induced effects in indigenous aquatic animals to the results of toxicity tests carried out concurrently in the laboratory on test organisms exposed to the ambient water and / or sediment. Results and recommendations will be presented. Financial support: COREM and NSERC Cooperative Research and Development Grant

Key words: mine effluents; biomonitoring; toxicity tests; metallothionein

Best Management Practices (BMP) Guide for Nutrient Management in Biological Treatment of Pulp and Paper Mill Effluents

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The December 2005 Smart Regulations report ¹ contained eight recommendations for government and industry to make the EEM program more effective and efficient. The Forest Products Association of Canada has documented its full support of all the reports' recommendations and as a demonstration of the industry commitment FPAC has recently released the BMP Guide to Nutrient Management in treating pulp and paper mill effluents. An overview of this guide is provided indicating its content and purpose. The guide is designed to be user friendly and to help effluent treatment specialists to develop their own action plan for pulp and paper mills exhibiting eutrophication.

Environment Canada is fully supportive of the intention of the guide and participated in the draft review process. EEM polices within Environment Canada are being developed to encourage mills that exhibit eutrophication downstream of their mills, to use the BMP guide and adopt a mill specific Nutrient Management Action Plan as part of their EEM Investigation of Cause / Investigation of Solution.

1. Improving the effectiveness of Pulp and Paper Environmental Effects Monitoring: a Smart Regulations Opportunity December 2005

Key Words: Best Management Practices; Nutrients; eutrophication; EEM

Eutrophication effect of pulp mill effluents: a possible pathway for investigation of solution studies

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Under the Pulp and Paper Effluent Regulations (PPER), mills have been going through four cycles of EEM since 1992. Although a very high rate of effluent compliance among the regulated community, a number of facilities have generated organic enrichment in their receiving aquatic environment. Moreover, some of those mill effluents have shown evidence of pronounced eutrophication during more than one cycle. This presentation will show the example of one TMP mill in Quebec. Cycle 2 and Cycle 3 benthos surveys have revealed higher invertebrate density in the exposure area compared to the reference area. The difference between the two areas was higher than the critical effect size i.e. >2 standard deviation from the reference mean. During Cycle 4, the mill recorded high phosphorous concentration in their effluent and were directed toward a phosphorous reduction study by the provincial government through their industrial waste reduction program. The mill proposed a research project for the optimization of phosphoric acid addition in the effluent treatment system. This presentation shows how a private research project could be incorporated in the EEM program through the IOC-IOS phase in trying to minimize the pronounced eutrophication effect of pulp and paper mills.

Keywords: Pulp and paper, phosphorus, pronounced eutrophication, benthos

Update on the national investigation of cause project on reduced gonad sizes in fish

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The first three cycles of Environmental Effects Monitoring (EEM) in the pulp and paper sector show two consistent national response patterns in fish; responses

related to nutrient enrichment, and metabolic disruption, typified by larger liver size, smaller gonad size and increased body weight. Effective and economical mitigating solutions for effects of mill effluents on fish reproduction have yet to be identified. In 2005 a team comprised of researchers from industry, government and academia was assembled and a five-activity plan to evaluate in-mill and endof-pipe treatment options for removing substances affecting fish reproductive capacity from mill effluents was formulated. The five activity plan is comprised of a review of the literature and EEM database, selection of appropriate diagnostic tools, investigation of cause, investigation of solution and field validation. Research got underway in 2006 determining which laboratory tests will provide the most appropriate measures of gonad size reductions seen in wild fish. The results of the first mill effluent studied show wild fish results can be reproduced with laboratory lifecycle experiments and that egg production in shorter term tests show promise for investigating causes and solutions.

Keywords: pulp mill effluent, fish reproduction, investigation of cause, investigation of solution, endocrine disruption

Investigation of solution (IOS) studies at FPInnovations Paprican

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The national assessment of the EEM fish data has indicated that the reproductive capacity of fish living downstream from mills may be jeopardized. This has led to the need for Investigation of Cause (IOC) and Investigation of Solution (IOS) studies. Ideally, IOS studies are undertaken once the causative agent(s) are known. As IOC studies may take some time, IOS studies were initiated without knowing the identity of the causative agent(s). The quality of the effluents was assessed by laboratory tests using the fathead minnow. The main endpoint of these tests was egg production. One study monitored the effect of changes in mill operating conditions (e.g., pulping, bleaching and treatment technologies) on effluent quality. A second study focused solely on the role of conventional effluent biotreatment. These studies showed that conventional effluent biotreatment and changes in mill operating conditions can be beneficial. Nonetheless, effluent-related effects persist at some mills. Hence, the third study investigated the outcome of i) refinements to effluent biotreatment (e.g., change in sludge age) in a pilot treatment plant as well as ii) ozone treatment. Neither eliminated the effects on fish reproduction indicating the refractory nature of the causative agent(s). Overall, while these IOS studies provided leads for reducing effects on fish reproduction but further efforts are needed to identify strategies that can achieve complete elimination of these effects. Keywords: process, treatment, reproduction, fish

Determining biologically active components in kraft mill chemical recovery condensates

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Previously, the condensate streams from the chemical recovery process in a kraft pulp and paper mill have been identified as depressing sex steroids in mummichog (Fundulus heteroclitus). Using solid phase extraction (SPE), a fractionation regime using two SPE cartridges in series was developed, with one particular eluted fraction (SPE2) being identified as containing the majority of biological activity. This fraction was then further separated using preparative scale reverse phase-HPLC. Unfortunately, exposure of HPLC separated fractions to mummichog showed little to no biological effect. Recent work has been to firstly ascertain why biological activity was lost in sample preparation and fractionation using preparative HPLC. It was determined that active compounds were being lost during solvent evaporation (water and acetonitrile) of samples in preparation for fish exposure studies. Experiments showed that compounds previously identified in the condensates were volatilised before all water was evaporated. Fractionation of this biologically active SPE2 fraction was tested using non-aqueous solvents of decreasing polarity to elute straight from the SPE2 cartridge. This produced similar fractions due to a washing effect, rather than separation based on polarity. Current work has focused on gaining a better understanding regarding how the condensates, and model compounds, behave under extraction conditions with the aim of producing chemically distinct fractions for further in-vivo and in-vitro bioassays.

Keywords: mummichog, condensate, endocrine disruptor, solid phase extraction

The effect of thermomechanical pulp mill effluent on reproduction in mummichog (*Fundulus heteroclitus*): an investigation of cause

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A study in 1997 exposing mummichog (*Fundulus heteroclitus*), an endemic fish species on the east coast of Canada, to final treated effluent of Irving Paper, Ltd. of Saint John, NB (a thermomechanical pulp mill; IP/TMP) showed a significant reduction in gonad size relative to body weight. Since 2007, we have been using an investigation of cause (IOC) approach to study the effect of IP/TMP effluent on fish reproduction. We have exposed mummichog to final effluent and different in-mill waste streams to: i) confirm the effect of the final effluent observed in the previous study, and ii) identify in-mill wastestream sources causing reproductive effects. Relative to reference, no significant differences in gonad size, plasma reproductive steroid levels nor egg production were observed in fish exposed to final treated effluent in three experiments spanning effluent collections over 12 months. No clear pattern of response was found when exposing mummichog to

different in-mill waste streams. These data indicate low potential for final effluent effects on fish reproduction. We therefore conclude that it is not appropriate to continue doing an IOC at this mill. Further studies are addressing the robustness of the mummichog bioassay for applications to other complex mixtures. *Key words*: Thermomechinal pulp mill (TMP-mill), endocrine disruption, investigation of cause, reproduction, *Fundulus heteroclitus*

Symposium Abstracts: Sublethal Toxicity Testing

Uses of Sublethal Toxicity Data in Pulp and Paper and Metal Mining EEM

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Since 1992 and 2002, pulp and paper mills and metal mining operations in Canada have been required to conduct sublethal toxicity (SLT) testing on samples of their primary effluent discharge under the EEM component of the Pulp and Paper Effluent and Metal Mining Effluent Regulations, respectively. Mills and mines must complete SLT testing using a battery of 3 or 4 tests at a frequency of once or twice per year. This presentation will discuss the various uses of EEM sublethal toxicity data and provide examples of data application at the national, regional or site-specific level that reflect these uses. *Key Words*: Sublethal, Toxicity, Uses, Data Application

Comparisons of the Ontario Metal Mining EEM benthic invertebrate community data with sublethal toxicity data using the Lab-To-Field rating system

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The Metal Mining Effluent Regulations (MMER) require metal mines to conduct benthic invertebrate surveys in effluent receiving waters and sublethal toxicity tests on final effluent. Metal mines are required to report four standard endpoints for the benthic survey: total benthic invertebrate density, taxa richness, Simpson's Evenness and the Bray-Curtis Index. Under the MMER, sublethal toxicity testing of final effluent is required using standard methods for four species: *Pimephales promelas, Ceriodaphnia dubia, Lemna minor* and *Pseudokirchneriella subcapitata*. The relationships between the benthic invertebrate community survey results and effluent sublethal toxicity data for twenty-two Ontario metal mines are examined using a qualitative lab-to-field rating system.

Keywords: Lab-To-Field, Sublethal Toxicity, Benthic Invertebrates

National, Regional and Mine-Type Trends of Sublethal Toxicity Testing in the Metal Mining EEM Program

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One component of the EEM program is the conductance of sublethal toxicity (SLT) testing on the principal effluent discharge. During the initial three year monitoring phase (2003-2005), mines completed SLT testing using a battery of tests on a fish, invertebrate, plant, and algae species at a frequency of twice per year. An analysis of 1648 toxicity tests was completed by staff of Environment

Canada's Biological Methods Division with input from all the regional EEM offices. Trends based on a national, regional and mine type basis will be presented for mines discharging to freshwater environments. At the time of this analysis there was only one mine in Canada who discharged to a marine environment so no trend analysis under the aforementioned categories could be performed. Test results were summarized based on specific definitions of sensitivity (i.e., number of times a test had the lowest IC25/EC25 in a test battery) and responsiveness (i.e., the test with the lowest number of no effect results, e.g., IC25>100). These quantitative analyses avoided arbitrary conclusions on what would be considered as "significant toxicity" (e.g., IC25<20% effluent is considered 'bad' for the environment), as these sorts of judgments should only be made with more information on a site-specific basis. *Key words*: metal mining, sublethal, toxicity, effluent quality

New Developments and Improvements in SLT Methods

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Improved scientific knowledge and the demand for new biological endpoints are key driving forces behind method development/improvement for current SLT methods. Using four examples (below), we'll describe how these forces shaped recently published and upcoming changes to biological test methods. Lemna, Pseudokirchneriella and Ceriodaphnia (recently published second editions): New statistical analysis; key methodology changes Fathead minnow (upcoming): Content of the amendment sheets (to be released this spring); long-term research goals centred on additional Investigation of Cause and lifecycle/reproduction test options Echinoid fertilization (upcoming): Methodology changes to further standardize the generic test; solid phase test option as a reference method Hyalella survival and growth (upcoming): Consideration of different feeding regimes and water-to-sediment ratios; water-only test option; possibilities for reproduction endpoint Collaboration is an essential component of method development and improvement but, depending on the circumstances, a variety of paths can be taken, including: expert input and focused research from government research scientists; employing surveys with experienced EEM private-sector labs; conducting literature review of existing international test methods; and round-robin testing between laboratories. Keywords: standardization, fish, invertebrates, toxicity testing

Relating sublethal toxicity to effluent chemistry using characterization data from the metal mining EEM program

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Sublethal toxicity and chemistry data are collected at operating metal mines across Canada to monitor effluent quality as part of the Metal Mining Effluent Regulations – Environmental Effects Monitoring Program (MMER-EEM). To identify potential toxicants, the chemistry data collected for the first cycle of the MMER-EEM were compared to available toxicity values, and correlation analyses were conducted on the chemistry and toxicity data. Factors that could limit the use of the data to identify specific toxicants were also studied, such as the use of two test methods for algae growth inhibition, the use of different clones for Lemna minor and the use of total metal concentrations. The analysis suggested elevated hardness and some metals as potential toxicants. The sensitivity of algal growth to metals is impacted by the reduction of the EDTA concentrations used in the test method. The two Lemna minor clones used in the 2007 test method had similar sensitivities to hardness and cadmium. In addition, the analysis indicated that for metals, the actual toxicity is much less than predicted based on total copper concentration, for example for copper and fathead minnow growth. Overall, the data review suggests that elevated hardness is a potential toxicant of general concern in mine effluents.

Keywords: mining effluent, effluent chemistry, effluent quality and sublethal toxicity

Utility of toxicity tests with *Hyalella azteca* in differentiating between current effluent quality and historical sediment contamination

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The objective of the study was to determine whether toxicity tests can be used to differentiate between current effluent quality and historical sediment contamination. The study involved a small headwater lake that currently receives industrial effluent, but has also received deposits from industrial activities carried out over the past 50+ years, and a reference lake, which has not been impacted by industrial, domestic or recreational activities. Both lakes have been subject to fish surveys and invertebrate community surveys. Additionally, effluent quality has been characterized for its chemical composition and aquatic toxicology. Results of benthic surveys provided evidence of impacts using the four benthic invertebrate community descriptors recommended by EC. However, this approach was unable to distinguish between historical effects and current effluent quality in these studies. Laboratory testing was conducted using the

freshwater amphipod, *Hyalella azteca*. Testing involved aqueous exposure of *H. azteca* to receiving water alone and to receiving water in presence of sediment. Receiving water samples were collected ~ 1M above the sediments. Receiving water tests were conducted under renewed static conditions (i.e., three times daily), whereas sediment tests were conducted under static conditions (i.e., no renewal of the overlying water). Controls for aqueous only tests consisted of dilution water adjusted to match the hardness of the receiving waters. Sediment tests were conducted using hardness adjusted dilution water as well as receiving water as the overlying water. Effects on survival and growth of *H. azteca* were determined after 14 days.

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