
WHOLE EFFLUENT TOXICITY TEST

Fontana/Walworth Water Pollution Control Walworth, Wisconsin

BACKGROUND

A one-year study of the efficacy of Neozyme's EcoSystem Plus non-bacterial, enzyme product was initiated on October 27, 1994 at the Fontana/Walworth Water Pollution Control Plant in Walworth, Wisconsin. This facility was chosen due to the completeness of historical data and quality of its operation.

Six weeks of additional baseline data was collected prior to the commencement of this study to establish various data points not being collected within the plant as a matter of course. These additional tests were run to produce an even more complete picture of the overall operation at various points throughout the plant. This study does include two years of extensive operation data as a backdrop for the study which will conclude on November 1, 1995.

As mandated by the Wisconsin Department of Natural Resources, and their permit under the National Pollution Discharge Environmental Section (NPDES), acute and chronic toxicity tests were conducted by an independent environmental consultant (CH2M Hill) for the Fontana/Walworth facility. These tests were run approximately eight months after the commencement of the Neozyme study.

RESULTS

Acute Toxicity/Survival shows the 100% Effluent Test passed for the three organisms tested; *Ceriodaphnia dubia*, *Daphnia magna* and Fathead Minnow.

Chronic Toxicity tests on 79% Effluent (IWC) passed for two species run; *Ceriodaphnia dubia* for Survival and Reproduction and Fathead Minnows for Survival and Growth. Estimated toxicity for the IC25 Test is estimated to be greater than 85% effluent.

CONCLUSION

The introduction of Neozyme's EcoSystem Plus does not appear to cause an increase of toxins or otherwise exhibit any demonstrable negative effect on the acute or chronic toxicity of the Fontana/Walworth effluent.

A copy of the complete CH2M Hill Test Summary is attached.

SUMMARY

CH2M HILL conducted acute and chronic toxicity tests on effluent samples provided by the Fontana-Walworth Water Pollution Control (WPC), Walworth, Wisconsin. The bioassays were conducted from June 20 through 27, 1995, as part of NPDES compliance monitoring for the State of Wisconsin. *Ceriodaphnia dubia*, *Daphnia magna*, and fathead minnows were used as the test organisms. The following is a summary of the test results:

Acute Toxicity/Survival

Test Media	<i>Ceriodaphnia dubia</i>	<i>Daphnia magna</i>	Fathead Minnow
Piscasaw Creek Control	Pass	Pass	Pass
100% Effluent	Pass	Pass	Pass
Laboratory Control	Pass	Pass	Pass

Chronic Toxicity

	<i>Ceriodaphnia dubia</i>		Fathead Minnow	
	Survival	Reproduction	Survival	Growth
Piscasaw Creek Control	Pass	Pass	Fail	Pass
79% Effluent (IWC)	Pass	Pass	Pass	Pass
IC ₂₅		> 85%		> 85%
Laboratory Control	Pass	Pass	Pass	Pass

For NPDES compliance purposes, the results of the tests show that:

- The effluent samples were not acutely toxic to *Ceriodaphnia dubia*, *Daphnia magna*, or fathead minnows at 100 percent concentrations using the 50 percent lethality criteria.
- The effluent samples were not chronically toxic to *Ceriodaphnia dubia* at the 79 percent IWC based on the 25 percent inhibition analysis for reproduction. The IC₂₅ analysis estimated toxicity at >85 percent effluent.

RESULTS

Photocopies of the laboratory data and computer printouts of the statistical analyses are found in Appendix A. There were no excursions from the protocols and all test conditions were within the limits required by the DNR. The results of the tests are summarized below.

ACUTE BIOASSAYS

Table 6 presents the results of the acute bioassays. No acute toxicity was demonstrated to *Ceriodaphnia dubia*, *Daphnia magna*, or fathead minnows in the 100 percent effluent concentration. Piscasaw Creek and laboratory control data were acceptable in all tests.

Table 6
Summary of Results of Acute Bioassays
Conducted for the Fontana- Walworth WPC
Walworth, Wisconsin
June 20 through 24, 1995

Test Media	Mean Percent Survival		
	<i>Ceriodaphnia dubia</i>	<i>Daphnia magna</i>	Fathead Minnow
Piscasaw Creek Control	100	100	100
50% Effluent	100	100	100
100% Effluent	100	100	100
Laboratory Control	100	100	100



CHRONIC BIOASSAYS

Table 7 presents the results of the chronic bioassays. Chronic toxicity was not indicated by *Ceriodaphnia dubia* at the 79 percent IWC using the IC₂₅ analysis. The point estimate of the concentration of effluent that is chronically toxic was greater than 85 percent.

Piscasaw Creek and laboratory control water data met test acceptability criteria in the *Ceriodaphnia* bioassay.

Piscasaw Creek water data did not meet the acceptable survival criterion in the fathead minnow chronic bioassay. Technically, the test is invalid because of the unacceptable control/dilution water. However, the higher effluent concentrations (70, 79, and 85 percent) did not appear to be chronically toxic to the fathead minnow. In comparison with the acceptable laboratory control data, the 79 percent IWC did not indicate chronic toxicity. The IC₂₅ was estimated at greater than 85 percent effluent.

Table 7
Summary of Results of Chronic Bioassays
Conducted for the Fontana- Walworth WPC
Walworth, Wisconsin
June 20 through 24, 1995

Test Media	<i>Ceriodaphnia dubia</i>		<i>Fathead Minnow</i>	
	Mean Survival (%)	Mean Number of Offspring per Adult	Mean Survival (%)	Mean Growth (mg)
Piscasaw Creek Control	100	24.8	40*	0.252
25% Effluent	70	19.9	62.5	0.471
50% Effluent	90	17.5	70	0.545
70% Effluent	90	25.2	100	0.635
79% Effluent (IWC)	100	28.2	94.5	0.618
85% Effluent	100	26.2	90	0.624
IC ₂₅		>85%		>85%
Laboratory Control	100	32.2	100	0.612

* Indicates data did not meet test acceptability criterion for survival.

PHYSICOCHEMICAL DATA

All physicochemical parameters measured satisfied the bioassay requirements (see Appendix A).

CONCLUSIONS

Based on the results of the laboratory bioassays, the effluent samples collected by the Fontana-Walworth WPC on June 19, 21, and 23, 1995, passed the DNR whole effluent acute and chronic toxicity criteria for all species tested for NPDES compliance purposes. In summary, the results of the tests show:

- Piscasaw Creek control water data were acceptable in all acute bioassays and the chronic *Ceriodaphnia dubia* test.
- Piscasaw Creek water data did not meet the acceptable survival criterion for use as a control in the fathead minnow test A5 a result, this test is technically invalid. However, greater survival and

growth values in the higher concentrations indicate that the effluent is not toxic. Additionally, the 79 percent effluent data did not demonstrate chronic toxicity when compared with the acceptable internal laboratory control data. The IC₂₅ analysis was performed estimating toxicity at ≥ 85 percent effluent.

- Laboratory control water data met test acceptability criteria in all bioassays.

REFERENCES

1. Weber, C.I. (ed.). 1993. *Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms* (Fourth Edition). EP A/600/4-90/027F. U.S. EPA., Environmental Monitoring and Support Laboratory, Cincinnati, Ohio. 293 p.
2. Denny, J.S. 1987. *Guidelines/or the Culture of Fathead Minnows, Pimephales promelas, for Use in Toxicity Tests*. EP A/600/3-87/001. U. S. EPA., Environmental Research Laboratory, Duluth, Minnesota. 42 p.
3. Weber, C.I., et al. 1989. *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms* (Second Edition). EPA/600/4-89/001. U.S. EPA, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio. 249 p.
4. Weber, C.I. et. al., 1989. *Supplement to Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms* (Revision 1). EPA/600/4-89/001a. U.S. EPA, Environmental Monitoring and Systems Laboratory, Cincinnati, Ohio. 42 p.