

Modified Phase I Toxicity Characterization Procedures for Use in Identifying Unknown Toxicants in Surface and Storm Water

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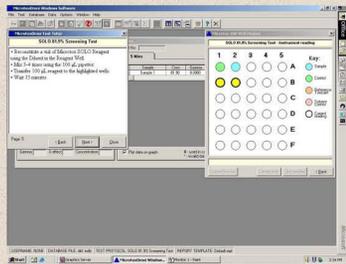
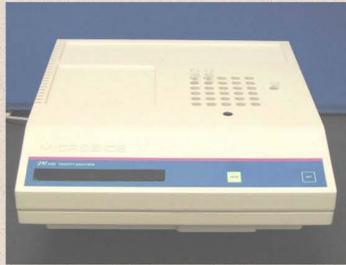
SCOPE/APPLICABILITY

The Microtox method of toxicity analysis utilizes a bioluminescent bacteria, *Vibrio fischeri*, that produces light as a by-product of cellular respiration. Rate of luminescence is directly related to the amount of cell respiration. Therefore, any toxicity to the cell will reduce respiration and the amount of light produced by the bacteria will be subsequently decreased.

The 81.9% SOLO screening test was used to determine the presence or absence of toxicity in surface water and storm water. Observable toxicity for the study was defined as any percent (%) greater than a 15% level of effect. The higher the % levels of effect, the more toxic the sample. A Modified Phase I Toxicity Identification Evaluation (TIE) protocol (USEPA 1991) was employed if toxicity was found in the SOLO screen. The Phase I TIE protocol was established to determine if a sample has toxicity due to pH, suspended particles, ionic metals, non-polar organics, or a chemical dissolved in solution.

Equipment/Supplies

- Microbiotics Model M500 Toxicity Analyzer
- Personal computer with Microtox Omni Software
- Strategic Diagnostics, Inc. (SDI) disposable glass cuvettes
- SDI Microtox Diluent
- SDI Microtox Osmotic Adjusting Solution
- SDI Microtox SOLO Reagent
- 100 ul-1 mL pipettor and pipette tips
- 1 ounce plastic cups (or similar)
- Nalgene 45 um disposable filter apparatus/hand pump
- 0.1 M EDTA solution
- SPE-C₁₈ (Solid Phase Extraction) columns
- NaOH (sodium hydroxide) and H₂SO₄ (sulfuric acid) for pH adjustments
- Surface water and/or storm water samples



PHASE I TIE PROTOCOL

Step 1: Measure the pH of the sample to be analyzed. If necessary, adjust the pH to between 6 and 8 using small amounts of NaOH to raise pH and H₂SO₄ or HCl to lower pH.

Step 2: Screen the sample using the SOLO test. If the % effect is below 15%, the sample is considered non-toxic and testing should end. If the % effect is greater than or equal to 15%, the sample is considered toxic. Initiate the manipulations in steps 3-5. All manipulations are tested with the SOLO screen.

Step 3: Using a Nalgene 45 um disposable filter apparatus (pre-rinsed with D.I. Water), filter a portion of the sample and re-test. If toxicity is eliminated or reduced, suspect that suspended particles are contributing to toxicity. If toxicity is still present, continue to step 4.

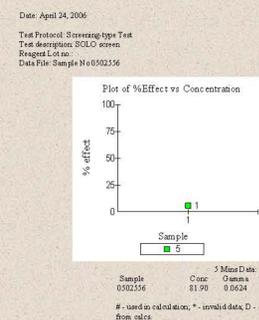
Step 4: Add 5 drops of EDTA solution to 20 milliliters of raw sample. Allow the sample to sit for 2 hours and then re-test. If toxicity is eliminated or reduced, ionic metals are likely contributing to toxicity. If toxicity is still present, continue to step 5.

Step 5: Suction a portion of raw sample (~20 mL) through a SPE-C₁₈ column and re-test. If toxicity is eliminated or reduced, non-polar organics are likely contributing to toxicity. If toxicity is still present, continue to step 6.

Step 6: If none of the above manipulations has an effect on toxicity, then suspect that the toxicity is attributed to a contaminant dissolved in solution.

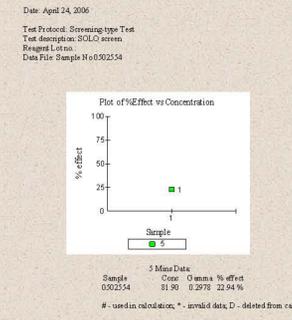


MicrotoxOmni Test Report



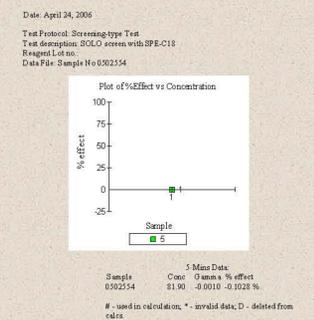
Example of SOLO test with no toxicity (5.874%).

MicrotoxOmni Test Report



Example of SOLO test with toxicity (22.94%).

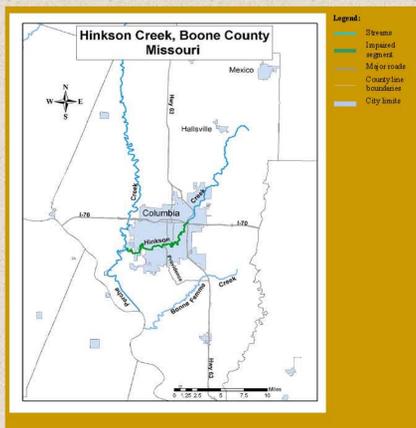
MicrotoxOmni Test Report



Example of SOLO test with SPE-C18. By looking at the reduced % effect, non-polar organics are a likely source of toxicity (-0.1028% effect).

Example: Hinkson Creek Study

In 1998 the Missouri Department of Natural Resources, Water Protection Program, Water Pollution Control Branch (WPCB) placed approximately fourteen-miles of Hinkson Creek on the impaired waters list designated under section 303(d) of the federal Clean Water Act. Hinkson Creek was listed as impaired for "unspecified pollutants" due to urban runoff. The impaired beneficial use was listed as "protection of warm water aquatic life." This means that Hinkson Creek does not meet the following criteria: "waters in which naturally occurring water quality and habitat conditions allow the maintenance of a wide variety of warm-water biota, including naturally reproducing populations of recreationally important fish species..." (Mo CSR 2004).



During the state fiscal year 2001, the Water Pollution Control Branch requested sampling of the aquatic macroinvertebrate community to determine the biological integrity of Hinkson Creek. During the fall of 2001 and spring of 2002, an aquatic macroinvertebrate community study was conducted (MDNR 2002a). Information obtained from the study showed a decline in the aquatic macroinvertebrate populations. Matrix comparisons were made against similar size, high quality streams within the same geographical area. The study results indicated that Hinkson Creek downstream of the Interstate 70-bridge (I-70) crossing was only "partially supporting" for aquatic life and confirmed stream impairment as summarized below.

- During the fall 2001 season, the number of invertebrates in the orders Ephemeroptera, Plecoptera, and Trichoptera taxa (EPT) were similar among stations. A slight increase in both the total numbers of taxa and EPT taxa occurred in downstream stations, likely due to an increase in water quantity downstream. The percent EPT (# of EPT taxa/total # of taxa present) tended to be slightly greater upstream of the impaired segment.
- During the spring 2002 season, there was a sharp decline of EPT taxa in the urban portion of Hinkson Creek, with a significant decline in the order Plecoptera. The total number of taxa declined substantially. Percent EPT was greater upstream of the impaired segment.

Because of the aquatic macroinvertebrate findings, further work was required to determine the nature and cause of impairment. The WPCB requested that the Environmental Services Program (ESP) conduct a comprehensive study of main-stem Hinkson Creek and major storm drainages located within the impaired segment of Hinkson Creek. This study consisted of additional biological sampling along with water quality and sediment monitoring, and toxicity testing.

Study Objectives

The overall objective for the three-phase study was to conduct a water quality assessment of the entire "impaired" 14-mile segment of Hinkson Creek in phases as summarized below.

- The first phase of the study was conducted during the 2004 state fiscal year and concentrated on an approximately 2.0 mile segment of Hinkson Creek between the I-70 and Broadway bridge crossings.
- The second phase of the study began during July 2004 and continued throughout the 2005 state fiscal year that ended June 30, 2005. The phase II portion of the study concentrated on an approximately 5-mile long segment of Hinkson Creek located between the Broadway bridge and Recreational Drive low-water bridge crossing (located just upstream of Providence Road).
- The third phase of the Hinkson Creek study began in July 2005 and continued through June 2006. The third phase focused on an approximately 7.5-mile long segment of Hinkson Creek from Recreational Drive low-water bridge crossing to Perche Creek.

The intent of the three-part study was to locate possible pollutant sources and identify contaminants contributing to impairment of the stream. Main-stem Hinkson Creek, major stormwater drainages, and major tributaries were monitored throughout each phase of the study.

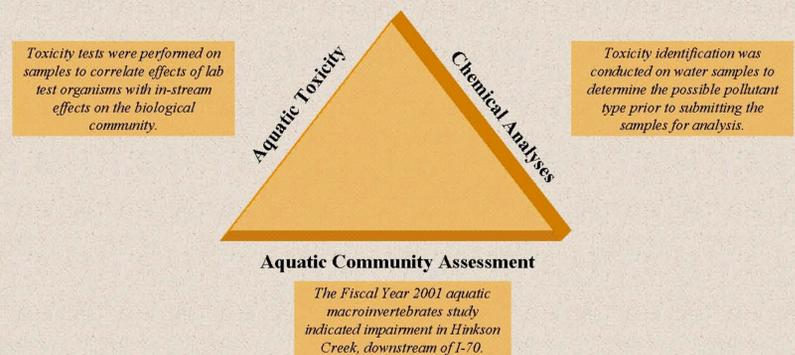


Study Design

The source and the type of pollutant(s) in Hinkson Creek were unknown. Therefore, a water quality triad was used to document impairments to the aquatic community and identify pollutants that are likely contributing to those impairments. The triad is a non-numeric, weight of evidence approach that is becoming frequently used as a regulatory tool for water quality impact assessment and management (Lee and Lee-Jones 2002, Burton and Pitt 2002). This approach is an integrated assessment of information obtained from the aquatic organism assemblages, chemical analyses, and toxicity testing.

The figure below summarizes how the water quality triad was implemented during this study. Because the macroinvertebrate data indicated impairment to Hinkson, it was necessary to collect a series of water samples for testing. Before the samples were submitted for chemical analysis, aquatic toxicity was determined using a Microtox test system. If the water samples were found to be toxic, a Toxicity Identification Evaluation procedure was conducted to determine the possible pollutant type(s) (e.g., organic, metals, etc). The water samples were then submitted for analysis based on the toxicity identification results.

The Water Quality Triad



Example of TIE protocol results and associated chemicals detected in water samples.

Sample Date	Sample Location	Level of Effect (%)				Chemicals Detected (from laboratory analyses)
		Raw	Filtered	EDTA	C ₁₈	
7/11/03	Wal-mart	46	--	56	-24	Carbaryl, Caffeine
10/27/03	Mo-DOT	54	--	60	4	TPH as Waste Oil
3/19/04	Mega Market	77	45	57	14	Oleyl alcohol; N-N-Diethyl-1-dodecanamine; N,N-Dimethyltetradecanamine; n-Hexadecanoic acid; 9-Octadecanoic acid; Bis (2-ethylhexyl) phthalate Total Recoverable (Cr, Cu, Ni, Pb, Zn)
3/24/04	Wal-Mart	82	47	18	15	Pyrene, Phenanthrene, Fluoranthene, bis(2-Ethylhexyl) phthalate, Benzoic acid, Benzo(a)anthracene
3/24/04	Mega Market	48	21	47	32	1,1,2,2-Tetrachloroethane; 2-Ethylhexanoic acid; Caffeine; n-Hexadecanoic acid; 9,10-Anthracene dione; 9-Octadecanoic acid; Octadecanoic acid
5/13/04	Mega Market	34	32	33	8	1,1,2,2-Tetrachloroethane; n-Hexadecanoic acid