St. Lawrence TECHNOLOGIES

INDUSTRIAL WASTEWATER

EVALUATION OF MICRO-SCALE BIOASSAYS AS TOXICITY MONITORING AND IDENTIFICATION TOOLS FOR PULP AND PAPER EFFLUENT

ABSTRACT

The use of four micro-scale assays for toxicity monitoring and identification was investigated to evaluate their performance and usefulness compared to conventional assays specified in the Canadian Pulp and Paper Regulations (i.e. *Daphnia magna* and rainbow trout tests). The micro-scale assays (Daphtoxkit F™, Daphnia I.Q.™, Thamnotoxkit F™ and Microtox®) were first evaluated and compared to the standard *Daphnia magna* assay using effluent samples from five different mill operations from the J. Ford & Co. Ltd. mill. The Thamnotoxkit F™ and the Daphtoxkit F™ were retained to pursue the evaluation and one type of effluent was selected to perform a Toxicity Identification Evaluation (TIE). The two micro-scale assays provided similar results to those of the *Daphnia magna* assay, indicating that they could prove useful for screening and monitoring toxicity of chemical products used in the mill.

HIGHLIGHTS

- **Technology**
  - Assays that can be used in mills for screening and monitoring purposes
  - Assays that can be used in a Toxicity Identification Evaluation (TIE) program.
- **Ecotoxicology**
  - Expansion of knowledge concerning toxicity of chemical products to aquatic organisms
  - Identification of micro-scale assays representative of conventional test responses.
- **Cost**
  - No costs associated with the culturing of organisms
  - Reduction in the time required to assess toxicity of mill effluents and chemical products.

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PROJECT OBJECTIVES

The goals of the project were as follows:

1. **Identify the micro-scale assays most representative of conventional *Daphnia magna* test responses that can be routinely used by mills to screen and monitor the toxicity of effluents and chemical products; and,**
2. **Reduce the costs and the time needed to perform Toxicity Identification Evaluation (TIE) tests by using micro-scale assays representative of conventional test responses to identify the toxic agents present in mill effluents.**

Phases:

I. Data collection: acquisition and evaluation of mill data and evaluation of mill operations.

II. Evaluation and selection of micro-scale assays: assays performed on five different types of effluents, data analysis, micro-scale assays and effluent selection.

III. Toxicity Identification Evaluation: characterization of the toxicity of the selected effluent; identification and confirmation of the toxic agent.

The project included a training program to transfer the technology to the technicians of the J. Ford & Co. Ltd. mill.

BACKGROUND

Many chemicals present in pulp and paper effluents are known to have toxicological effects on aquatic organisms. The Toxicity Reduction Evaluation (TRE) and Toxicity Identification Evaluation (TIE) procedures were developed to identify and reduce chemicals of concern in industrial effluents.

Testing methods for acute toxicity identification evaluation generally involve the use of *D. magna* and fathead minnow larvae. We investigated the use of four micro-scale tests as surrogates for *D. magna* and fathead minnow larvae in a TIE program and to monitor toxicity in the effluent of the J. Ford & Co. Ltd. mill in Portneuf, Québec.

METHODOLOGY

The four micro-scale assays and the *D. magna* assay were performed on effluent samples from five different mill operations. This mill produces specialty papers, corrugated paper and felt board, with no bleaching or de-inking operations.

The two micro-scale assays most representative of the toxicity for *D. magna*, and the most toxic effluent, were selected to perform the TIE.

A series of characterization tests was performed on the effluent samples to identify the physical and chemical properties of the toxic agents. Each treated sub-sample was analysed using two conventional assays (*D. magna* and fathead minnow assays) and the two micro-scale assays previously selected. A sub-sample of the effluent was separated by distillation into two fractions and chemical analyses and toxicity tests were performed to evaluate their toxicity.

These same tests were also done on the product used by the mill that contained the chemical identified as the toxic agent (Table 1).

TABLE 1.
DESCRIPTION OF THE METHODOLOGY

Phase I **Data collection**	**Phase II** **Evaluation and selection of micro-scale assays**	**Phase III** **Toxicity Identification Evaluation (TIE)**
• Acquisition and evaluation of mill data (physico-chemistry, toxicity of the effluents, chemistry of the products, frequency and period of use). • Evaluation of mill operations (mill visit). ➔ Identification of the mill operation that produces the most toxic effluents and for which biological assays will be done.	• Assays performed on mill effluents: *Daphnia magna*, Thamnotoxkit F™, Microtox®, Daphtoxkit F™ and Daphnia IQ™. • Data analysis and selection of micro-scale assays: correlation between *Daphnia magna* assays and the four micro-scale assays. ➔ Selection of the micro-scale assay representative of toxicity for *Daphnia magna*, and selection of the mill operation found to be the most toxic.	• Characterization of the toxicity of the selected effluent with *Daphnia magna*, rainbow trout, fathead minnow and the micro-scale assays selected in Phase II. • Identification of the toxic agent. • Confirmation of the toxic agent. ➔ Identification of the toxic agent.

RESULTS

Phases I and II: Data Collection, Evaluation and Selection of Micro-scale Assays

The best correlation between the micro-scale assays and the conventional assay was obtained with Thamnotoxkit F™, followed by Daphtoxkit F™ and then Daphnia IQ™. The first two tests were therefore selected as being the most representative micro-scale assays of the toxicity for *Daphnia magna* (Table 2).

Phase III: Toxicity Identification Evaluation

Toxicity Characterization

Based on the results obtained with the conventional assays and with the micro-scale assays performed on the treated samples, organic substances appear to be responsible for the observed toxicity of the effluent produced by machine C. The only treatment that reduced the toxicity of the effluent was the activated carbon column.

Identification of the Toxic Agent

The two fractions obtained by distillation were analysed using gas chromatographic/mass spectrometric analysis to identify the chemicals responsible for the toxicity. The toxicity of the two fractions was also assessed using both the conventional and the micro-scale assays.

The results of the micro-scale assays were similar to those obtained with the conventional assay, but were slightly more sensitive than the conventional assays, particularly Daphtoxkit F™ (Table 2). Fraction 2 seemed to be the most toxic fraction, although the most potentially toxic substance was found in greater concentration in fraction 1 (Table 2). Although formaldehyde initially appeared to be responsible for the toxicity, analysis of the fractions did not support this hypothesis. Rather, either the formaldehyde is not the cause of the toxicity or it is not being accurately measured by the chemical analyses.

Confirmation Phase

Two products used commercially in the process of machine C contain formaldehyde. Conventional assays and micro-scale assays were performed on formaldehyde and on one of the two secret products. The formaldehyde appeared non-toxic, but the product caused toxicity to all the organisms. This product may be the toxic agent in the machine C effluent.

TABLE 2.
TOXICITY RESULTS FOR CONVENTIONAL ASSAYS AND MICRO-SCALE ASSAYS PERFORMED ON THE FIVE EFFLUENTS (PHASE II: EVALUATION OF MICRO-SCALE ASSAYS), ON THE WHOLE EFFLUENT AND ON THE TWO FRACTIONS (PHASE III: TOXICITY IDENTIFICATION EVALUATION)

Effluent	*Daphnia magna* (LC_{50} - 48 h %, v/v)	Fathead minnow (LC_{50} - 96 h %, v/v)	Thamnotoxkit F™ (LC_{50} - 24 h %, v/v)	Daphtoxkit F™ (LC_{50} - 48 h %, v/v)	Daphnia IQ™ (LC_{50} - 1 h 15 min %, v/v)	Microtox® (LC_{50} - 15 min %, v/v)
Phase II: Evaluation of micro-scale assays						
Final effluent – Regular operation	86.07	–	> 100	23.17	27.5	> 90
Final effluent – Irregular operation	> 100	–	> 100	> 100	22.1	> 90
Machine A	70.71	–	> 100	76.25	44.63	44.37
Machine B	64.77	–	70.71	17.95	24.4	> 90
Machine C	0.52	–	1.43	0.68	4.2	> 90
Phase III: Toxicity Identification Evaluation						
Whole effluent	12	15	7.8	2	–	–
Fraction 1 (8.3 mg/L formaldehyde)	35	71	29	21	–	–
Fraction 2 (3.9 mg/L formaldehyde)	19	20	12	5	–	–

LC_{50}: lethal concentration for 50% of organisms. –: assays not performed.

POTENTIAL AND LIMITATIONS

Potential

This project demonstrated that the Thamnotoxkit F™ and Daphtoxkit F™ micro-scale assays provide results that are representative of the results obtained with the conventional assay on *Daphnia magna*. Due to the simplicity, speed of execution and representativeness of conventional assays, these micro-scale assays could be used by mill personnel to assess the toxicity of various effluents from mill operations.

Limitations

Two constraints were identified regarding the use of the micro-scale assays in a TIE program. First, the pH of the samples was impossible to adjust during the test, due to the small sample volume. The treatments applied to a sample can change the buffer capacity of the effluent, thereby making it necessary to adjust the pH during the test. Second, the presence of suspended matter seemed to hinder the movement of the test organisms. Whether this could be a cause of mortality remains inconclusive, however.

INFORMATION

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St. Lawrence TECHNOLOGIES

St. Lawrence Technologies data sheets are intended for all companies, industries, organizations and individuals interested in new environmental technologies. They are produced by the Eco-Technology Innovation Section, Environment Canada, as part of St. Lawrence Vision 2000. They serve to disseminate the results of technology development and demonstration projects conducted in the following five sectors: industrial wastewater; contaminated soil; hazardous wastes; contaminated sediment and innovative tool.

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