FLORIDA INTERNATIONAL UNIVERSITY
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DRINKING WATER RESEARCH CENTER

Introduction:

Tests were performed to determine the biodegradability of Microsolve, a degreasing product design for use where dilute solutions can enter the soil or bodies of water. Static microcosms and dynamic columns of environmental materials (i.e., water and soil) were constructed and injected with know concentrations of Microsolve. After a suitable period, samples of the water phase were removed from the test systems and analyzed by gas chromatography to determine the amounts of Microsolve that remained unchanged.

Methods and Materials:

Static microcosms of a typical ground water environment were constructed in 50ml septum bottles using pond water filtered through Whatman 100 to remove large suspended particles, and glacial till consisting of 35% sand, 34% clay and 31% silt. The soil-water ratio was 15-35 on a volume/volume basis.

Microsolve was added to the microcosms to bring the final concentration to 10mg/L. Controls consisted of Microsolve in sterile distilled water in identical septum bottles.

Dynamic test systems consisted of one-inch internal diameter glass columns eight inches long filled with glacial till and glass beads, 50-50 on a volume/volume basis. Three columns were arranged in parallel, two of which were test columns and one a control column. All were fed by a syringe pump at the rate of 50ml per day. The control column was sterilized and fed sterilized water containing Microsolve. One of the two test columns was kept under ambient atmosphere with the solubility of oxygen in the feed water at approximately 8.4mg/L. The other test column was maintained in an anaerobic state (i.e., no detectable dissolved oxygen) under a nitrogen atmosphere.

Microcosms and columns were foil wrapped to exclude light and prevent photo oxidation. All systems were incubated at 25C for a period of four weeks following analysis to determine time-zero concentrations of Microsolve in the water plane.

Analysis for Microsolve was done using a Hewlett-Packard gas chromatograph equipped with a flame ionization detector and a megabore column. Samples were taken from the water phase of the static microcosms and extracted with pentane prior to injection into the gas chromatograph. The fifty-ml daily effluent volumes from the columns were analyzed in the same way at time-zero and after four weeks operation. All chromatograms were compared with chromatograms of solutions of Microsolve in regent grade water made concurrently.

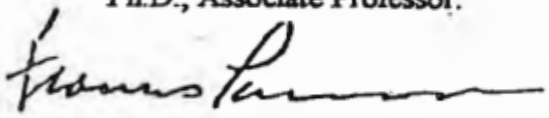
Results and discussion:

After four weeks incubation, 60% of the Microsolve remained in the water phase of the static microcosms; 40% had degraded to compounds not identified with the original added Microsolve.

The aerobic column degraded 82% of the Microsolve that was added daily at 10 mg/L. Eighteen percent of the daily dose could be recognized as unchanged Microsolve. The anaerobic system degraded 21% daily of the added 10 mg/L Microsolve and 79% remained unchanged.

The columns and microcosms represent environmental systems with active microbiota. The columns are dynamic; the microcosms static. Hence, real world situations bear a resemblance to both systems. The microcosms were dosed once with Microsolve and the columns received continual feed water containing a fixed concentration. The results indicate that Microsolve is biodegradable, especially under aerobic conditions.

These tests were performed by Pedro Lorenzo, Chemist, under the supervision of Frances Parsons, Ph.D., Associate Professor.



, Frances Parsons

V. The Biodegradability and Toxicity of Micro-Solve®

Micro-Solve® is totally and completely non-toxic to waste water systems, when used as directed, whether in grease straps, the collection system or in an activated sludge treatment system. Three sets of testing have been completed that illustrate this point. First, the Drinking Water Research Center of Florida International University performed two tests, biodegradability and chronic/acute toxicity. Law & Company, Consulting and Analytical Chemists, of Tucker, Georgia performed priority pollutant testing utilizing a bench scaled grease trap.

The biodegradability testing, contained on the next few pages, indicate that in an aerobic system, Micro-Solve® is biodegradable in concentrations of up to 10 mg/1. The chronic toxicity testing, contained on the next few pages, indicates that Micro-Solve® at 10 mg/1 and lower should not interfere with reproduction and growth of Fathead Minnows in natural waters and that a 5 mg/1 should not interfere with the growth of water flea, Daphnia Pulex. Of course, one must point out that the concentrations used will never occur in natural waters. The normal dosage rates of Micro-Solve® our applied to waste water stream, between dilution and biodegration, the final concentration in the waste water plant will be near zero, therefore, after treatment at the plant, no Micro-Solve® will be released into natural waters.

As can be seen, Micro-Solve®, when applied according to the manufacturers use instructions, is not toxic to the environment or the local wastewater treatment plant. We, at Bio-tech Distribution, Inc., pride ourselves in making sure that our product is safe and non-toxic.

Bio-Tech Industries

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Attn: All Sales and Technical Support Personnel

Subject: Toxicity Studies of Micro-Solve

Please find attached reports of the short term method used to determine the toxicity of Micro-Solve to fresh water fish.

Methods used for determination of toxicity were based on those published by the US-EPA, environmental monitoring and support laboratory, Cincinnati, Ohio (short-term methods for estimating the chronic toxicity of effluents and receiving waters to fresh water organisms, edited by W.B. Horning II and Cornelius I. Weber).

Attached reports were performed by Frances Parsons, Ph.D., Associate Professor and Pedro Lorenzo, Chemist, with the Drinking Water Research Center at Florida International University in Miami, Fl., 1-305-348-2826.

Sincerely,

David J. McGarva
President
LL/DM
cc: Mr. J. Zanin
Mr. J. Devane

Short Term Method
Used to Determine Toxicity

The method used to determine the toxicity of Micro-Solve® and freshwater fish was based on those published by the U.S. EPA, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio (Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, edited by Cap W. B. Horning, II and Cornelius I. Weber). Wall-eyed eggs were obtained, soon after fertilization from a commercial supply house and place in several concentrations of Micro-Solve® made with filtered, natural, upon water has outlined in Table 1.

Results of the tests are shown in Table 2. The larvae emerged during the second day of observation and were observed for period of six days following complete hatching.

No differences were seen between the test chambers the contained 10 mg/L Micro-Solve® and test chambers the contained clean, filtered, pond water. All eggs hatched and the larva appeared healthy and lively during the period of observation.
Forty percent (total of 24 eggs) hatched in the test chambers the contained 100 mg/L of the test material in pond water. All 24 died within one hour of hatching. The remaining unhatched eggs kept their fresh appearance for two days, then became opaque and disintegrated over the following two days. Higher concentrations prevented hatching.

These results indicate the concentrations of 10 mg/L and lower should not interfere with reproduction and growth of fathead minnows in natural waters. In natural surface waters, volatilization, photolysis, and absorption can be expected to moderate the effect of low concentrations of such materials on the biota. Biodegradation can be expected to lower the concentration that would adversely affect higher life forms.

TABLE 1

SUMMARY OF TEST CONDITIONS FOR FATHEAD MINNOW EMBRYO-LARVAL SURVIVAL IN MICRO-SOLVE (WORKING TITLE)

Test Organism:	Fathead minnow (Pimephales promelas)
Test type:	Static
Duration:	Eight days (two days pre-hatch, six days post-hatch).
Temperature:	25c (+/- 2c)
Light:	Laboratory lighting; 16 h light, 8 h dark
Test Chamber Size:	250 mL
Test Solution Vol.:	100 mL
Test Concentrations:	10-, 100-, 1000-, 10,000-mg/L Micro-Solve and clean water control.
Replicate Tests per Concentration:	4
Organisms/Test Chamber:	15 eggs
Aeration:	Intermittent, to maintain DO at 50% saturation.
Dilution Water:	Filtered, natural, pond water. Ph, 7.2; total hardness, 250 (as carbonate); TOC, 16 mg/L.
Effects Observed:	Hatch and survival (for six days) of larvae.

TABLE 2

HATCH OF FATHEAD MINNOW EGGS AND SURVIVAL OF LARVAE EXPOSED FOR EIGHT DAYS TO MICRO-SOLVE (WORKING NAME)

Test Concentration mg/L	% Hatched	% Survived (6 days)
10	100	100
100	40	0*
1,000	0	0
10,000	0	0
Control	100	100

* Died within one hour of hatching.

TABLE 3

RESULTS OF ACUTE TOXICITY TEST OF MICRO-SOLVE
ON
DAPHNIA PULEX AND PIMEPHALES PROMELAS

Concentration Micro-Solve, mg/L	Percent survived after 48 hours	
	Daphnia pulex	Pimephales promelas
1.0	100	100
2.5	100	100
5.0	100	90
7.5	50	50
8.0	25	5
9.0	5	5
10.0	0	0

All individuals in control vessels remained viable for the test period and beyond.