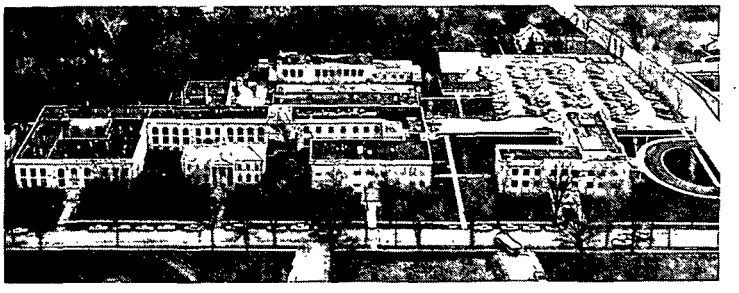

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EFFLUENT BIOMONITORING IN WISCONSIN

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Effluent Biomonitoring in Wisconsin

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EFFLUENT BIOMONITORING IN WISCONSIN

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ABSTRACT

A biomonitoring requirement in the form of short-term chronic toxicity assays using the fathead minnow (Pimephales promelas) and a water flea (Ceriodaphnia dubia) accompanied the 1986 discharge permit renewals for the pulp and paper industry in Wisconsin. Acute toxicity values and the NOEC (no observed effect concentration) for water flea survival and reproduction and for fish survival and growth were estimated under this program.

Test results are intended to indicate specific effluent quality and potential impact upon receiving waters. Acute toxicity values (based on survival in 100% effluent) ranged from 0 to 100% for both Ceriodaphnia and fathead minnows. Effluent NOECs using Ceriodaphnia reproduction ranged from 1 to 100%. Effluent NOECs using Pimephales growth ranged from 0 to 100%.

Preliminary indications from the Wisconsin Department of Natural Resources are that those mills showing acute toxicity (mortality) problems at end of pipe or chronic toxicity at the edge of the mixing zone will require additional problem definition. In addition, at least quarterly effluent bioassays are being considered as a permit special condition.

INTRODUCTION

In September, 1985, the Wisconsin Department of Natural Resources (WDNR) announced a biomonitoring requirement as part of the reapplication process for pulp and paper wastewater discharge permits (1). This requirement called for effluent bioassays employing the United States Environmental Protection Agency's (EPA) Ceriodaphnia survival and reproduction test and the larval fathead minnow survival and growth test (2).

WDNR Biomonitoring Program

The details of the WDNR testing requirement and the specific language used for its implementation (frequency and criteria) changed dramatically from the initial reapplication exercise (3) to present permit drafting (4). What began as a gathering of information on paper industry effluents using new biomonitoring techniques is now appearing as permit conditions/limitations. The required frequency of testing progressed from a one-time test to quarterly. The criteria for data analysis and test interpretation (5) have been modified from the original EPA chronic toxicity end points (NOEC = no observed effect concentration; LOEC = lowest observed effect concentration; and ChV = the geometric mean between the NOEC and LOEC) to the following:

1. Fifty percent or less survival of Ceriodaphnia in 100% effluent at 48 hours, or
2. Fifty percent or less survival of larval fathead minnows in 100% effluent at 96 hours, or
3. A NOEC less than the IWC (instream waste concentration).

Other pertinent language to the present program includes retesting within three weeks of a bioassay where toxicity is demonstrated and the initiation of a toxicity reduction evaluation program when directed by the WDNR.

Wisconsin Pulp/Paper Repermitting Bioassays

The Aquatic Biology Group of The Institute of Paper Chemistry (IPC) conducted 27 sets of Ceriodaphnia and larval fish bioassays between December, 1985 and August, 1986 under the Wisconsin paper industry reapplication process. The major objectives of these tests were:

1. Estimate the chronic toxicity of effluent to Ceriodaphnia and larval fathead minnows according to EPA methods, and
2. Determine the acute toxicity of effluent to Ceriodaphnia and larval fathead minnows as stipulated by the WDNR.

METHODS

All methods, including culturing, quality assurance, sample handling, test procedures, and data analyses were in accordance with the EPA and/or WDNR bioassay procedures (2-4,6-11).

Screening Test

Approximately three weeks prior to the definitive bioassay, a receiving water sample was collected upstream from the test mill outfall and prescreened at IPC for animal acceptance as control/dilution water to be used in the bioassay.

Sample Collection and Preparation

The mill effluents used in these tests were initial 24-hour composite samples. The control/dilution waters were initial grab samples collected above the outfalls.

Laboratory Analysis

Summaries of test conditions for each bioassay are presented in Tables 1 and 2.

Data Analysis

Bioassay Validity

The acceptable bioassay criterion was 80% survival in the controls or in any test solution for the Ceriodaphnia test and larval fish test, respectively.

Bioassay Data

The statistical analyses used were: Fisher's Exact Test on Ceriodaphnia survival and Dunnett's Procedure and/or Steel's Many-One Rank Test for Cerio-

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daphnia reproduction and fathead minnow survival and growth data.

Table 1. Summary of test conditions for Ceriodaphnia bioassay.

1.	Test organism:	Ceriodaphnia dubia (Crustacea: Cladocera)
2.	Test type:	Static renewal
3.	Age of test organisms:	Less than 24 hours, all released within a four-hour period, same generation from even-aged parents
4.	Test chamber size:	1 oz (plastic cup)
5.	Test solution volume:	15 mL
6.	Renewal of test solutions:	Daily
7.	Number of replicate chambers per treatment:	10
8.	Number of test organisms per chamber:	1
9.	Control/dilution water:	Receiving water
10.	Effluent test concentrations:	5(1,3,10,30, and 100%)
11.	Temperature:	25 ± 1°C
12.	Feeding regime:	0.1 mL culture food/15 mL/day
13.	Aeration:	Initial, when necessary
14.	pH adjustment:	When necessary (differences greater than 0.5 unit), with HCl or NaOH
15.	Test duration:	7 days
16.	Effects measured:	Survival and reproduction

The end points determined for these bioassays included the NOEC and the acute toxicity value as described above.

For the purpose of interpretation, the instream wastewater concentration (IWC) of an effluent in a receiving water was determined by the WDNR (12) or calculated accordingly as follows.

$$\% \text{ IWC} = \frac{\text{Mean effluent discharge}}{0.25 \times 7Q10} \times 100$$

where: 7Q10 = ten year lowest river flow seven-day average.

Table 2. Summary of test conditions for fish bioassay.

1.	Test organism:	Fathead minnow (Pimephales promelas)
2.	Test type:	Static renewal
3.	Age of test organisms:	Larval, less than 24 hours
4.	Test chamber size:	1 L
5.	Test solution volume:	500 mL
6.	Renewal of test solutions:	Daily
7.	Number of replicate chambers per treatment:	4
8.	Number of test organisms per chamber:	10
9.	Control/dilution water:	Receiving water
10.	Effluent test concentrations:	5 (1,3,10,30, and 100%)
11.	Temperature:	25 ± 1°C
12.	Feeding regime:	0.1 mL newly hatched brine shrimp nauplii three times daily, at four-hour intervals
13.	Aeration:	Initial or continuous when necessary
14.	Cleaning:	Siphon daily
15.	Test duration:	7 days
16.	Effects measured:	Survival and growth (biomass)

Passing Test Criteria

The passing test criteria for the bioassays, as defined by the WDNR, are:

1. Greater than 50% survival of the test organisms in 100% effluent, at their respective exposure times, and
2. The NOEC greater than the IWC.

RESULTS

Screening Test

Only one case (with Ceriodaphnia) in 52 river water screens experienced a problem which led to the substitution of IPC culture media, for receiving water. However, a later concurrent control test with the definitive bioassay proved successful using that receiving water.

Bioassay Validity

All 54 bioassays met the EPA's acceptable test survival criterion.

Ceriodaphnia Bioassay

A summary of Ceriodaphnia survival from the bioassays is presented in Table 3. The acute toxicity value (survival in 100% effluent at 48 hours) ranged from 0 to 100%. The chronic (seven-day) survival NOEC ranged from 1 to 100% effluent. The NOEC frequencies are as follows: 1 NOEC of 1%, 1 NOEC of 3%, 3 NOEC's of 10%, 5 NOEC's of 30%, and 17 NOEC's of 100%.

Ceriodaphnia reproduction data are summarized in Table 4. The reproduction NOEC ranged from 1 to 100% effluent. The distribution of the NOEC was: 3 NOECs of 1%, 2 NOEC's of 3%, 10 NOEC's of 10%, 8 NOEC's of 30%, and 4 NOEC's of 100%.

Fathead Minnow Bioassay

Table 5 lists the larval fish survival information. The acute toxicity value (survival in 100% effluent at 96 hours) ranged from 0 to 100%. The seven-day survival NOEC ranged from 0 to 100% effluent. The NOEC frequencies were: 1 NOEC of 0%, 1 NOEC of 3%, 6 NOEC's of 10%, 5 NOEC's of 30%, and 14 NOEC's of 100%.

A summary of fish growth data is presented in Table 6. The NOEC ranged from 0 to 100% effluent. The NOEC distribution follows: 1 NOEC of 0%, 4 NOEC's of 3%, 9 NOEC's of 10%, 10 NOEC's of 30%, and 3 NOEC's of 100%.

DISCUSSION

Bioassay Results

Examination of Ceriodaphnia bioassay results in Tables 3 and 4 indicates that the reproduction parameter was more sensitive than 7-day survival. Only four effluents demonstrated no effect upon reproduction, while seventeen effluents had no effect upon survival.

Likewise, fish growth was affected more by effluent than was 7-day survival. From Tables 5 and 6 one can see that only three effluents had no effect upon growth, whereas fourteen effluents had no effect upon survival.

Based upon these 54 bioassays, both test organisms appear to be equally sensitive to effluent effects despite their different trophic level and the parameters measured (Fig. 1). However, variability between the effects on the test species within a single effluent was often present.

Using the more sensitive parameters (Ceriodaphnia reproduction and fish growth data), approximately 54% of the Wisconsin mills tested had an effect below a 30% effluent concentration, with 33% of the mills showing an effect between 30 and 100% effluent. Approximately 13% of the mills' effluent had no effect upon the test organisms.

Table 3. Summary of Ceriodaphnia bioassay survival data and results.

Effluent	48-hour percent survival (in 100% effluent)	NOEC (%) (based on 7-day survival)
1	100	100
2	100	10
3	100	100
4	0	10
5	40	30
6	100	100
7	100	100
8	100	100
9	100	100
10	0	10
11	100	100
12	60	30
13	100	100
14	0	3
15	0	1
16	90	100
17	100	100
18	100	100
19	0	30
20	20	30
21	0	100
22	90	100
23	100	100
24	100	100
25	100	100
26	90	100
27	100	30

Table 4. Summary of Ceriodaphnia bioassay reproduction results.

Effluent	NOEC (%)
1	10
2	30
3	30
4	10
5	10
6	30
7	30
8	100
9	3
10	1
11	100
12	10
13	30
14	1
15	1
16	30
17	10
18	30
19	10
20	10
21	3
22	10
23	100
24	10
25	100
26	30
27	10

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Table 5. Summary of larval fathead minnow bioassay survival data and results.

Effluent	96-hour percent survival (in 100% effluent)	NOEC (%) (based on 7-day survival)
1	100	100
2	97.5	100
3	100	100
4	0	10
5	77.5	10
6	67.5	30
7	65	30
8	100	100
9	92.5	100
10	87.5	30
11	95	100
12	0	10
13	62.5	10
14	97.5	100
15	0	0
16	95	100
17	95	100
18	87.5	100
19	0	3
20	60	30
21	0	10
22	0	10
23	100	100
24	97.4	100
25	100	100
26	0	30
27	100	100

Table 6. Summary of larval fathead minnow bioassay growth results.

Effluent	NOEC (%)
1	30
2	30
3	10
4	10
5	3
6	10
7	30
8	10
9	3
10	10
11	100
12	3
13	10
14	30
15	0
16	30
17	10
18	100
19	3
20	100
21	10
22	30
23	30
24	30
25	30
26	10
27	30

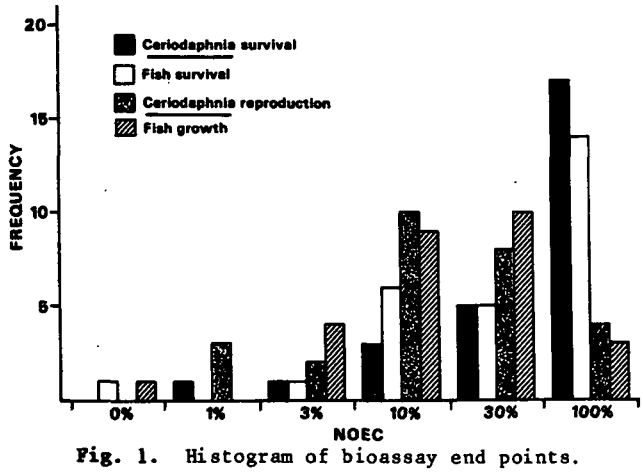

Fig. 1. Histogram of bioassay end points.

Bioassay Interpretation

A summary of the bioassay results, interpreted using the WDNR passing test criteria is presented in Table 7. It is apparent that the WDNR acute toxicity criterion was more stringent than that for chronic toxicity. Fifteen cases of failures versus five cases were noted for acute toxicity and chronic toxicity, respectively. The net result was fifteen of the mills tested (representing 55.6%) passed; while twelve mills (44.4%) failed the effluent bioassays.

Table 7. WDNR interpretation of bioassay results.

	Ceriodaphnia		Fish		Overall
Effluent	Acute	Chronic	Acute	Chronic	Toxicity
1	P	F	P	P	F
2	P	P	P	P	P
3	P	P	P	P	P
4	F	P	F	P	F
5	F	P	P	P	F
6	P	P	P	P	P
7	P	P	P	P	P
8	P	P	P	P	P
9	P	P	P	P	P
10	F	F	P	P	F
11	P	P	P	P	P
12	P	P	F	P	F
13	P	P	P	P	P
14	F	F	P	P	F
15	F	F	F	F	F
16	P	P	P	P	P
17	P	P	P	P	P
18	P	P	P	P	P
19	F	P	F	P	F
20	F	P	P	P	F
21	F	P	F	P	F
22	P	P	F	P	F
23	P	P	P	P	P
24	P	P	P	P	P
25	P	P	P	P	P
26	P	P	F	P	F
27	P	P	P	P	P
Total P	19	23	20	26	15
Total F	8	4	7	1	12

P = pass, F = fail.

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Future WDNR Biomonitoring Program

At the time of this writing, the WDNR and EPA Region V personnel were negotiating future biomonitoring requirements to be part of the Wisconsin Pollution Discharge Elimination System permits. Direct communication with the WDNR and various Wisconsin pulp/paper mills provided information to make the following assumptions:

1. Wisconsin pulp/paper mill discharges will be required to conduct quarterly chronic bioassays (using Ceriodaphnia and larval fathead minnows) at least for the first year of their permit, and annually thereafter.

2. If toxicity (as defined by the WDNR) is demonstrated, a retest must be conducted within three weeks.

3. When toxicity continues to be demonstrated, a toxicity evaluation-reduction program must be developed (to identify and correct the problem).

4. A possible effluent limitation, based on a toxicity value, may be included in the discharge permit.

CONCLUSIONS

The 54 bioassays conducted on Wisconsin pulp/paper mill effluents are but a fraction of the data necessary to draw any conclusions on this industry's effluent quality. All of these tests were conducted on a one-point-in-time basis. Seasonal differences in both effluent and receiving water quality are variables which influence bioassay results and should be factored into the interpretive process. Additionally, validation research is needed to relate these laboratory findings to the receiving waters condition. Instream biological monitoring/assessments should also remain an active part of all environmental programs.

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