Willis et al 1994

United States Department of the Interior

FISH AND WILDLIFE SERVICE
1875 Century Boulevard
Atlanta, Georgia 30345

IN REPLY REFER TO:

March 21, 1994

Memorandum

To: Field Supervisor, FWS, Asheville, NC
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Field Supervisor, FWS, Charleston, SC
Field Supervisor, FWS, Clemson, SC
Field Supervisor, FWS, Daphne, AL
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Field Supervisor, FWS, Panama City, FL
Field Supervisor, FWS, Raleigh, NC
Field Supervisor, FWS, Vero Beach, FL
Field Supervisor, FWS, Vicksburg, MS

From: Acting Assistant Regional Director (ES), FWS, Atlanta, GA (TS/EC)

Subject: Contaminant Study Report

Attached is a copy of the contaminant study report entitled "An Investigation of Sediment Toxicity In Horse Lick Creek (Upper Cumberland Drainage)" which was prepared by our Cookeville, Tennessee, Field Office. We hope that this report will not only serve to keep you abreast of contaminant problems in the Southeast Region, but will also prove helpful in evaluating contaminant impacts within your respective areas.

RECEIVED
MAR 23 1994

Nancy C. Coon

Attachment

USFWS
ASHEVILLE NC

3/24	BPC	NAM	
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	JAF	LR	
	JLN	SE	
	VGH		

CO-91-4261

TENNESSEE

AN INVESTIGATION OF SEDIMENT TOXICITY IN HORSE LICK CREEK (UPPER CUMBERLAND DRAINAGE)

U.S. FISH AND WILDLIFE SERVICE/SOUTHEAST REGION/ATLANTA, GEORGIA

U.S. Fish and Wildlife Service

Southeast Region

AN INVESTIGATION OF SEDIMENT TOXICITY IN THE HORSE LICK CREEK SYSTEM (UPPER CUMBERLAND RIVER DRAINAGE)

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Cookeville, Tennessee

November 1993

ABSTRACT

Microtox tests were used to assess the toxicity of water and sediment pore water samples collected from the Horse Lick Creek system in southeastern Kentucky. A variety of mussels, including federally listed species, are known to occur in this system, which drains areas where surface mining of coal has occurred. All samples were tested using the sodium chloride and sucrose protocols for Microtox. Although no toxicity was observed in any of our samples, influx of sediment and other indications of surface mining runoff to the stream were evident at several sites. Because our samples were collected at low flow conditions, it is recommended that follow-up studies include samples from higher flows, and that larval, juvenile or caged adult mussels be used to provide a more direct application to the species of concern.

ACKNOWLEDGEMENTS

Dr. Donald Shultz, former Environmental Contaminants Program Coordinator in the U.S. Fish and Wildlife Service's (USFWS) Southeast (Atlanta) Regional Office, reviewed the Horse Lick Creek study plan, helped to secure funds and process samples. Dr. Charles Facemire, Senior Environmental Contaminants Specialist at the USFWS Regional Office, reviewed the draft reports. Dr. Lee Barclay, Field Supervisor for the USFWS Ecological Services Field Office, Cookeville, Tennessee, also reviewed the draft reports. Sandra Silvey provided assistance with word processing and report duplication. Barbara Bohanon provided assistance with the artwork on the report cover. Jason Duke prepared the map showing the collection sites.

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INTRODUCTION

Studies conducted through Tennessee Technological University (Anderson 1989; Layzer and Anderson 1992) indicated recent severe declines in both the diversity and density of freshwater mussels inhabiting several Cumberland Plateau streams. The most severe declines occurred at sites thought to have been impacted by surface coal mine runoff, although concurrent water quality analyses did not indicate abnormal physical or chemical water quality parameters. Although Anderson (1989) implicated coal mining as a probable factor in declining mussel populations, he did not indicate which facet of mining operations was specifically harmful to mussels.

Surface coal mining operations are one of several land uses that have been implicated as factors in recently observed, sharp declines of mussel fauna, particularly in those streams draining the Appalachian coal fields. Present coal mining activities have the potential to further degrade the habitat of several threatened and endangered mussel species endemic to the Cumberland Plateau region. The Kentucky Department of Surface Mining Reclamation and Enforcement and the Department of the Interior's Office of Surface Mining have objected to assertions that mining is the problem, and have stated that factors other than mining (e.g., oil and gas development, forestry practices, etc.), or the combined effects of various land use practices other than mining, may be the cause of observed declines in mussel numbers and species diversity.

This study was conducted to determine if the observed declines in mussel fauna could be associated with stream sediment toxicity, as opposed to effects produced by physical habitat perturbations. Horse Lick Creek, located in southeastern Kentucky, was selected for this study because of the available historical information (DiStefano 1984; Anderson 1989). The stream has a diverse mussel fauna which includes the federally listed species Villosa trabalis, Pegias fabula and Alasmidonta marginata. The stream system is relatively small and easily accessible. It also includes areas upstream and downstream of coal surface mining operations. This study was conducted to supplement the work of Layzer and Anderson (1992).

STUDY AREA

The Horse Lick Creek drainage lies within the Eastern Kentucky Coalfield Region of the Appalachian Plateau Physiographic Province, and is in the Western Allegheny Plateau Ecoregion. The stream originates in northeastern Jackson County, Kentucky, approximately 300 meters east of the Rockcastle-Jackson County

Line, and flows southward 26.2 kilometers (16.3 miles) to join the Rockcastle River near the boundaries of Jackson, Laurel and Rockcastle Counties (Figure 1). It is a low-gradient, fourth-order stream, characterized by extensive deep pools separated by intermittent, shallow riffles (Carter and Jones 1969). This area is underlain primarily by limestone deposits interspersed with shale, siltstone and sandstone bedrock.

Human impacts such as agriculture, logging and mining are limited within the drainage basin. The watershed is 65 percent forested and is almost totally contained within the Daniel Boone National Forest. About 10 percent of the Clover Bottom Creek watershed, the largest tributary to Horse Lick Creek, is disturbed by strip mining. Although Horse Lick Creek has experienced overall severe declines in its mussel fauna, a few isolated beds of mussels with high diversity and abundance still exist (DiStefano 1984; Anderson 1989). Horse Lick has been described as one of the highest quality streams in the upper Cumberland River drainage (Harker et al. 1979, 1980; DiStefano 1984).

Description of Collection Sites

Four locations on Horse Lick Creek, five tributary sites, and two Rockcastle River locations were sampled in this study. They are described as follows:

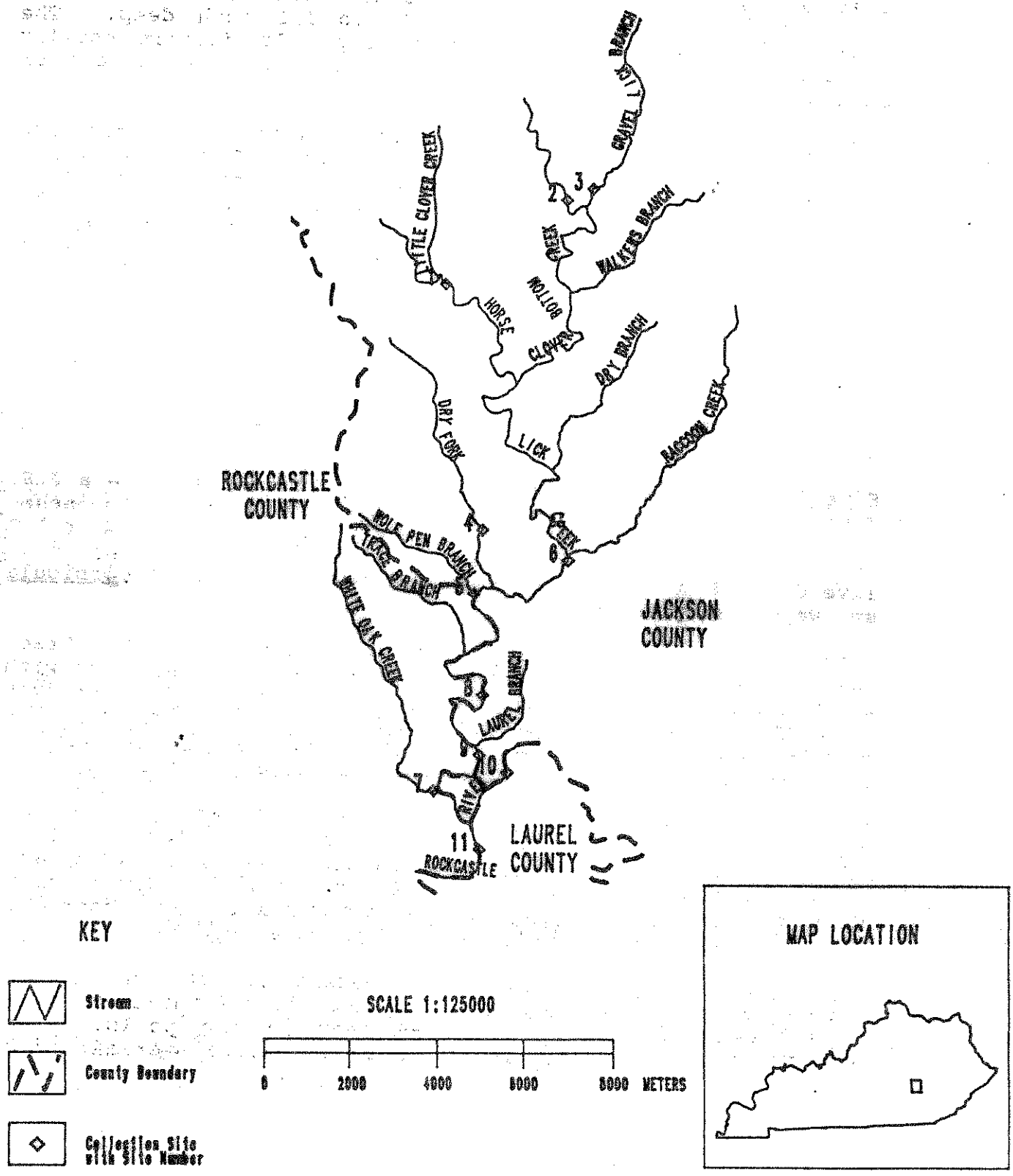
Site 1: Horse Lick Creek immediately downstream of the bridge on Highway 1955. Water depth was approximately 6-8 inches over a substrate of silt and gravel. No mussels or relict shells were observed.

Site 2: Clover Bottom Creek, approximately 100 meters upstream from its confluence with Gravel Lick Creek. Water depth was approximately 3 inches over a substrate of sand and gravel mixed with a small amount of clay. No mussels or relict shells were observed.

Site 3: Gravel Lick Branch adjacent to an unpaved road, approximately 100 meters upstream from the confluence with Horse Lick Creek. This is a very small tributary to Horse Lick Creek. Water depth was approximately 8-12 inches over a red silt and sand substrate. The appearance of the substrate suggested that the tributary might be receiving runoff from an abandoned coal mining site. No mussels or relict shells were observed.

Site 4: Dry Fork, a small tributary to Horse Lick Creek. The site was located immediately below a series of strip mine settling ponds, approximately 300 meters upstream from the

Figure 1. Map Showing Collection Sites



confluence with Horse Lick Creek. Water depth was approximately 3-4 inches over a gravel and cobble substrate overlain with a heavy layer of silt. No mussels or relict shells were observed.

Site 5: Wolf Pen Branch immediately upstream of its confluence with Horse Lick Creek. Water depths varied from 3-24 inches over a gravel and cobble substrate. The entire area was overlain with a layer of silt 1/4 to 1/2 inch deep. The sediment sample was taken from a deep silt deposit showing evidence of mining effluent ("yellow-boy"). No mussels or relict shells were observed.

Site 6: Horse Lick Creek immediately below the confluence with Raccoon Creek. Water depths varied from 4-12 inches over a gravel, cobble, and sand substrate. The entire stream bottom was overlain with a layer of silt 1/4 to 1/2 inch deep. The site was located at a ford and showed evidence of extensive use by all-terrain vehicles. A few relict mussel shells were found, but no live specimens were observed.

Site 7: White Oak Creek immediately upstream from its confluence with Horse Lick Creek. Water depths averaged approximately 4-6 inches over a rock and sand substrate. Sediments were composed almost entirely of sand with an orange tinge, indicating the possible presence of high levels of iron. No mussels or relict shells were observed.

Site 8: Horse Lick Creek approximately 25 meters above a U.S. Forest Service road ford. Water depths varied from 5-10 inches over a substrate of cobble and sand. A layer of silt 1/4 to 1/2 inch deep covered all substrate. A few scattered, individual live Unionid mussels were found. A few specimens of Corbicula sp. were also present.

Site 9: Horse Lick Creek 25 meters above Sweet Towhee Trace Ford, the first ford upstream from the creek's confluence with the Rockcastle River. Water depths varied from 6-18 inches over a substrate of cobble overlain with 1/4 to 1/2 inch of silt. A few scattered, individual live Unionid mussels and some relict shells were observed. Corbicula sp. was also present.

Site 10: Rockcastle River approximately 200 meters above its confluence with Horse Lick Creek. Water depth varied from 8-10 inches in the riffles to 4-6 feet in the pools. The substrate was cobble overlain with 1/4 to 1/2 inch of silt. Significant numbers of live Unionid mussels and relict shells were observed, as well as large numbers of relict Corbicula sp. shells.

Site 11: Rockcastle River 100 meters downstream of its confluence with Horse Lick Creek. Water depths varied from 8-10 inches in the riffles to 4-6 feet in the pools. The substrate was mainly sand with scattered cobble overlain with

1/4 to 1/2 inch of silt. No live mussels were found; however, a large number and variety of relict mussel shells were observed on the banks adjacent to the stream.

METHODS AND MATERIALS

Sampling was conducted July 22-23, 1991. Water levels were low at the four Horse Lick Creek collection stations, the five tributary stations and the two reference sites on Rockcastle River (Figure 1). However, there was no difficulty in obtaining both surface water and sediment pore water (water lying within the interstitial spaces between sediment particles) samples at all sample sites.

Collection Procedures

Surface water samples were collected in 10 ml plastic syringes, with a short length of PVC flexible tubing (1/8" I.D. x 1/4" O.D.) attached to the tips. The tubes were placed under the water surface approximately 8-10 inches when possible and the samples were drawn into the syringes. When filled, the syringes were capped, placed in a plastic bag bearing a sample site location number, and placed on wet ice in a cooler.

At each location, sediments containing high percentages of clay and organic matter were collected for this study, as they tend (by virtue of their small particle size and electrostatic charge) to adsorb metals and other contaminants much better than larger, uncharged silica particles (sand) and gravel. Sediment samples were collected with a 4-foot long piece of 1.5 inch diameter PVC pipe. The pipe was withdrawn after being pushed into stream sediment deposits containing the highest percentage of organic matter that could be found at the sample site. If the sediment contained enough clay and organic matter, a plug of sediment would remain in the pipe after it was withdrawn from the stream bottom. The pipe was then inverted and the surface water above the sediment was decanted out of the pipe, leaving the sediment plug. This technique would not work with very sandy or gravelly sediments, because they would not remain in the pipe when it was withdrawn from the stream bottom.

Sediment samples were composited into a chemically cleaned 500 ml glass jar. An aquarium airstone attached to one end of a length of surgical tubing was then submerged in the sample, and pore water was separated from the sediment by drawing it through the stone using a 10 ml plastic syringe attached to the other end of the tubing. When full, the syringes were capped, placed in a plastic bag bearing a sample site location number, and placed on wet ice in a cooler. Before leaving the sample site,

the PVC pipe used for the sediment sample collection was thoroughly rinsed in the stream surface water. Surface and pore water samples were kept refrigerated until the Microtox assays were performed. All assays were completed within ten days after collection.

Laboratory Analyses

A Microtox Model 500 Toxicity Test System (Microbics Corporation, Carlsbad, CA) was used to test the water samples for toxicity. The Microtox Acute Toxicity Assay technique involves exposing bioluminescent bacteria species (Photobacterium phosphoreum) to a test substance. The Microtox instrument is a temperature-controlled photometer that measures the light level of the bacteria reagent before and after exposure to the potentially toxic sample. The amount of decrease in light indicates the degree of toxicity of the sample.

The Microtox assays were conducted by two different protocols. The standard Microtox protocol utilizes a commercially prepared sodium chloride (NaCl) solution as a diluent (available from Microbics, Inc.). A newer protocol utilizes an ACS-grade sucrose solution which enhances the sensitivity of the Microtox reagent to metal toxicity.

The Microtox unit contains an integral statistical analysis software package that was linked with an office microcomputer to automatically calculate levels of significance and other statistical information. Samples were analyzed to determine (1) if there was any variation in observable toxicity among the reaches of stream sampled; (2) if any measurable variations in toxicity among the sample sites were statistically significant; and (3) if toxicity might correlate with those reaches of Horse Lick Creek which were believed to have been impacted by surface coal mining. A detailed description of the Microtox assay is given in Appendix A.

RESULTS

None of the surface or sediment pore-water samples collected from Horse Lick Creek, its tributaries, or the Rockcastle River demonstrated any detectable toxicity using Microtox with either the NaCl or the sucrose protocols. Sediment and water quality analyses were not conducted as a part of this study.

Although species richness and mussel density were not measured, each site was carefully inspected for both live specimens and relict shells. Very few of either were observed in Horse Lick

Creek or its tributaries. The Rockcastle River site (#10) upstream of Horse Lick Creek clearly had greater mussel diversity and density than the site (#11) located downstream of Horse Lick Creek.

Our Site No. 6 was in the same general area as Site No. 18 sampled by Layzer and Anderson (1992) during 1988-1990. They observed the greatest variety of mussels (17 species) at this site, which was approximately 10 km downstream from known surface mining areas. Although we observed very few mussels approximately one year later, our site was about 400 m upstream from theirs.

DISCUSSION

Relationships between Microtox test results and instream effects on freshwater mussels are not clear. Although Ankley et al. (1990) included discharges from four mining operations, larval or juvenile mussels were not used in their evaluation of Microtox. With two mining effluents, they found that Microtox-NaCl and Microtox-sucrose results (EC_{∞}) agreed with LC50 values for Ceriodaphnia dubia and fathead minnows (Pimephales notatus), respectively. With the third mining effluent, all three test organisms indicated no toxicity, and with the fourth, only C. dubia responded (Ankley et al. 1990).

We are uncertain whether our Microtox test results reliably indicate the absence of surface water and sediment pore water toxicity to the endemic mussel fauna in Horse Lick Creek. Our sampling was conducted during low flow conditions with a minimum of surface-water runoff occurring. The mussel fauna are likely exposed to substantially different water quality conditions during higher flow events. Any future studies on Horse Lick Creek, or similar studies on other streams, should also include: (1) larval or juvenile mussel tests as described by Jacobson et al. (1993) and (2) field enclosures with endemic mussel species as used by Layzer and Anderson (1992) in other streams within this area. Glochidia and juvenile mussel bioassays have been used by the Tennessee Valley Authority and other agencies, and are very sensitive indicators of contamination. Since mussels are the species of concern in Horse Lick Creek, this would allow a more direct application of test results.

It would also be useful to collect water samples during the initial flush of higher flow conditions. Changes in pH, alkalinity, hardness and other general water quality parameters would be useful in evaluating the potential for acid stress on mussels and the bioavailability of heavy metals. Suspended sediment loading and instream heavy metals concentrations are likely to be greatly increased during higher flows, thus

resulting in a pulsed exposure to both physical (siltation) and chemical (acid mine drainage, metals) stressors.

The heavy layer of silt found covering the substrate at all sample locations is a result, at least in part, of strip mining or related activities, such as the construction of access roads. Erosion of these roads by heavy precipitation and vehicle use likely results in silt and sediment in the stream. Siltation is known to be detrimental to most, if not all, species of mussels, particularly those species inhabiting flowing waters (Ellis 1936). Based on our observations, siltation is considered to be a major factor related to the documented declines (Anderson 1989) in mussel populations in Horse Lick Creek.

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APPENDIX A

MICROTOX RECOMMENDED 100% ASSAY PROCEDURE FOR THE U.S. FISH AND WILDLIFE SERVICE

The standard Microtox procedure tests four sample dilutions, of which the highest possible concentration is 45%. Some Microtox users have a need to test complex samples without dilution (100% sample). A Microtox Assay procedure was developed to provide a convenient assay for screening samples for levels of toxicity.

NOTE: This procedure is faster and easier than the Standard Assay; also, it can be more technique-sensitive than the standard procedure. The area that makes it more technique-sensitive is pipetting of 10 uL of reagent using a 10 uL micropipettor. The pipetting is greatly improved by using 20 uL reagent, 2X the volume, and a repeat pipettor. Repeat pipettors are less technique-sensitive, and this protocol for the U.S. Fish and Wildlife Service uses both 20 uL of reagent and a repeat pipettor.

ANALYZER AND SAMPLE PREPARATION

1. Place cuvettes in incubator wells A1 to A5 and a cuvette in the Reagent Well.

2. Add 1 mL Recon Solution to cuvette in Reagent Well. This cuvette will be used to reconstitute the reagent.

3. Add 1 mL Diluent to cuvettes A1 to A5. These cuvettes will be used for making serial dilutions of the sample.

4. Add 250 mL MOAS to cuvette A5. MOAS is used to osmotically adjust the sample to 2% NaCl.

5. Add 2.5 mL sample (2500 mL or 5 additions using the 500 mL pipettor) to cuvette A5. Mix A5 two times with the pipettor.

6. Discard 750 uL from A5, so that the volume in A5 is 2 mL.

7. Transfer 1 mL from A5 to A4, then mix A4 two times with the pipettor.

8. Transfer 1 mL from A4 to A3, then mix A3 two times with the pipettor.

9. Transfer 1 mL from A3 to A2, then mix A2 two times with the pipettor.

10. Discard 1 mL from A2.

NOTE: That 1:2 serial dilutions of the samples have been made, and that the volume in cuvettes A1 through A5 is the same, 1 mL per cuvette. The sample concentration in percent (%) in the following cuvettes is: A1=Blank, A2=11.25, A3=22.5, A4=45, A5=90.

11. Wait 5 minutes for temperature incubation. While waiting, perform steps in Computer Preparation.

REAGENT PREPARATION

1. Just prior to reagent reconstitution (recon.), take a vial of Microtox Reagent from the freezer. Remove the seal and the stopper and discard them.

2. If the reagent pellet is not seated on the bottom of the vial, tap the vial until the pellet is seated.

3. Take the cuvette of recon. solution from the Reagent Well. Place the tip of the cuvette on top of the reagent vial. Then, as quickly as you can, empty the recon. solution into the reagent vial.

4. Swirl the reagent vial two times, then pour the reagent back into the reagent cuvette and place the cuvette back in the Reagent Well.

5. Place a new pipette tip on the 500 uL micropipettor and mix the reconstituted reagent 20 times with the pipettor by filling and dispensing.

REPEAT PIPETTOR PREPARATION

1. Place a 0.06 mL syringe on the repeat pipettor.
NOTE: Read carefully the repeat pipettor instructions.

2. Set repeat pipettor volume to 20 mL, a setting of 2 on the pipettor adjustment dial.

NOTE: If 10 uL of reagent is used, a setting of 1 on the pipettor adjustment dial is required.

3. Fill repeat pipettor with reagent from Reagent Well, by pushing up on the Piston Lever.

4. Dispense the first shot of reagent (possibly inaccurate) back into the Reagent Well cuvette, by pressing down on the Thumb Knob.

ASSAY PROCEDURE

1. Set/start timer for 5 and 15 minutes (change initial concentration from 40% to 91% and units from mg/L to percent.

2. Add 20 uL of reagent to each cuvette in the following order: A1, A2, A3, A4, A5, by pressing down 1 time on the Thumb Knob of the repeat pipettor for each cuvette.

   NOTE: There is no I(O) light level reading with 100% assays, and the assay starts with the addition of the reagent to cuvette A1.

   NOTE: Add the 20 uL of reagent to each cuvette without letting the reagent hit the side of the cuvette or placing the syringe tip down into the liquid (sample). This is easily done by placing a finger approximately half-way over the top of the cuvette, then resting the syringe tip against the finger and down into the center of the cuvette. This will keep the pipette tip in the center of the cuvette while pressing down on the Thumb Knob of the repeat pipettor. When moving the repeat pipettor to the next cuvette, keep the finger against the pipette tip. This will speed up the pipetting process.

3. Dispense the rest of the reagent back into the Reagent Well cuvette.

4. Mix each cuvette by shaking it 2-3 times, in the same order of sample addition.

5. When the timer alarm sounds, place the A1 cuvette into the Read Well. Press the Set button. DO NOT press the set button again during this assay.

6. When the Microtox Ready green light comes on, read and record on paper the I(T) light level of A1 by pressing the Read button.

7. Cycle cuvettes in the following order into the Read Well, reading and recording on paper the I(T) light levels: A2, A3, A4, A5.

8. Reduce data using the standard assay software by entering the "INPUT DATA FROM KEYBOARD" (see procedure below, from "How To Use The Microtox Standard Assay Calculation Program For 100% Assay On An IBM PC Computer").

HOW TO USE THE MICROTOX ASSAY CALCULATION PROGRAM FOR 100% ASSAYS ON AN IBM PC COMPUTER

NOTE: With the 100% Assay, there is no I(O) light reading. For this reason, special software is being developed to interface with your computer. At this time, Microbics does not have the 100% Assay software ready. Below is a method using the software for standard assay.

1. Perform a 100% assay, collecting the 5 and 15 minute data.

2. Call up the calculation program.

3. When asked for a I(O) light value (for the blank and all sample dilutions), enter the BLANK I(T) light reading.

4. When asked for a I(T) light value, enter the appropriate I(T) light reading.

Example data of a 5 minute assay:

Concentration	Light Level
BLANK	90
11.25%	60
22.50%	50
45.00%	40
90.00%	30

Simulated computer screen for Microtox 500 software:

Test Data	BLANK	1	2	3	4
I(O) Input	90	90	90	90	90
I(T) Input	90	60	50	40	30

ABBREVIATED MICROTOX 100% ASSAY PROCEDURE

ANALYZER AND SAMPLE PREPARATION

1. Add 1 mL Recon. Solution to cuvette in Reagent Well.
2. Add 1 mL Diluent to cuvettes in A1 to A4.
3. Add 250 uL MOAS to cuvette A5.
4. Add 2.5 mL sample to cuvette A5, and mix A5.
5. Discard 750 uL from A5.
6. Transfer 1 mL from A5 to A4, and mix A4.
7. Transfer 1 mL from A4 to A3, and mix A3.
8. Transfer 1 mL from A3 to A2, and mix A2.
9. Discard 1 mL from A2.
10. Wait 5 minutes.

REAGENT PREPARATION

1. Reconstitute a vial of reagent.

ASSAY PROCEDURE

1. Set/start timer for 5 and 15 minutes.
2. Add 20 uL of reagent to each cuvette in the following order: A1, A2, A3, A4, A5.
3. Mix each cuvette by shaking it 2-3 times, in the same order of sample addition.
4. When the timer alarm sounds, read the I(T) light level for A1, A2, A3, A4, and A5.
5. Reduce the data.