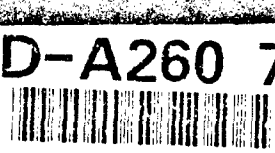

U.S. ARMY BIOMEDICAL RESEARCH AND DEVELOPMENT LABORATORY AQUATIC BIOMONITORING TRAILER VERSION 1.0: OPERATIONS MANUAL

FINAL REPORT

Randall S. Herriott, B.A.
Dennis T. Burton, Ph.D.
University of Maryland System
Wye Research and Education Center
P.O. Box 169, Queenstown, MD 21658

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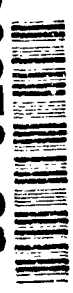

Project Officer: Tommy R. Shedd, M.S.
U. S. Army Biomedical Research and
Development Laboratory
Fort Detrick, Frederick, MD 21702-5010

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The U.S. Army Biomedical Research and Development Laboratory (USABRDL) Aquatic Biomonitoring Trailer Version 1.0 is a mobile laboratory which employs several biological biomonitoring systems for hazard assessment of potentially contaminated wastewater, complex effluents, and groundwater. This operations manual has been designed to provide a specific overview of the Aquatic Biomonitoring Trailer. Included in the manual is a general description of the trailer; initial utility, diluent water, test material, and waste drainage hook-up of the trailer; types of tests/assays performed; recommended water quality tests to be performed; daily trailer maintenance/operations; and complete shutdown of the trailer for site relocation. The manual also contains several procedures which explain in detail the operation of the various pieces of equipment and systems that are utilized durin testing in the trailer. In addition, several diagrams are included as visual representations of the trailer and its associated equipment.

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EXECUTIVE SUMMARY

The U.S. Army Biomedical Research and Development Laboratory (USABRDL) Aquatic Biomonitoring Trailer Version 1.0 is a mobile laboratory which employs several biological biomonitoring systems for hazard assessment of potentially contaminated wastewater, complex effluents, and groundwater. This operations manual has been designed to provide a specific overview of the Aquatic Biomonitoring Trailer. Included in the manual is a general description of the trailer; initial utility, diluent water, test material, and waste drainage hook-up of the trailer; types of tests/assays performed; recommended water quality tests to be performed; daily trailer maintenance/operations; and complete shut-down of the trailer for site relocation. The manual also contains several procedures which explain in detail the operation of the various pieces of equipment and systems that are utilized during testing in the trailer. In addition, several diagrams are included as visual representations of the trailer and its associated equipment.

ACKNOWLEDGEMENTS

We thank Mr. Tommy R. Shedd, Mr. Henry S. Gardner, and Dr. Robert A. Finch of the Research Methods Branch/Health Effects Research Division of the U.S. Army Biomedical Research and Development Laboratory, Fort Detrick, Frederick, Maryland, for their continual support and helpful discussions throughout the development of the manual. We thank Dr. William H. van der Schalie, currently at the U.S. Environmental Protection Agency, for initiating the study, from which the manual evolved. We acknowledge the Research Methods Branch/Health Effects Research Division of the U.S. Army Biomedical Research and Development Laboratory for supporting the study via Navy Contract No. N00039-89-C-0001 to The Johns Hopkins University Applied Physics Laboratory. We would also like to thank the University of Maryland System, Maryland Institute for Agriculture and Natural Resources, Agricultural Experiment Station, Wye Research and Education Center for partial support during the writing of this manual.

TABLE OF CONTENTS

LIST OF FIGURES

SECTION 1

INTRODUCTION

The U.S. Army Biomedical Research and Development Laboratory (USABRDL) Aquatic Biomonitoring Trailer Version 1.0 is a mobile laboratory which employs several biological biomonitoring systems for hazard assessment of potentially contaminated wastewater, complex effluents, and groundwater. As a mobile laboratory, the trailer may be easily transported to various test sites which have adequate diluent water and power services. The trailer is specifically designed for the evaluation of water soluble compounds; the laboratory is not equipped with a ventilation system for testing highly volatile compounds. Several tests may be conducted within the Aquatic Biomonitoring Trailer in order to examine toxicological effects at several levels of biological organization. Tests routinely conducted include: Microtox®, teratogenicity, carcinogenicity, and continuous biological monitoring (fish ventilatory early warning system).

The biological early warning system is a continuous, real time automated fish ventilation monitoring system developed by W. H. van der Schalie and T. R. Shedd of USABRDL for rapid detection (<15 min is some cases) of acute toxicity changes in aquatic media. In addition to the continuous monitoring of changes that may occur in fish ventilatory activity, changes in acute toxicity may be monitored with grab samples or composite samples up to 24 hours in duration via Microtox®. Teratogenicity is examined by a 96-hour flow-through frog embryo teratogenesis assay (FETAX) involving the African clawed frog (Xenopus laevis). Carcinogenicity is evaluated through six month exposure tests involving the Japanese medaka (Oryzias latipes). Routine water quality is conducted during all tests. Composite samples are taken for off-site mutagenicity (Ames) testing and chemical analyses.

This operations manual has been designed to provide a very specific overview of the Aquatic Biomonitoring Trailer. Included in the manual is a general description of the trailer, initial utility, diluent water, test material, and waste drainage hook-up of the trailer, types of tests/assays performed, recommended water quality tests to be performed, daily trailer maintenance/operations, and complete shut-down of the trailer for site relocation. Incorporated into the manual are several procedures which explain in detail the operation of the various pieces of equipment and systems that are utilized during testing in the trailer. In addition, several diagrams are included as visual representations of the trailer and its associated equipment.

SECTION 2

GENERAL DESCRIPTION OF THE BIOMONITORING TRAILER

The aquatic biomonitoring trailer version 1.0 is an 8'x 24' mobile laboratory. The laboratory is divided into two compartments (See Figure 1). The small room (8'x 5') is used primarily to isolate fish for the ventilatory biological monitoring test. The large room (8'x 19') is a two-tiered multipurpose room used for carcinogenicity, teratogenicity, and water quality testing, storage of test materials, and data acquisition.

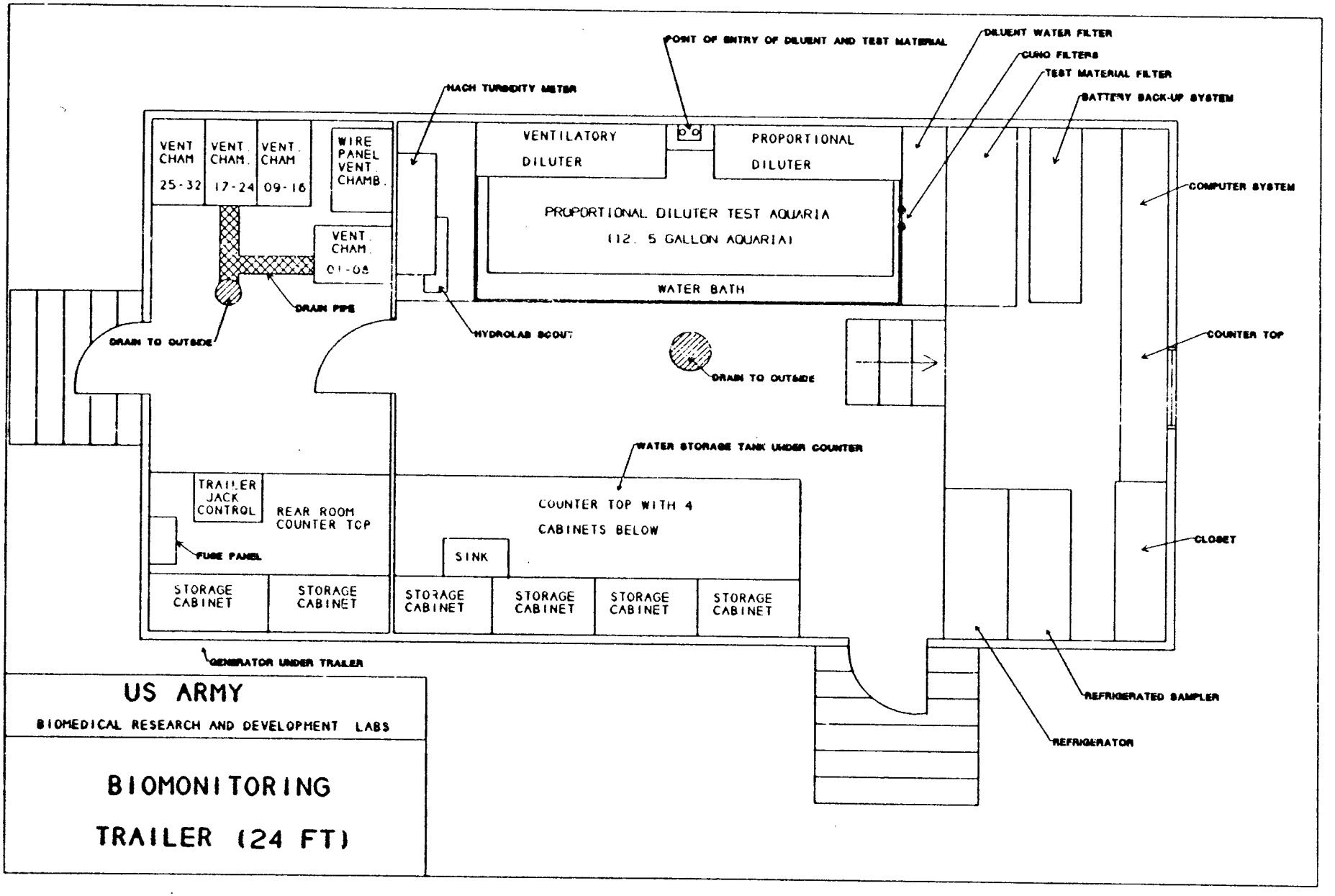
The small room contains the ventilatory chamber holding boxes, ventilatory chambers, storage cabinets, control panel for the trailer leveling jacks, the fuse box, and a door for outside access.

The large room contains the proportional and ventilatory biological monitoring dilutors, automated water quality testing/sampling equipment, constant temperature water bath, battery back-up system for the computer which collects data from the ventilatory test, particle filtration filters for the diluent and test material systems, a refrigerator, storage cabinets, and two doors (one for outside access, the other for access to the small room).

The drain system is located beneath the trailer. There are a number of locations inside the trailer, where the drains are plumbed through the floor (i.e., water bath and the center of the floor in the large and small rooms). These provide discharge of the diluent water and test material used during testing.

The diluent water system, which includes: the diluent water source, 10 micron fine particle filters, Culligan® carbon filtration system, 150-gallon aeration/equilibration tanks, aeration system, and centrifugal pump are housed separate from the trailer due to its lack of space. The test material system, which includes: the test material source, submersible pump, and stainless steel "countercurrent heat exchanger" is also housed separately from the trailer for the same reasons. Both the diluent water and test material are delivered to the trailer via 3/4" PVC pipe from the building housing the diluent and test material water systems. For additional explanation see Section 3.

Figure 1. Internal layout of the aquatic biomonitoring trailer.


POINT OF ENTRY OF DILUENT AND TEST MATERIAL
DILUENT WATER FILTER
CUNO FILTERS
TEST MATERIAL FILTER
BATTERY BACK-UP SYSTEM
HACH TURBIDITY METER
VENT CHAM 25-32
VENT CHAM. 17-24
VENT CHAM 09-16
WIRE PANEL VENT CHAMB.
VENTILATORY DILUTER
PROPORTIONAL DILUTER
COMPUTER SYSTEM
PROPORTIONAL DILUTER TEST AQUARIA
(12. 5 GALLON AQUARIA)
VENT CHAM. 01-08
WATER BATH
DRAIN PIPE
DRAIN TO OUTSIDE
HYDROLAB SCOUT
DRAIN TO OUTSIDE
COUNTER TOP
WATER STORAGE TANK UNDER COUNTER
TRAILER JACK CONTROL
REAR ROOM COUNTER TOP
COUNTER TOP WITH 4 CABINETS BELOW
FUSE PANEL
SINK
CLOSET
STORAGE CABINET
STORAGE CABINET
STORAGE CABINET
STORAGE CABINET
STORAGE CABINET
STORAGE CABINET
GENERATOR UNDER TRAILER
REFRIGERATED SAMPLER
REFRIGERATOR
US ARMY
BIOMEDICAL RESEARCH AND DEVELOPMENT LABS
BIOMONITORING
TRAILER (24 FT)

SECTION 3

INITIAL TRAILER SITE INSTALLATION

This section describes the procedures followed during the initial utility installation process. Included are electrical specifications, diluent water plumbing, test material plumbing, and waste drainage hook-up.

3.1 Electrical Specifications

The biomonitoring trailer requires a 240 volt (single phase), 100 amps power supply, which is provided on site by direct hookup to an established source of electricity.

3.2 Diluent Water Plumbing

Freshwater (normally potable tap water is used; however, other sources may be used) is routinely routed through two 10 micron filters, a Culligan® carbon filtration system, two 150-150-gallon aerated diluent water holding tanks to achieve greater than 90% dissolved oxygen saturation, and then pumped into the trailer (via a centrifugal pump capable of delivering a minimum of 2 gallons/minute) through 3/4 inch PVC line.

3.3 Test Material Plumbing

Test material is pumped from the source (via a submersible, deep well pump capable of delivering a minimum of 2 gallons/minute at 0 ft head) through 3/4 inch PVC, then through 3/8 inch I.D. coiled stainless steel tubing which is submerged in one of the 150-gallon diluent water aeration tanks for heat equilibration purposes, and then into the trailer through 3/4 inch PVC tubing.

3.4 Waste Drainage Hook-up

The method of water disposal from the biomonitoring trailer is dependent upon the characteristics of the test water at the study site. The waste disposal method that is to be employed should be specified in the study protocol.

SECTION 4

TESTS/ASSAYS PERFORMED

This section describes the various tests/assays that are routinely conducted in the aquatic biomonitoring trailer.

4.1 Microtox®

4.1.1 Purpose

The Microtox® test is a rapid acute toxicity test that may be completed in less than one hour. This section describes the procedures followed when using the Microtox® Model 500 system to measure a specific test sample(s) level of toxicity.

4.1.2 Utilizing the Microtox® Computer Program - Version 5.20

1) Integrate a computer (See Section 4.1.4) with the Microtox® Model 500 unit by connecting the cable provided between the RS232 port on the back of the Microtox® unit and COM port on the computer.

2) Turn the computer on and insert a working copy (DO NOT INSERT THE ORIGINAL COPY; THE ORIGINAL COPY PROVIDED BY MICROBICS IS WRITE PROTECTED) of the Microtox® Data Capture and Recapture Program Version 5.20 (MDCRP v 5.20) into the disk drive.

3) At the prompt type: gwbasic mtx, and wait for the MDCRP v 5.20 screen to appear. Once the screen has appeared press the space bar, thus accessing the Microtox® menu screen.

4) Once you are in the Microtox® menu screen select number 1 to begin testing. Information describing the test to be done will now be required. The computer will ask for the following information:

   1. Number of assays (1-4) _____
   2. Number of dilutions (3-7) _____
   3. Assay times: time 1 _____ time 2 _____
   4. File name _____
   5. Description _____

A typical standard, single assay procedure for a sample of treated wastewater effluent taken at Site X on December 6, 1990 would be entered as follows:

1. Number of assays (1-4) 1
2. Number of dilutions (3-7) 4
3. Assay times: time 1 5 time 2 15
4. File name 1206901
5. Description SITE X EFFLUENT DEC 06 1990

5) After entering the aforementioned information, a screen displaying the current default parameters will appear. The parameters should appear as follows:

1. Number of dilutions: 4
2. Initial concentration: 45
3. Dilution factor: 2
4. Units: Percent
5. Ionic adjustment: MOAS
6. Procedure: Standard
7. Default ON/OFF: OFF

In the event that the parameters are not as they are depicted above, they may be corrected by typing in the number of the parameter and then replacing it with the appropriate information. Once the parameters are set press enter.

6) The MDCRP v 5.20 is now ready to accept data from the Microtox® unit.

4.1.3 Microtox Standard Single Assay Procedure

1) Plug in the Microtox® Model 500 unit and allow it to warm up until the red temperature light goes out and the green temperature light comes on.

2) While waiting for the Microtox® unit to warm up, fill a 1000 ml Nalgene bottle with the test sample collected over the previous 24 hours by the ISCO® sampler.

3) Place Microtox® glass cuvettes in rows A (1-5), B (1-5), and one cuvette in the Reagent well. For example:

```
1     2     3     4     5

<-------5 cuvettes-------->   A
<-------5 cuvettes-------->   B
-------------------------     C   Reagent Well
-------------------------     D
-------------------------     E
-------------------------     F

          Read Well
```

4) Once the green temperature light has come on, indicating that the Microtox® unit is warmed up, the assay procedures may begin.

5) Using a 1000 µl micropipetter equipped with the proper disposable pipette tip, place 1000 µl of Microtox® Reconstitution Solution into the glass cuvette in the Reagent Well. Discard pipette tip.

6) Using a 500 µl micropipetter equipped with the proper disposable pipette tip, place 500 µl of Microtox® Diluent into each of the 5 cuvettes in row B (1-5). Discard disposable tip.

7) Using a 1000 µl micropipetter place 1000 µl of Microtox® Diluent into cuvettes A1, A2, A3, and A4. Discard disposable tip.

8) Using a 250 µl micropipetter equipped with the proper disposable pipette tip, place 250 µl of Microtox® Osmotic Adjusting Solution into cuvette A5. Discard disposable tip.

9) Using a 1 - 5 ml macropipetter equipped with the proper disposable pipette tip, place 2.5 ml of the sample to be tested (e.g., effluent) into cuvette A5. Discard disposable tip.

10) Mix the sample in cuvette A5 10 times using a 500 µl micropipetter. Discard disposable tip.

11) Using a 1000 µl micropipetter, transfer 1000 µl of the sample in cuvette A5 to cuvette A4. Discard disposable tip.

12) Mix the sample in cuvette A4 10 times using a 500 µl micropipetter. Discard disposable tip.

13) Using a 1000 µl micropipetter, transfer 1000 µl of the sample in cuvette A4 to cuvette A3. Discard disposable tip.

14) Mix the sample in cuvette A3 10 times using a 500 µl micropipetter. Discard disposable tip.

15) Using a 1000 µl micropipetter, transfer 1000 µl of the sample in cuvette A3 to cuvette A2. Discard disposable tip.

16) Mix the sample in cuvette A2 10 times using a 500 µl micropipetter. Discard disposable tip.

17) Wait 5 minutes for temperature equilibration.

18) At the end of the 5-minute waiting period, obtain a vial of the Microtox® Reagent stored in a freezer. Open the vial and pour the Microtox® Reconstitution Solution contained in the cuvette in the Reagent Well into the vial. Swirl, and then carefully pour this solution back into the cuvette in the Reagent Well. Mix the solution contained in the cuvette in the Reagent Well 20 times using a 500 µl micropipetter. Discard disposable tip.

19) Using a 10 µl micropipetter equipped with the proper disposable pipette tip, place 10 µl of the solution contained in the Reagent Well into cuvettes B1 - B5. Discard disposable tip after each transfer.

20) Mix the solutions contained in cuvettes B1 - B5 5 times each using a 250 µl micropipetter. Discard disposable tip after mixing each cuvette.

21) Wait 15 minutes for temperature equilibration.

22) At the end of the 15-minute waiting period, place cuvette B1 into the Read Well of the Microtox® unit. Press the set button (DO NOT PRESS THE SET BUTTON AFTER THIS STEP). Once the cuvette reappears, press the read button. After the reading is taken on cuvette B1, place it back into the B1 slot. Now take readings on cuvettes B2 - B5 respectively by placing each one of the cuvettes into the Read Well and then pressing the read button. Assuming a computer has been integrated with the Microtox® unit, the values on the Microtox® unit's digital display will automatically be entered into the computer. If a computer is not integrated with the Microtox® unit, the data must be recorded on a data sheet so that it may be entered manually after the test has been completed.

23) When all of the readings are completed, the Microtox® unit will begin to beep. At this point several sample transfers must be executed.

24) Using a 500 µl micropipetter, transfer 500 µl from cuvette A1 to cuvette B1. Mix 3 times. Discard disposable tip.

25) Using a 500 µl micropipetter, transfer 500 µl from cuvette A2 to cuvette B2. Mix 3 times. Discard disposable tip.

26) Using a 500 µl micropipetter, transfer 500 µl from cuvette A3 to cuvette B3. Mix 3 times. Discard disposable tip.

27) Using a 500 µl micropipetter, transfer 500 µl from cuvette A4 to cuvette B4. Mix 3 times. Discard disposable tip.

28) Using a 500 µl micropipetter, transfer 500 µl from cuvette A5 to cuvette B5. Mix 3 times. Discard disposable tip.

29) Press the space bar on the computer keyboard immediately after the sample transfers are completed.

30) Once the computer timer reaches the 5-minute mark it will beep 3 times. When the word "enter" appears on the computer screen place cuvette B1 into the Read Well, and then press the read button. After the reading is taken, place cuvette B1 back into its respective slot. Follow the same procedure to take readings for cuvettes B2 - B5.

31) When the computer timer reaches the 15-minute mark it will beep 3 more times. Follow the same procedures outlined in step number 30 to take readings for cuvettes B1 - B5.

32) The assay is now complete. Unplug the Microtox® unit (it is not equipped with an on/off switch) and discard the glass cuvettes.

33) The screen will now revert back to the menu screen. In order to review the test results, press number 5 (run statistics on a data file), and then type in the file to be reviewed. For example, to review the results of a sample taken on December 6, 1990 type: 1206901.05 (12=month, 06=day, 90=year, 1=number of sample, and .05= 5-minute assay) to review the 5-minute assay or 1206901.015 to review the 15-minute assay. Any file that is created when running a test sample will automatically be stored in the MDCRP v 5.20 memory.

34) The MDCRP v 5.20 when integrated with the Microtox® Model 500 unit allows up to 3 samples to be tested at once. Please refer to the Microtox® Manual for further instructions when testing multiple samples.

4.1.4 Apparatus and Materials

1) Hardware - A DOS-based PC computer; either a 3 1/2" and/or 5 1/4" disk drive; monitor; computer cable capable of connecting the Microtox® Model 500 unit to disk dri ; and a printer (in the event that the printing of test results is required).

2) Software - Microtox® Data Capture and Recapture Program Version 5.20.

3) Microtox® test materials - 50 ml bottle of Microtox® Reconstitution Solution; 50 ml bottle of Microtox® Osmotic Adjusting Solution; 1000 ml bottle or 8-16 50 ml bottles of Microtox® Diluent; 40 vials of Microtox® Reagent; and 2 boxes of 360 each Microtox® disposable glass cuvettes.

4) Pipetters and related supplies - 10 µl micropipetter; 250 µl micropipetter; 500 µl micropipetter; 1000 µl micropipetter; and a 1 - 5 ml macropipetter. Non-sterile; disposable pipette tips.

4.1.5 References

1) Microtox® Manual, 1988. How To Reduce Microtox Test Data. Microbics Corporation, Carlsbad, CA.

2) Microtox® Manual, 1988. How To Run Toxicity Tests Using The Microtox® Model 500. Microbics Corporation, Carlsbad, CA.

4.2 Ames

4.2.1 Purpose

The Ames test is a microbial genotoxicity assay that is conducted on a periodic basis to predict chemical mutagenic activity in the test media and diluent water. Both unconcentrated and samples concentrated 10-fold are normally analyzed. The Ames test is not conducted in the biomonitoring trailer.

4.2.2 Sampling Method

Twentyfour-hour composite samples (amount to be specified by laboratory) of test media and diluent water are taken via refrigerated ISCO® samplers.

4.2.3 Sample Preparation for Delivery to Laboratory

The samples are divided into appropriate subsamples which are specified by the laboratory performing the Ames assays and placed on ice for delivery to the laboratory.

4.3 FETAX

4.3.1 Purpose

The frog embryo teratogenesis assay - Xenopus (FETAX) is a short-term (96-hour) quantitative teratogen assay used to screen for developmental toxicants in aquatic media. The assay can be conducted under continuous flow or static renewal test conditions.

4.3.2 Laboratory Preparations

Ninetysix-hour FETAX tests are normally scheduled to begin on a Monday and end on a Friday. In order to begin the test on a Monday, 3 Xenopus breeding pairs must be injected with human chorionic gonadotropin (HCG) during the early afternoon (e.g., 2:00 P.M.) on the day prior to test initialization. By 8:00 A.M. Monday morning, the frogs will have laid and fertilized the eggs. Each breeding pair should produce approximately 2000 eggs (6000 total eggs between the 3 breeding pairs). A total of 2000 eggs, from a single breeding pair only, are needed to provide embryos for a test. Eggs from the remaining 2 pairs should be disposed of properly. Injecting 3 breeding pairs rather than 1 breeding pair ensures that approximately 2000 eggs will be obtained, in the event that 1 or more of the breeding pairs have trouble with egg production. The eggs are then placed in an Erlenmeyer flask containing FETAX to begin test egg selection by 10:00 A.M. Monday morning. At this time the eggs should be between Normal Stage 8 Blastulae and Normal Stage 11 Gastrulae. Approximately one hour remains to prepare and select the eggs to be used in the test.

4.3.3 On-Site Test Egg Selection

1) The 2000 eggs to be selected from should be suspended in FETAX solution (See Section 4.3.6 for reference) in a 250 ml Erlenmeyer flask.

2) Prepare 200 ml of a 2.0% L-cysteine solution (2.0 g of Cysteine per 98.0 ml of FETAX solution) in order to de-jelly the eggs.

3) Raise the pH of the L-cysteine solution to 8.10. This is accomplished by adding 10 N NaOH drop by drop, swirling the solution, and checking the pH after each drop. If necessary, once the pH approaches 8.10 add 1 N NaOH drop by drop and swirl until the pH = 8.10.

4) Decant the FETAX solution from the 250 ml Erlenmeyer flask, while making sure not to pour off any of the eggs.

5) Pour the L-cysteine solution into the 250 ml Erlenmeyer flask containing the eggs. Swirl the eggs for approximately 2.5 minutes (not more than 3 minutes or the eggs will begin to disintegrate). The solution should appear more and more cloudy as the jelly coat surrounding each egg is removed. At the 2.5 minute mark, stop swirling and allow the eggs to settle on the bottom of the flask. Immediately pour off the L-cysteine solution.

6) Add FETAX solution to the eggs, swirl briefly, and decant. Repeat this procedure 2-4 times to ensure that the L-cysteine solution is completely removed from the eggs and the flask. After the final egg rinse, re-suspend the eggs in FETAX solution.

7) It is now time to begin the double selection process. 300 of the approximately 2000 eggs will be needed for the test (12 beakers/1 per tank @ 25 eggs per beaker = 300 eggs). Separate the eggs into 4 or 5 large plastic petri dishes containing FETAX solution (approximately 400 eggs per dish). Immediately remove any eggs which are white, inconsistent in color or shape, or deformed in any way. Next, using a dissecting microscope equipped with a light source carefully but quickly remove all Normal Stage 8 Blastulae. Place them in their own plastic petri dish filled with FETAX solution. Set the bad eggs aside to be disposed of later. Three hundred eggs must now be chosen from the Normal Stage 8 Blastulae initially selected (thus the double selection process).

8) The test eggs are placed in 12, 250 ml fine plastic mesh bottomed beakers (25 eggs per beaker) when the organisms are going to be tested under continuous flow conditions. The beakers (1 beaker per aquarium) are suspended by a stainless steel wire harness in the 5 gallon aquaria utilized in the 6-month carcinogenicity test (Section 4.4). The 12 aquaria are normally divided into 3 test concentrations (the concentrations will be specified in the study protocol).

4.3.4 Post-Test Procedures

1) At the end of the 96-hour test the Xenopus tadpoles are anesthetized in 12 separate petri dishes containing MS-222. Examine the tadpoles for heart abnormalities after exposure to the MS-222, but before formalin fixation. Abnormalities are easier to visualize while blood is still circulating through the heart. Next, place the tadpoles into 12 separate 20 ml scintillation vials containing a 3.0% formalin solution. Each vial

should be clearly marked with the beginning and ending test date and the specific tank concentration. The 3.0% formalin solution preserves the Xenopus tadpoles, for morphological analysis which can be performed in the biomonitoring trailer or off-site.

4.3.5 Apparatus and Materials

1) The following materials are needed to perform a FETAX test in the biomonitoring trailer: 2000 Xenopus eggs between Normal Stage 8 Blastulae and Normal Stage 11 Gastrulae; 1 liter of FETAX solution; 2 g of L-cysteine; 20 ml of 1 N and 10 N NaOH; pH meter; 250 ml Erlenmeyer flask; dissecting microscope equipped with light source; 12 250 ml beakers with the bottoms cut out and replaced with a fine mesh plastic screen; stainless steel wire used to suspend the 250 ml beakers from the sides of the 5 gallon aquaria; 20 ml scintillation vials with teflon screw on caps; 3.0% formalin solution; 10 ml disposable pipets; disposable petri dishes; disposable eye droppers; disposable rubber gloves; lab coat; and protective eyewear.

4.3.6 References

1) Bantle, J.A., J.N. Dumont, R.A. Finch, and G.J. Linder. 1991. Atlas of Abnormalities - A Guide for the Performance of FETAX. Oklahoma State Publications Department, Stillwater, Ok.

4.4 Carcinogenicity

4.4.1 Purpose

The Japanese medaka (Oryzias latipes), which has been shown to be a sensitive laboratory carcinogen model, is used to test for environmental pollutants which may induce neoplasms. Both unexposed and embryos initiated with diethylnitrosamine (DEN) are used in 6-month continuous exposure assays.

4.4.2 Pre-Test Procedures

1) See Section 8.1 - Proportional Dilutor.

2) Place a temperature probe connected to a continuous strip chart temperature recorder into the centermost tank in the water bath.

4.4.3 Test Organism Delivery to the Trailer

Both unexposed and embryos exposed to DEN are reared off-site until the test is started in the biomonitoring trailer. Japanese medaka, 22-25 days old, are transported to the trailer in 12 separate 1000 ml mesh bottomed beakers (approximately 60 fish per beaker), which are suspended in 10 gallon aquaria. The water bath in the biomonitoring trailer is maintained at 25°C. In addition, a temperature probe from a strip chart temperature recorder constantly monitors/records the temperature of the water bath. In the event the temperature fluctuates, the laboratory rearing the organisms is asked to acclimate the fish to the current temperature to avoid unnecessary acclimation procedures in the trailer.

4.4.4 Loading Test Organisms

The 1000 ml beakers containing the fish delivered to the trailer are labeled 1-12, respectively. Upon delivery, the tanks in the trailer are labeled to match the beakers (1 beaker per tank). The beakers are suspended into the 5 gallon aquaria by a wire. The fish should remain in the beakers for one week, in order to allow them to adjust to their new environment and to monitor the small fish for mortality. In addition, keeping them in the beakers also prevents these small test organisms from being sucked through the screen which covers the drain opening. After the one week "acclimation period," the Japanese medaka are released into the tanks.

4.4.5 Daily Test Operations

1) The number of test organisms alive in each tank is monitored and recorded daily.

2) Record the temperature of the centermost tank on the strip chart recorder (Be certain to record the temperature, current date and military time, and strip chart block hour directly onto the strip chart recorder's thermograph paper).

3) Calculate the dilutor cycle time. When necessary, adjust the cycle time by following the instructions outlined in Section 8.1.2.

4) Measure the amount of diluent water or test material each tank receives once a week. Volumes delivered to each stainless steel flow splitting cup and each 5 gallon aquarium may be adjusted by following the instructions outlined in Section 8.1.2.

5) When noticeable flow restrictions occur, clean out the silicon and stainless steel tubes and the solenoid valves.

6) Clean all algae or test material debris from the dilutor when a build-up on the glass fixtures of the dilutor occurs.

7) The remaining daily test operations are discussed in Section 5 - Water Quality Tests.

8) Preserve any fish that die or appear moribund during the 6-month period (See Section 7.5).

4.4.6 Test Organism Husbandry

See Section 7.2 - Feeding Japanese Medaka; Section 7.3 - Brine Shrimp Cultures; Section 7.4 - Aquarium Maintenance; and Section 7.5 - Preserving Dead or Moribund Japanese Medaka.

4.4.7 Post-Test Procedures

1) Follow the clean-up procedures that are outlined in Section 8.1.3.

2) All test organisms are returned to USABRDL, Health Effects Research Division, Ft. Detrick, Frederick, Maryland 21701-5010 for further testing and analysis.

3) For additional information see the specific test protocol, which outlines test procedures, provided by USABRDL prior to testing.

4.4.8 Apparatus and Materials

1) Proportional dilutor and associated equipment - 7 stainless steel flow splitting cups; 10 pairs of flow reducers; and ≈ 100′ of 3/16" I.D. silicon tubing.

2) Water bath and associated equipment - 12-15 5 gallon aquaria; drains for the aquaria; 2 Little Giant® circulation pumps; 2 heater/circulators; 2 strip-chart temperature recorders equipped with temperature probes.

3) Aeration system - 12-15 air stones (1.5"x 0.5" silica; glass bonded air diffusers); 3 5 prong aquaria gang valves; 3/16" O.D. Tygon air tubing; 3 standard aquarium air pumps.

4.5 Ventilatory Biological Monitoring

4.5.1 Purpose

The ventilatory biological monitoring system is a real-time continuous monitoring system designed to detect unexpected abrupt changes in water quality or episodic events which may be harmful to the environment. The system uses changes in fish ventilation frequency, opercular amplitude, and cough frequency to predict acute toxicological effects. Individual fish in two control and two experimental groups of 8 fish/group (total of 32 fish) are monitored for a period of 21 days during a typical ventilatory test.

4.5.2 Pre-Test Procedures

See Section 8.2 - Ventilatory Biological Monitoring Dilutor.

4.5.3 Test Organism Delivery to the Trailer

Approximately 70 bluegill (Lepomis macrochirus) are delivered to the biomonitoring trailer in an aerated container no less than two weeks prior to a ventilatory test. The fish are acclimated for two weeks in the diluent water prior to the test.

4.5.4 Test Organism Husbandry

See Section 7.1 - Feeding Bluegill and Section 7.4 - Aquarium Maintenance.

4.5.5 Loading Test Organisms

1) Select 32 bluegills, between 2.5"- 3.5" in length, to be used as test subjects.

2) Randomly place (follow a randomization program or a random number chart) 1 bluegill in each of the 32 ventilation chambers. Preferred position is facing toward the water input side of the test chamber (See Figure 9).

3) Place the removable cover associated with each chamber over the top of the bluegill and carefully attach a strip of tape over the cover to prevent the fish from jumping out of the test chamber.

4) Place a temperature probe from a continuous temperature strip chart recorder into the water input section of one of the test chambers. The temperature can be monitored throughout the test from the recorder.

5) Connect each test chamber to their designated leads (leads are numbered from 1 to 32), in order to ensure that individual gill ventilation from each test chamber is continuously monitored by the aquatic biomonitoring program during the test.

6) The signal sent by each test chamber should be checked for crispness and clarity on the computer and oscilloscope before beginning the test. Refer to USABRDL's Aquatic Biomonitoring Program User's Manual - Version 2.0 for information on signal detection.

7) Once all signals are found to be test worthy, place the specially designed covers onto the ventilation chamber holding boxes. The ventilation chambers should not be touched after this point in time.

8) The 21-day ventilatory test can now be started. Refer to USABRDL's Aquatic Biomonitoring Program User's Manual - Version 2.0 for directions on the implementation of the test.

4.5.6 Daily Test Operations

1) Check computer and printer operation. Print computer screen upon entering trailer (Shift Prt Sc). Log the entry time (military and block hour) of the most recent printout in the trailer entry/exit log.

2) Check the ventilatory data for the 32 bluegills. Turn on the oscilloscope, type ea, and then b1,1 on the computer keyboard. This allows the ventilatory response of each specific test subject to be viewed on the oscilloscope. In order to view each successive fish, type n and then press the enter key. Enter observations of each test subject's breathing patterns into the Ventilatory Test log.

3) Calculate and time the cycle times for each of the 4 dilutors (range 00:00:55.00 to 00:01:05.00). Enter all required information into the specific cycle time logs for each dilutor. Adjust cycle times as needed by following directions given in Section 8.2.2.

4) Inspect the high and low electrodes located in each mixing chamber. Clean as needed.

5) Ensure that all solenoids are working, and that all lines are flowing properly.

6) If a group of fish is out of control, pull a water sample from the section of the ventilatory biological monitoring dilutor which supplies the out of control group for further analysis. In addition, refer back to steps 1-5 of this section to ensure that the out of control response is not due to a mechanical error.

7) Print screen when leaving the trailer (Shift Prt Sc). Log the exit time (military and block hour) of the most recent printout in the trailer entry exit log.

8) Refer to Section 5 - Water Quality Tests, for the remaining daily test operations.

4.5.7 Post-Test Procedures

1) Weigh and measure each bluegill. Record this information on the designated data sheet.

2) Measure the volumes of diluent water and test material delivered to each ventilation chamber. Record this information on the designated data sheet.

3) Follow the procedures outlined in USABRDL's Aquatic Biomonitoring Program User's Manual - Version 2.0 for final test shutdown of the computer.

4) Follow the clean-up procedures outlined in Section 8.2.3.

4.5.8 Apparatus and Materials

1) Ventilatory biological monitoring dilutor and associated equipment: 40 ventilatory chambers; 4 ventilatory chamber holding boxes; 16 stainless steel flow splitting cups; 20 pairs of flow reducers; ≈ 200' of 3/16" I.D. silicon tubing or ≈ 20 10' sections of 1/4" O.D. stainless steel tubing (the type of tubing to be used will be specified in the test protocol).

2) Hardware - A DOS-based PC computer; either a 3 1/2" and/or 5 1/4" disk drive; monitor; and a printer (completely integrated computer system).

3) Software - USABRDL's Aquatic Biomonitoring Program (See Section 4.5.9).

4) Strip-chart temperature recorder equipped with a temperature probe.

4.5.9 References

1) Aquatic Biomonitoring Program User's Manual - Version 2.0, 7 August 1991. U.S. Army Biomedical Research and Development Laboratory (USABRDL), Ft. Detrick, Frederick, MD.

4.6 Chemical Analyses

4.6.1 Purpose

Chemical analyses are conducted on a periodic basis to quantify the chemicals present in the test media. Identical tests are normally performed on both the diluent water and test media. The following chemical analyses are normally performed: manganese, sulfate, alkalinity, aluminum, arsenic, barium, boron, cadmium, calcium, cobalt, copper, iron, lead, beryllium, magnesium, mercury, molybdenum, nickel, potassium, selenium, sodium, silver, cyanide, fluoride, sulfide, volatile organics, acid/base/neutral organics, pesticides/PCB, and herbicides.

4.6.2 Sampling Method

Twentyfour-hour composite samples (volume to be specified by the laboratory performing the analyses) of test media and diluent water are taken via refrigerated ISCO® samplers.

4.6.3 Sample Preparation for Delivery to Laboratory

The samples are divided into appropriate subsamples which are specified by the laboratory performing the chemical analyses and placed on ice for delivery to the laboratory.

SECTION 5

WATER QUALITY TESTS

Both manual and automated water quality measurements are made when toxicity studies are being conducted in the aquatic biomonitoring trailer. The manual water quality tests are conducted primarily on grab samples taken from various carcinogenicity aquaria or from the dilutor supplying a ventilatory test. Section 5 describes the manual water quality tests. Section 8 describes the automated water quality tests. The following manual tests are performed on both the diluent water and test material: alkalinity, ammonia-nitrogen, chlorine, conductivity, dissolved oxygen, hardness, pH, and temperature. Also included is the frequency of testing, recommended testing schedule, and recording of test results.

5.1 Tests Performed and Methods Employed

5.1.1 Alkalinity

Alkalinity may be measured by following Standard Method 2320 B. Titration Method (APHA et al. 1989).

5.1.2 Ammonia-Nitrogen

Ammonia-Nitrogen may be measured by following Standard Method 4500-NH_3. Ammonia Selective Electrode Method (APHA et al. 1989).

5.1.3 Chlorine

Chlorine may be measured by following Standard Method 4500-Cl G. DPD Colormetric Method (APHA et al. 1989).

5.1.4 Conductivity

Conductivity may be measured by following Standard Method 2510 B. Laboratory Method (APHA et al. 1989).

5.1.5 Dissolved Oxygen

Dissolved Oxygen may be measured by following Standard Method 4500-O G. Membrane Electrode Method (APHA et al. 1989).

5.1.6 Hardness

Hardness may be measured by following Standard Method 2340 C. EDTA Titrimetric Method (APHA et al. 1989).

5.1.7 pH

pH may be measured by following Standard Method 4500-H^+ B. Electrometric Method (APHA et al. 1989).

5.1.8 Temperature

Temperature may be measured by following Standard Method 2550 B. Laboratory and Field Methods (APHA et al. 1989).

5.2 Frequency of Testing

Alkalinity, ammonia-nitrogen, chlorine, conductivity, and hardness are usually measured twice a week (all tests should be performed together on the same days), with an allotment of 3-4 days between sample measurements. Dissolved oxygen, pH, and temperature should be measured once daily.

5.3 Recommended Testing Schedule

1) Sunday through Saturday - DO, pH, and temperature should be measured in:

   - the 12 aquaria used in a carcinogenicity test
   - one stainless steel flow splitting cup containing diluent water (ventilatory test)
   - one stainless steel flow splitting cup containing test material (ventilatory test)

2) Tuesday - Alkalinity, ammonia-nitrogen, chlorine, conductivity, and hardness should be measured in:

   - the 6 even numbered aquaria used in a carcinogenicity test
   - one stainless steel flow splitting cup containing diluent water (ventilatory test)
   - one stainless steel flow splitting cup containing test material (ventilatory test)

3) Friday - Alkalinity, ammonia-nitrogen, chlorine, conductivity, and hardness should be measured in:

   - the 6 odd numbered aquaria used in a carcinogenicity test
   - one stainless steel flow splitting cup containing diluent water (ventilatory test)
   - one stainless steel flow splitting cup containing test material (ventilatory test)

4) Note - Microtox® samples taken on Tuesday and Friday should also be measured for their ammonia-nitrogen and

chlorine contents.

5.4 Recording Test Results.

1) Carcinogenicity Test - All water chemistry results should be entered into the carcinogenicity test individual tank water chemistry data sheet (Figure 2) by date, cumulative test day, and time of sample measurement.

2) Ventilatory Test - All water chemistry results should be entered into the 21-day ventilatory biological monitoring test water chemistry data sheet (Figure 3) by date, cumulative test day, and time of sample measurement.

3) Microtox® Sample - Ammonia-nitrogen and chlorine values obtained from the Microtox® sample(s) should be entered into the Microtox® test results and associated chlorine and ammonia-nitrogen values data sheet (Figure 4) by date and cumulative test day.

5.5 References

1) APHA et al. (American Public Health Association, American Water Works Association, and Water Pollution Control Federation). 1989. Standard Methods For The Examination Of Water And Wastewater. 17th ed. American Public Health Association, Washington, DC.

Figure 2. Carcinogenicity test individual tank water chemistry data sheet.

CARCINOGENICITY TEST
INDIVIDUAL TANK WATER CHEMISTRY DATA SHEET

Test Designation: _____ Tank Concentration: _____

Tank No.: _____ DEN Exposure: Yes_____ No_____

Date	Cummu-lative Test Day	Time (Mil)	Temp (°C)	pH	DO (mg/L)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)	Conductivity (μmhos/cm)	Total Residual Chlorine (mg/L)	Free Available Chlorine (mg/L)	Total Ammonia-Nitrogen (mg/L)	Total Unionized Ammonia-Nitrogen (mg/L)	Initials

Figure 3. 21-day ventilatory biological monitoring test water chemistry data sheet.

21-day VENTILATORY BIOLOGICAL MONITORING TEST
WATER CHEMISTRY DATA SHEET

Test Designation: _____ **Stainless Steel Cup No.:** _____

Ventilation Dilutor No.: _____ **Feed For Ventilatory Chambers:** _____ **And** _____

Test Material: _____

Date	Cummulative Test Day	Time (Mil)	Temp (°C)	pH	DO (mg/L)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)	Conductivity (μmhos/cm)	Total Residual Chlorine (mg/L)	Free Available Chlorine (mg/L)	Total Ammonia-Nitrogen (mg/L)	Total Unionized Ammonia-Nitrogen (mg/L)	Initials

Figure 4. Microtox® test results and associated chlorine and ammonia-nitrogen values data sheet.

MICROTOX® TEST RESULTS AND ASSOCIATED CHLORINE AND AMMONIA-NITROGEN VALUES DATA SHEET

Test Designation: _____

Test Material: _____

Date	Cummulative Test Day	Microtox 5-min EC50 (% Test Material by Volume)	Microtox 15-min EC50 (% Test Material by Volume)	Total Residual Chlorine (mg/L)	Free Available Chlorine (mg/L)	Total Ammonia-Nitrogen (mg/L)	Total Unionized Ammonia-Nitrogen (mg/L)	Initials

SECTION 6

DAILY TRAILER MAINTENANCE AND OPERATIONS

This section outlines the procedures to be followed during routine maintenance and daily operation of the Exide Electronics Uninterrupted Power Source, Cuno Auto-Klean® filters, in-line diluent water system filters, and Culligan® carbon filtration system.

6.1 Exide Electronics Uninterrupted Power Source

6.1.1 Purpose

The Exide Electronics Series 1000 (S1000) Uninterrupted Power Source (UPS) serves as a back-up source of power for the computer system which monitors and collects data for the Aquatic Biomonitoring Program during a ventilatory test.

6.1.2 Exide Operation

All relevant information for the operation of the S1000 UPS may be found in the Series 1000 Operators Manual, Part # 164-200-035 (REV. A), Exide Electronics, Raleigh, NC.

6.2 Cuno Auto-Klean® Filters

6.2.1 Purpose

The 80 micron Cuno Auto-Klean® filters serve as coarse particle filtration devices for both the diluent and test material water systems. Their primary function is to prevent clogging of the dilutors. This describes the procedures followed when cleaning and/or replacing the filters (CUNO, Inc., Meriden, CT).

6.2.2 Daily Filter Maintenance

1) Both the diluent water system and the test material system are equipped with flow through CUNO Auto-Klean® filters. The filters should be cleaned separately once a day on a daily basis. The cleaning process is a relatively easy task; however, specific directions need to be followed in order to avoid damaging the filters.

2) Open the ball valve located directly below the filter until pressure in the line decreases to 5 psi. This increases the flow of the diluent water or test material through the filter to the drain.

3) Each filter is equipped with a handle located directly above the filter unit. In addition, an adapter which fits over the handle should be setting on top of one of the filters (the handles are easily turned using the adapter). Place the adapter over the handle, and carefully turn the handle in a counterclockwise direction. The handle should be turned 2 complete counterclockwise rotations. It is very important not to force the handle to turn. When resistance is felt, back off in a clockwise direction and then proceed slowly back in a counterclockwise direction

4) After completing the 2 rotations, close the ball valve beneath the filter until a reading of 15 psi is achieved on the pressure gauge located in line in that specific system. Follow the same procedures when cleaning the other filter.

6.2.3 Post-Test Maintenance Procedures

1) Two CUNO Auto Klean® filters are located in the trailer. One filter serves the diluent water system, while the other serves the test material system. Both filters need to be thoroughly cleaned and/or replaced at the end of a 6-month carcinogenicity test. It is much easier to clean and/or replace one filter at a time because they are bolted back to back on a steel frame.

2) Before beginning the cleaning and/or replacement process the diluent water and test material systems must be completely shut off. In addition, the PVC lines running to the trailer should be disconnected at their point of attachment to the trailer.

3) Use a socket, open end wrench, or an adjustable wrench to disconnect the more easily accessible filter from the steel frame.

4) Each filter is plumbed in with 3/4" 40 schedule PVC pipe. There are 3 locations on the filter where the PVC pipe screws into the filter. From the aforementioned locations, the filter is connected directly to 3 PVC lines (1 incoming line, 1 outgoing line, and 1 drain line) by three 3/4" PVC couplings. Once all three 3/4" PVC couplings are unscrewed, the entire filter may be removed from the system.

5) Unscrew the threaded 3/4" PVC male adapters complete with PVC pipe and the couplings from both sides and the bottom of the filter.

6) The filter is now ready to be cleaned and/or replaced. Remove the 4 bolts which secure the casing around the internal filter unit, and loosen the brass bolt located on top of the filter at the base of the handle. Carefully remove the outer casing from the internal filter unit.

7) The filter should now be thoroughly cleaned inside and out. Items which may be helpful in cleaning the filter include: wire brush, flexible aquarium brush, flathead screwdriver, and a hose.

8) After cleaning, re-assemble the filter so it may be re-inserted into the system. The threads of the 3/4" female PVC adapters should be wrapped with new teflon tape before screwing and hand tightening them back into the filter. If the old filter is to be replaced with a new filter, save the old filter so that it may be used as a backup (in the event that the new filter malfunctions).

9) It is advantageous to take the second filter out of line, clean and/or replace it, and then reinsert it into its respective system before placing the first filter back in line. The second filter (located to the right of the steel frame) cannot be bolted into or unbolted from the steel frame with the first filter (located to the left of the steel frame) in line.

6.2.4 Apparatus and Materials

1) Filters - 2 CUNO, Model EG, Auto-Klean® Filters (80 micron) equipped with mounting brackets and mounting bolts.

2) PVC attachments - 6 3/4" PVC male adapters; 10'-20' of 3/4" 40 schedule PVC pipe; 6 3/4" PVC couplings; teflon tape or teflon paste; and PVC cement glue.

6.3 In-Line Diluent Water Filters

6.3.1 Purpose

The 10 micron in-line diluent water filters, which serve as fine particle filtration devices, are located between the diluent water source and the Culligan® carbon filtration system. This describes the procedures followed when replacing the filter cartridges.

6.3.2 Replacing the Filter Cartridge

1) The water filters should be replaced once a month, or whenever a noticeable restriction of water flow occurs (the rate of flow in psi may be observed on the pressure gauge located on the outlet side of the filter). The current filter type used is the AMETEK® Model CP-10 medium fine sediment cartridge (AMETEK, Sheboygan, WI.). The filters may be changed separately, in order to keep the diluent water system running at all times.

2) Close both the inlet (left-hand side) and outlet (right-hand side) water supply valves located on either side of the filter unit to be replaced.

3) Open the air valve located on top of the filter unit by turning the brass nut counterclockwise.

4) Next, using both hands, turn the filter case clockwise until it has been removed from the top part of the unit.

5) Remove the old filter cartridge and throw it away. Pour off any remaining water and clean the inside of the filter case thoroughly. Place a new filter cartridge into the filter case and screw the filter case back into the top part of the unit.

6) Slowly open the inlet water supply valve located on the left side of the filter unit, and allow the filter case to fill up with water. As soon as a small flow of water can be noticed exiting the air valve located on the top of the filter unit, close the air valve. Immediately open the outlet water supply valve located on the right side of the filter unit. Make sure that both the inlet and outlet water supply valves are now fully opened. The same procedure should be followed when changing the other filter cartridge.

6.3.3 Apparatus and Materials

1) AMETEK® medium fine sediment cartridges (Model CP-10: nominal 10 micron filter).

6.4 Culligan® Carbon Filtration System

6.4.1 Purpose

The Culligan® carbon filtration system is placed in the diluent water feed line to remove residual chlorine and trace organics. This describes the procedures followed when back-washing and replacing the existing carbon inside of the Culligan® carbon filtration tanks.

6.4.2 Back-Washing the Carbon Filtration Tanks

1) The carbon filtration tanks need to be back-washed once a week. The plumbing configuration of the system allows the 2 tanks, which are placed in series, to be back-washed separately. Therefore, the diluent water system may be kept running at all times. Instructions for back-washing are located on both tanks; however, in the event that the instructions are not included with the system the following procedures should be followed.

2) Close the inlet water supply ("push to bypass") valve on the tank to be back-washed. The inlet water supply valve is marked by a red sticker labeled "push to by-pass" on the right side of the tank and a blue sticker labeled "push for soft water" on the left hand side of the tank.

3) Attach a garden hose to the drainage outlet on the tank, and run the hose to drain.

4) Carefully move the shift lever to the top or number 1 position (back-wash position).

5) Open the inlet water supply valve ("push for soft water"). Allow the tank to back-wash until the water from the drain hose appears to be as clear as the unfiltered diluent water (approximately 20 minutes).

6) Once the tank is finished back-washing, close the inlet water supply valve ("push to by-pass"). Carefully move the shift lever to middle or number 2 position (rinse position).

7) Open the inlet water supply valve ("push for soft water"), and allow the tank to rinse for 2 minutes.

8) Once the tank is finished rinsing, close the inlet water supply valve ("push to by-pass"). Carefully move the shift lever into the bottom or number 3 position (service position).

9) Open the inlet water supply valve ("push for soft water"), to return the filter to service. The same procedures should be followed when back-washing the other filtration tank.

6.4.3 Replacing the Carbon in the Filtration Tanks

1) The carbon inside the filtration tanks should be replaced every 3 months. A 50 lb bag of replacement carbon is sufficient to re-fill both tanks. The carbon in the tanks may be changed separately, in order to keep the diluent water system in service at all times.

2) Close the inlet water supply valve ("push to by-pass"). Carefully move the shift lever into the top or number 1 position (back-wash position).

3) Disconnect the tank from the incoming and outgoing water line valve apparatus by lifting up the two metal bushings, located directly above the filtration tank, at their point of connection and then carefully slide the tank out from underneath of the valve apparatus.

4) Once the tank has been disconnected, remove the fixture which screws into the top of the tank. This may be accomplished by turning the fixture counterclockwise until it is unscrewed. Carefully lift the fixture out of tank by twisting it back and forth while pulling it up and out of the tank.

5) The tank should then be moved to a location where the old carbon can be removed and properly disposed of according to site regulations. Thoroughly, rinse out the inside of the tank.

6) Add one-half of the 50 lb bag of new carbon to the tank. Now fill the tank about half-full of water, and place the fixture back into the tank. Pressure may have to be applied in a downward direction while twisting the fixture back and forth. Once the fixture has been screwed back into the tank, the tank may be reconnected to the incoming and outgoing water line valve apparatus. It is important that the 2 rubber bushings/washers located on the inside of the incoming and outgoing water line valve apparatus are in place before the tank is reconnected.

7) After the tank has been reconnected allow it to back-wash for approximately 30 minutes (See Section 6.4.2). Once the first tank has completed its back-wash cycle, the remaining charcoal may be replaced in the second tank.

6.4.4 Apparatus and Materials

1) Culligan® water system - 2 carbon filtration tanks; 2 incoming/outgoing water valve apparatuses; 50 lb bag of carbon; and 1 garden hose.

SECTION 7

TEST ORGANISM HUSBANDRY

Strict test organism husbandry guidelines must be followed when caring for the various test organisms used during testing in the biomonitoring trailer. This section specifically outlines the following husbandry procedures: bluegill and Japanese medaka feeding, brine shrimp cultures, aquarium maintenance, and preserving dead or moribund Japanese medaka.

7.1 Feeding Bluegill

7.1.1 Purpose

This describes the feeding procedures followed for bluegill during the acclimation period prior to a ventilatory test.

7.1.2 Feeding Regime

1) Bluegill feeding takes place during periods of pre-test acclimation only. Test fish are not fed during 21-day ventilatory tests.

2) Bluegill should be fed trout chow (2 times a day; Sunday through Saturday) or frozen brine shrimp (2 times a day; Sunday through Saturday). They may also be given 1 feeding of trout chow and 1 feeding of frozen brine shrimp.

3) Bluegill should be fed Ad libitum for 10 minutes. Leftover food should be siphoned from the bottom of the tank to prevent fungal growth.

7.2 Feeding Japanese Medaka

7.2.1 Purpose

This describes the feeding procedures followed during a carcinogen test involving the Japanese medaka.

7.2.2 Pre-Adult Fish (15-22 Days Old)

1) Microworms - 2 feedings per day.

2) Brine shrimp - 2 feedings per day; 30 brine shrimp per fish.

7.2.3 Pre-Adult Fish (23-30 Days Old)

1) Tetramin® flake food - 2 feedings per day Monday, Wednesday, Friday, Saturday, and Sunday; 1 feeding per day Tuesday and Thursday.

2) Brine shrimp - 1 feeding per day; 40 brine shrimp per fish.

3) Ground ocean plankton - 1 feeding per day Tuesday and Thursday.

7.2.4 Adult Fish (31 Days or Older)

1) Tetramin® flake food - 2 feedings per day Monday through Friday; 1 feeding per day Saturday and Sunday.

2) Brine shrimp - 1 feeding per day Monday, Wednesday, and Friday. The amount of brine shrimp per fish is listed in Section 7.3.3.

3) Ground ocean plankton - 1 feeding per day Tuesday, Thursday, Saturday, and Sunday.

7.2.5 Guidelines

1) Japanese medaka should be fed Tetramin® flake food and ground ocean plankton Ad libitum during the designated feeding periods for 10 minutes.

2) Leftover food should be siphoned from the bottoms of all of the tanks after each feeding of Tetramin® flake food and ground ocean plankton, in order to prevent fungal growth.

3) When questions arise concerning the feeding of the Japanese medaka refer to the following: Japanese Medaka Feeding Schedule, Section 7.3 - Brine Shrimp Cultures,

or the Medaka Breeding Colony Protocol provided by USABRDL.

4) Japanese Medaka Feeding Schedule:

	Brine Shrimp	Ocean Plankton	Tetramin Flake
Monday	01	--	02
Tuesday	--	01	02
Wednesday	01	--	02
Thursday	--	01	02
Friday	01	--	02
Saturday	--	01	01
Sunday	--	01	01

The numbers listed in the columns (e.g., 01, 02) represent the number of feedings per day.

7.3 Brine Shrimp Cultures

7.3.1 Purpose

This describes the procedures followed when raising and maintaining brine shrimp cultures to be used in the feeding of the Japanese medaka during a carcinogenicity test.

7.3.2 Water Bath Set-Up

1) Fill a 10 gallon aquarium approximately 2 inches from the top with diluent water.

2) Construct a removable lid for the aquarium out of plexi-glass.

3) Measure the diameter of a 1000 ml Nalgene polypropylene pear-shaped separatory funnel at its widest point. Record this measurement.

4) Drill two holes in the aquarium lid slightly smaller than the widest point on the funnel. This will allow two funnels to be suspended directly into the 10 gallon aquarium. In addition, cut a hole in the lid near the side of the aquarium through which a small aquarium heater may be placed.

5) The aquarium heater should be set to keep the temperature of the water bath between 25-27°C.

7.3.3 Preparation of Cultures

1) Cultures should be prepared 48 hours prior to a designated feeding day requiring live brine shrimp.

2) Fill a 1000 ml Nalgene polypropylene pear-shaped separatory funnel equipped with a screw cap with 1000 ml of diluent water.

3) Add approximately 38 g of artificial sea salts (e.g., Instant Ocean®) to the diluent water.

4) Drill a hole large enough in the middle of the screw cap for a 1 ml disposable pipet to fit through. Connect a piece of tygon air tubing to the large end of the pipet. Attach the other end of the tygon tubing to an aquarium gang valve. Connect the gang valve to an air pump. Begin aerating the mixture in the funnel.

5) Allow the diluent water and artificial sea salts mixture to aerate for 24 hours.

6) After the 24 hour aeration period, measure the salinity of the solution. The salinity should be approximately 28-30 ppt. The salinity of the mixture may easily be adjusted by either adding more artificial sea salts (to increase salinity) or siphoning out some of the mixture and adding more diluent water (to decrease salinity).

7) The amount of brine shrimp eggs to be added to the saltwater solution in the funnel will vary according to the cumulative test day and the number of Japanese medaka still alive in each of the 12 tanks. Refer to the following to determine the amount of brine shrimp needed per fish:

Test Day	Brine Shrimp Per Fish
003	0010
008	0020
015	0030
023	0040
031	0060
035	0080
040	0120
045	0150
050	0250
055	0300
060	0375
065	0400
070	0450
080	0500
090	0600
100	0650
110	0750
125	1000
200	1300

8) Calculate the number of fish still alive by adding the totals from each of the 12 tanks together.

9) The label on the container of brine shrimp eggs should list the approximate number of cysts per gram and the percent hatchout of the cysts. It is recommended that Argentemia® Certified Grade 1 brine shrimp eggs be used, which are supplied by Argent Chemical Laboratories, Inc. (Redmond, WA.). An Argent Chemical Laboratories 500 g can contains approximately 280,000 cysts/g. The hatchout rate is listed as 93%.

10) Therefore, if it were day 35 of a 6-month carcinogenicity test and 696 Japanese medaka were still alive, approximately 56,000 live brine shrimp would be

needed to feed the fish. The calculations for determining the number of brine shrimp eggs to be added to the saltwater solution in the funnel for a feeding on Test Day 35 is as follows:

80 brine shrimp per fish x 696 fish = ≈56,000

93% hatchout of x = ≈56,000

56,000 ÷ 0.93 = ≈60,000 are added to the funnel

280,000 cysts/g

60,000 ÷ 280,000 = 0.214 g of brine shrimp eggs are added to the funnel

11) Add the brine shrimp eggs to the funnel, swirl the funnel in order to suspend the eggs in the saltwater solution, and aerate for 48 hours to obtain the maximum brine shrimp hatchout.

12) After the 24-hour incubation period is completed, the live brine shrimp should be separated from the hatched and unhatched cysts. Turn the aeration off and remove the 1 ml pipet from the funnel. Allow the unhatched cysts to settle to the bottom of the funnel and the hatched cysts to float to the top (approximately 15 minutes). Three layers should now be present in the funnel: a top layer of hatched cysts, a middle layer of reddish-orange live brine shrimp, and a bottom layer of unhatched cysts. Carefully drain off the bottom layer of unhatched cysts from the funnel. Dispose of the unhatched cysts. Slowly drain the reddish-orange layer of live brine shrimp into a brine shrimp net or an alternative fine-meshed filtration device. Do not allow any of the hatched cysts in the top layer to escape from the funnel. Rinse the live brine shrimp thoroughly with diluent water to wash away any saltwater. Re-suspend the live brine shrimp in 1000 ml of diluent water in a 1000 ml beaker.

13) Place a small stirring magnet in the beaker. Use a magnetic stirring device to keep the brine shrimp suspended in the diluent water (live brine shrimp tend to settle on the bottom of the beaker).

14) Pipet 1 ml out of the beaker and count the number of brine shrimp in that 1 ml sample (a dissecting scope equipped with a light source may be needed to count the brine shrimp). Multiply that number by the 1000 ml of diluent water. The value obtained will be the approximate number of brine shrimp contained in the beaker.

15) Now determine the amount of brine shrimp solution that needs to be pipetted into each tank. For example:

Tank 1 contains 58 fish

On Test Day 35 each fish needs 80 brine shrimp

58 fish x 80 brine shrimp = 4,640 brine shrimp

The number of brine shrimp needed per tank should now be divided by the number of brine shrimp per 1 ml of diluent water in the beaker. The resulting value will be the number of ml of brine shrimp solution that should be pipetted into tank 1. If the amount of brine shrimp contained in the beaker is not enough to feed all of the fish, then ground ocean plankton should be substituted for the brine shrimp feeding.

7.3.4 Equipment Clean-Up

1) The separatory funnel and 1000 ml beaker should be thoroughly scrubbed with a bottle brush before starting a new culture.

2) The brine shrimp net should be thoroughly rinsed with diluent water in order to remove any remaining salt deposits.

7.3.5 Maintaining Cultures

1) Japanese medaka are given brine shrimp every Monday, Wednesday, and Friday. Therefore, new cultures need to be started on: the previous Saturday for a Monday feeding, the previous Monday for a Wednesday feeding, and the previous Wednesday for a Friday feeding.

2) Add diluent water to the water bath as needed.

7.3.6 Apparatus and Materials

1) Water bath - 10 gallon aquarium; plexi-glass lid; aquarium heater; standard aquarium air pump; aquarium gang valve; and tygon air tubing.

2) Brine shrimp culture - Two 1000 ml Nalgene polypropylene pear-shaped separatory funnels with screw-on caps; 500 g can of Argentemia® Certified Grade 1 brine shrimp eggs from Argent Chemical Laboratories, Inc.; 16 lb bag of Instant Ocean®; 1 ml disposable pipets; brine shrimp net; two 1000 ml beakers; stirring magnet; magnetic stirring device; and 25 ml disposable pipets.

7.3.7 References

1) Hatchout Techniques for the Brine Shrimp, *Artemia*: An overview of methods. Argent Chemical Laboratories, Inc., Redmond, WA.

7.4 Aquarium Maintenance

7.4.1 Purpose

This describes the procedures followed when cleaning the 5 gallon aquaria used in the carcinogenicity test and the 10 gallon aquaria used as bluegill acclimation tanks prior to the ventilatory test.

7.4.2 Carcinogenicity Test Tanks

1) Carcinogenicity tanks should be cleaned and siphoned 1-2 times daily.

2) Remove any algae, sediment, or fecal material from the sides of the tanks by scrubbing them with an aquarium brush or sponge. Allow the material to settle to the bottom of the tank, and then siphon all of the unwanted material out. Use a small siphoning hose to remove any unwanted debris, in order to avoid siphoning any fish from the tank.

3) The tank drains should also be cleaned 1-2 times daily. Each drain should be scrubbed with an aquarium brush. After scrubbing, the drains should be squirted with distilled water to ensure that they are free and clear of debris.

7.4.3 Ventilatory Test Acclimation Tanks

1) Ventilatory test acclimation tanks should be cleaned once a week.

2) Place all of the bluegills in one tank while the others are being cleaned. The tanks should be completely drained and then scrubbed with a sponge to remove all algal and fecal material. Once the tanks are cleaned, the bluegills should be equally divided by size and numbers across the 4 acclimation tanks. Note: try to keep the smaller fish separated from the larger fish in order to reduce competition for food; thus, creating a more unified sample size.

3) The drain hoses should also be cleaned with an aquarium brush to ensure a clear pathway for all drainage material. The PVC drainpipe which the acclimation tanks drain into should be blown out daily using a shop-vac in the exhaust mode or by back-flushing the drain line with water under pressure. Clearing out the PVC drainpipe daily is a precautionary measure taken to avoid unnecessary flooding.

7.5 Preserving Dead or Moribund Japanese Medaka

7.5.1 Purpose

This describes the procedures followed when preserving dead or moribund Japanese medaka for histopathological observation.

7.5.2 Storage of Test Materials

1) Bouin's solution should be stored in a cabinet away from any source of light.

2) 70% ethyl alcohol solution should be refrigerated at all times.

3) 10% neutral buffered formalin should be stored in a cabinet or an area by itself.

7.5.3 Fixing Procedures

1) Obtain a 20 ml glass scintillation vial equipped with a teflon screw on cap.

2) Place a strip of tape on the outside of the vial which lists the test designation, year, julian date, tank number, and fish number. For example, during carcinogen test (T) a medaka from tank 5 (the 3rd fish to die in tank 5) was found dead on January 1, 1991. The information to appear on the label is as follows:

Test Designation	(T)	Test T
Year	(91)	1991
Julian Date	(001)	1st Day Of The Year
Tank Number	(5)	Tank 5
Fish Number	(3)	3rd Mortality In Tank 5

The label should read: T-91-001-5-3.

3) Always wear protective gloves, eyewear, and a mask while handling any of the materials used to preserve the fish.

4) Fill the vial with 10 ml of Bouin's solution. After removing the dead or moribund fish from its tank, use a pair of forceps to carefully place it into the vial. Screw the cap on the vial and allow it to set for 24 hours.

5) When the 24-hour period is completed, drain the Bouin's solution from the vial and fill the vial with 10 ml of a 70% ethyl alcohol solution. Screw the cap back on the vial and refrigerate for 24 hours.

6) When the 24-hour period is completed, drain the 70% ethyl alcohol solution from the vial and fill the vial with 10 ml more of the 70% ethyl alcohol solution. Screw the cap back on the vial and refrigerate for 24 hours.

7) When the 24-hour period is completed, drain the 70% ethyl alcohol solution from the vial and fill the vial with 10 ml of a 10% neutral buffered formalin.

8) The fish is now fixed for histopathological observation.

7.5.4 Materials

1) Preservatives - 1 liter bottle of Bouin's solution; 1 liter bottle of 70% ethyl alcohol solution; 10% neutral buffered formalin.

2) Remaining materials - 50 20 ml glass scintillation vials with teflon screw on caps; 1 pair of forceps; disposable rubber gloves; protective eyewear; mask; and disposable 10 ml pipets.

SECTION 8

DILUTOR OPERATION

This section describes the operation and maintenance of the dilutors used during the carcinogenicity and ventilatory biological monitoring tests.

8.1 Proportional Dilutor

8.1.1 Purpose

This describes the procedures followed when utilizing the proportional dilutor to run a 6-month carcinogenicity test. Please refer to Figure 5 - The proportional dilutor and Figure 6 - The stainless steel flow splitting cup for additional information.

8.1.2 Pre-test Preparations and Adjustments

1) Activate the solenoids to be used during the test by depressing the corresponding button located on the control box. Normally, the solenoids on the dilutor are not all used during a test. Note that solenoids Fill 1, 2, and 3 are always used during testing.

2) Set the desired volumes to be delivered to each stainless steel flow splitting cup by adjusting the height of the stainless steel tubes located inside of each of the partitioned glass distribution chambers. Place a piece of labeling tape on each glass partition noting the specific amount of diluent water or test material which they are to contain.

3) Place 2 flow reducers in each flow splitting cup and connect 2 silicon tubes to the outlets located on the bottom of the stainless steel cups (the tubing runs from the flow splitting cups to the 5 gallon aquaria).

4) Adjust the height of the flow reducers located in each flow splitting cup, so that each 5 gallon aquaria receives 250 ml of either diluent water or test material per 2.5-3.5 minute cycle.

5) Adjust the dilutor cycle time to the desired 2.5-3.5 minute range by increasing and/or decreasing the flow of the diluent water and test material into the dilutor. This is accomplished by carefully opening or closing the fill 1 and fill 2 solenoid valves located on the top left hand side of the proportional dilutor. It is very important to remember that the test material

which flows into the far left partitioned section of the upper distribution chamber should always overflow to drain before the diluent water reaches the diluent water electrode located in the far right partitioned section of the upper distribution chamber. In addition, the predetermined amount of test material which is delivered from the dosing chamber to the lower distribution chamber, should be completely received before the diluent water triggers the diluent water electrode in the upper distribution chamber.

8.1.3 Cleaning the Dilutor

1) Close the test material water line and re-route the diluent water through the PVC pipe which delivers test material to the proportional dilutor.

2) Allow diluent water to run through the dilutor for 24 hours.

3) After 24 hours, shutdown the dilutor to prepare for an acid rinse.

4) Remove the electrodes from the effluent chamber and far right partition (diluent chamber) of the upper distribution chamber. Scrub the electrodes with an abrasive pad to remove any debris, and wipe electrodes clean.

5) Add 500 ml of a 10% nitric acid (HNO_3) solution to the dosing chamber. Fill each partitioned section of the upper and lower distribution chambers and each stainless steel flow splitting cup with HNO_3. Let stand for 5 minutes.

6) Scrub the dosing chamber, partitioned sections, and flow splitting cups with an abrasive pad or aquarium brush.

7) Siphon the HNO_3 and debris from the aforementioned areas. Rinse generously with diluent water from a squirt bottle. Siphon.

8) Rinse with acetone from a squirt bottle. Allow to air dry.

9) Disassemble all stainless steel standpipes, solenoid valves, flow reducers, and silicon tubing and clean with a 10% HNO_3 solution. Use a long, flexible snake-brush to scrub out the inside. Rinse with diluent water and reassemble all parts. Replace any parts which are damaged or unable to be properly cleaned.

10) Activate the dilutor and rinse with diluent water for 24 hours to ensure that no acid residue remains.

11) Completely siphon all diluent water from dosing chamber, partitioned sections, and stainless steel flow splitting cups.

12) Turn the main power switch located on the dilutor control/power box off. Steps 11 and 12 should be followed between testing or during shutdown for site relocation only.

Figure 5. Proportional dilutor.

1. Test material line with fill 2 solenoid valve.
2. Diluent water line with fill 1 solenoid valve.
3. Test material electrode.
4. Diluent water electrode.
5. Upper distribution chamber.
6. Dilutor control/power box.
7. Cycle counter.
8. Hour counter.
9. Overflow drain line from the test material section of the upper distribution chamber.
10. Overflow drain line from the diluent water section of the upper distribution chamber.
11. Fill 3 solenoid valve.
12. Dosing chamber.
13. Stir plate (optional).
14. Lower distribution chamber.
15. Stainless steel flow splitting cup.
16. Silicon tubing which delivers diluent water or test material from the dilutor to the 5 gallon test aquaria.
17. 5 gal aquaria in the water bath trough.

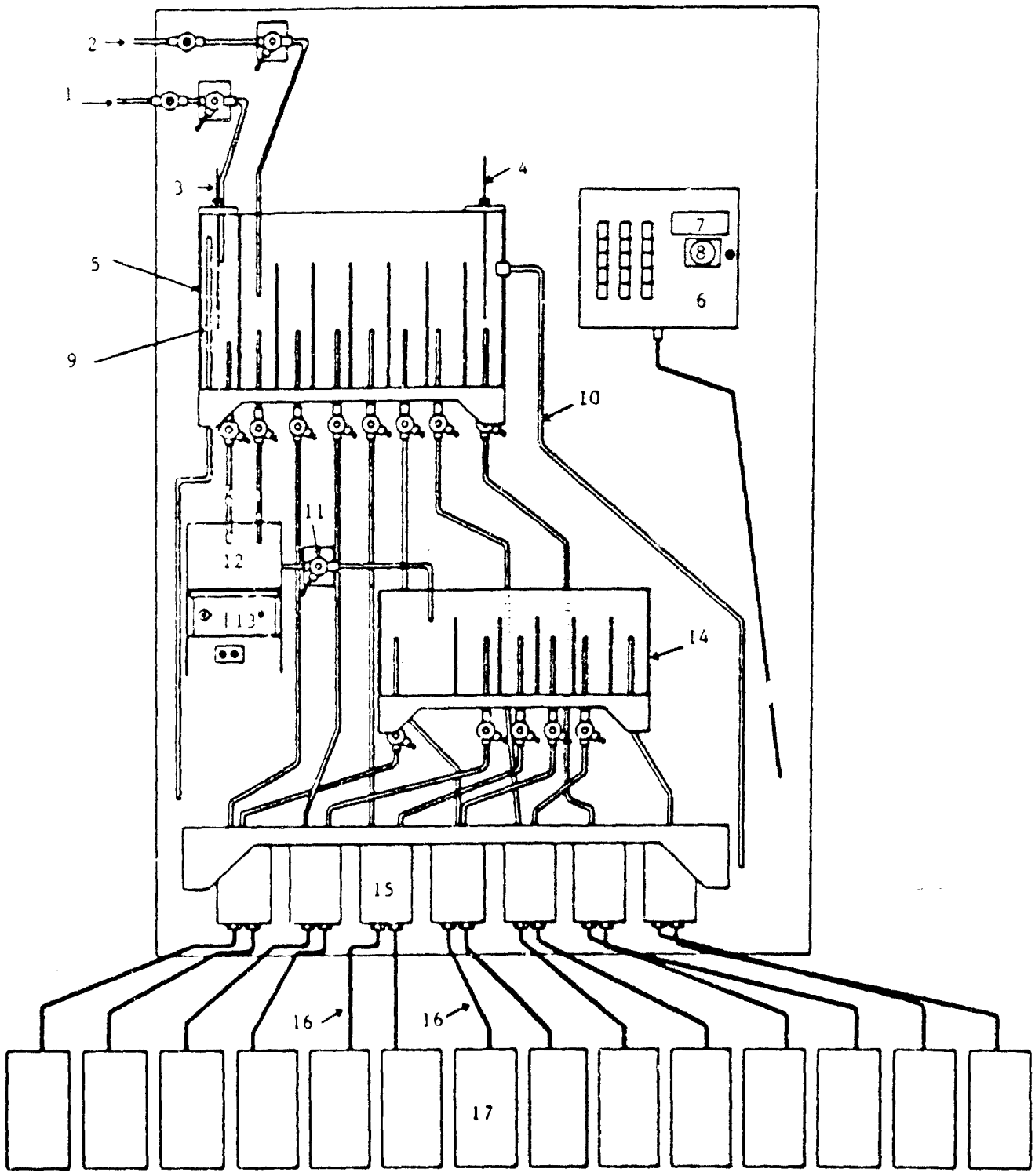

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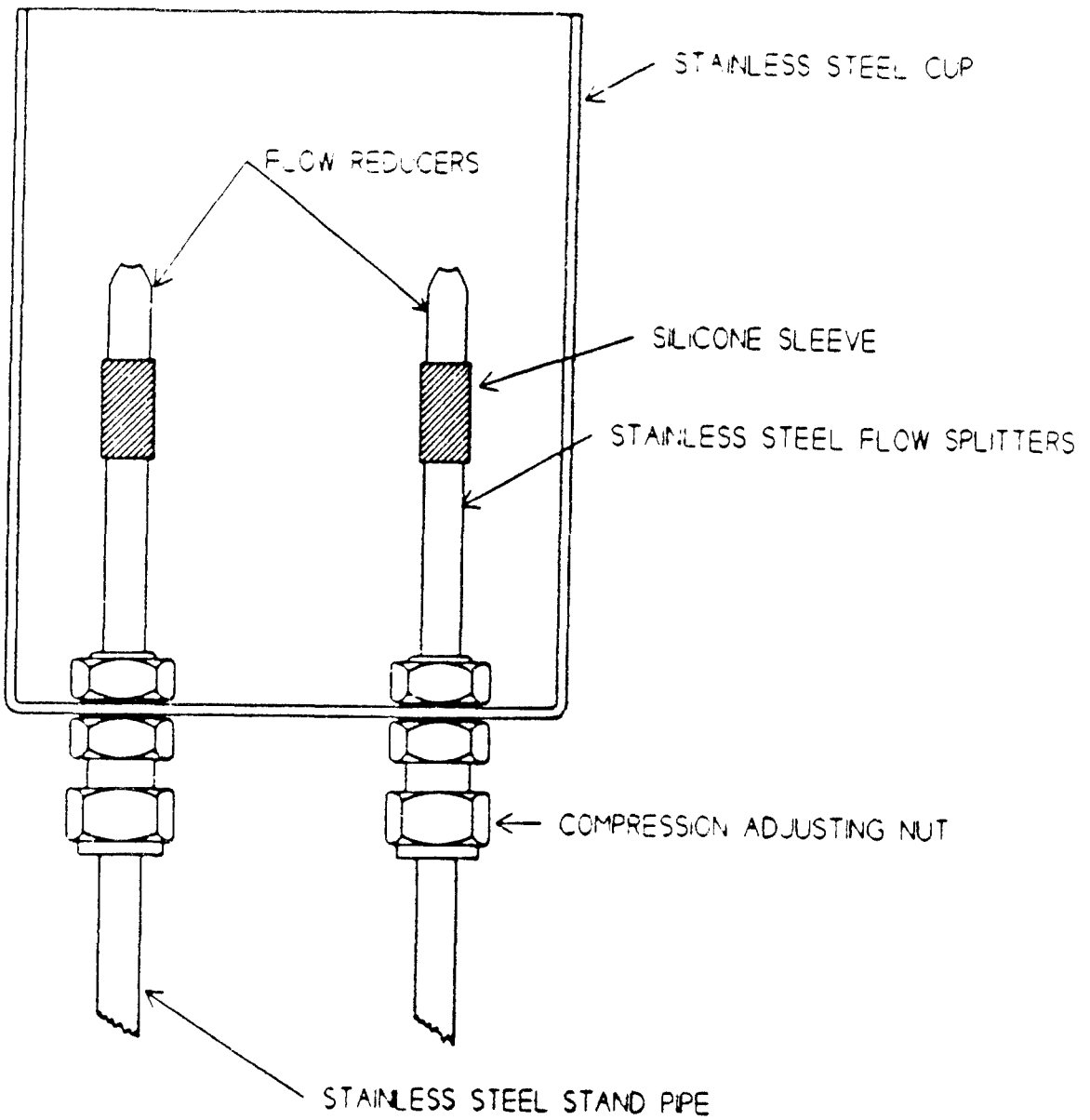
Figure 6. Stainless steel flow splitting cup.


STAINLESS STEEL CUP
FLOW REDUCERS
SILICONE SLEEVE
STAINLESS STEEL FLOW SPLITTERS
COMPRESSION ADJUSTING NUT
STAINLESS STEEL STAND PIPE

8.2 Ventilatory Biological Monitoring Dilutor

8.2.1 Purpose

This describes the procedures followed when utilizing the ventilatory biological monitoring dilutor to run a 21-day ventilatory test. Please refer to Figure 6 - Stainless steel flow splitting cup, Figure 7 - Ventilatory biological monitoring dilutor, Figure 8 - Ventilatory chamber holding box, and Figure 9 - Ventilatory chamber for additional information.

8.2.2 Pre-Test Procedures and Adjustments

1) Activate the solenoids to be used during the test by depressing the corresponding buttons located on the dilutor control/power box.

2) Set the desired volumes to be delivered to each of the 4 diluent water or test material mixing chambers by adjusting the height of the high and low level electrodes.

3) Set the desired volumes to be delivered from the 4 diluent water or test material distribution chambers to each of the 16 stainless steel flow splitting cups, by adjusting the height of the 4 stainless steel tubes located inside each of the distribution chambers.

4) Place 2 flow reducers in each flow splitting cup and connect the silicon and glass tubing combinations to the outlets located on the bottom of each cup.

5) Adjust the height of the flow reducers located in each stainless steel cup, so that each ventilation chamber receives 100 ml of diluent water or test material. This amount should be measured at the point in which the glass tubing enters the ventilation chamber holding box.

6) Measure the cycle times of the 2 diluent water dilutors and the 2 test material dilutors. Cycle times for each of the 4 dilutors should be between 00:00:55.00 and 00:01:05.00. When the cycle times do not fall within the designated time parameters, the flow of the diluent water or test material may need to be adjusted. The flow of diluent water or test material into the mixing chambers may be increased or decreased by slightly opening or closing the stainless steel valves for flow regulation of the diluent water or test material, located next to each of the 4 mixing chambers on each of the 4 dilutors.

7) Measure the volumes delivered to each diluent water or test material distribution chamber, flow splitting cup, and ventilation chamber. Record this information on the designated data sheet.

8.2.3 Cleaning the Dilutor

1) Close the test material water lines and reroute the diluent water through the stainless steel pipes which supply test material to the mixing chambers.

2) Allow diluent water to run through the dilutor for 24 hours.

3) After 24 hours, shutdown the dilutor to prepare for an acid rinse.

4) Add 1000 ml of a 10% nitric acid (HNO_3) solution each of the diluent water and test material mixing chambers, 500 ml to the distribution chambers, and 200 ml to each stainless steel flow splitting cup. Let stand for 5 minutes.

5) Scrub the aforementioned parts of the dilutor with an abrasive pad or aquarium brush.

6) Siphon the acid solution and debris from the dilutor. Rinse generously with diluent water from a squirt bottle. Siphon.

7) Rinse with acetone from a squirt bottle. Allow to air dry.

8) When necessary, disassemble all stainless steel standpipes, solenoid valves, flow reducers, and silicon tubing/glass rod combinations and clean with a 10% HNO_3 solution. Use a long, flexible snake-brush to scrub out the inside. Rinse with diluent water and reassemble all parts. Cleaning of this magnitude may not be required after each 21-day test.

9) Activate the dilutor and rinse with diluent water for 24 hours to ensure that no acid residue remains.

10) Completely siphon all diluent water from the mixing chambers, distribution chambers, and flow splitting cups.

11) Shut the power to dilutors 1-4 off.

Figure 7. Ventilatory biological monitoring dilutor.

1. Diluent water line.
2. Test material water line.
3. Valves which regulate diluent water or test material supply to a mixing chamber.
4. Solenoid.
5. Mixing chamber.
6. Flow splitting chamber.
7. Stainless steel standpipe.
8. Dilutor control/power box.
9. Cycle counter.
10. Hour counter.
11. High and low electrodes.
12. Wooden holding rack for electrodes.
13. Stir plate (optional).
14. Two rows of stainless steel flow splitting cups.
15. Compression fitting nut.
16. Silicon tubing and glass rod combination which delivers diluent water or test material to the ventilation chambers.
17. Standpipes which supply diluent water or test material to the two rows of stainless steel cups.

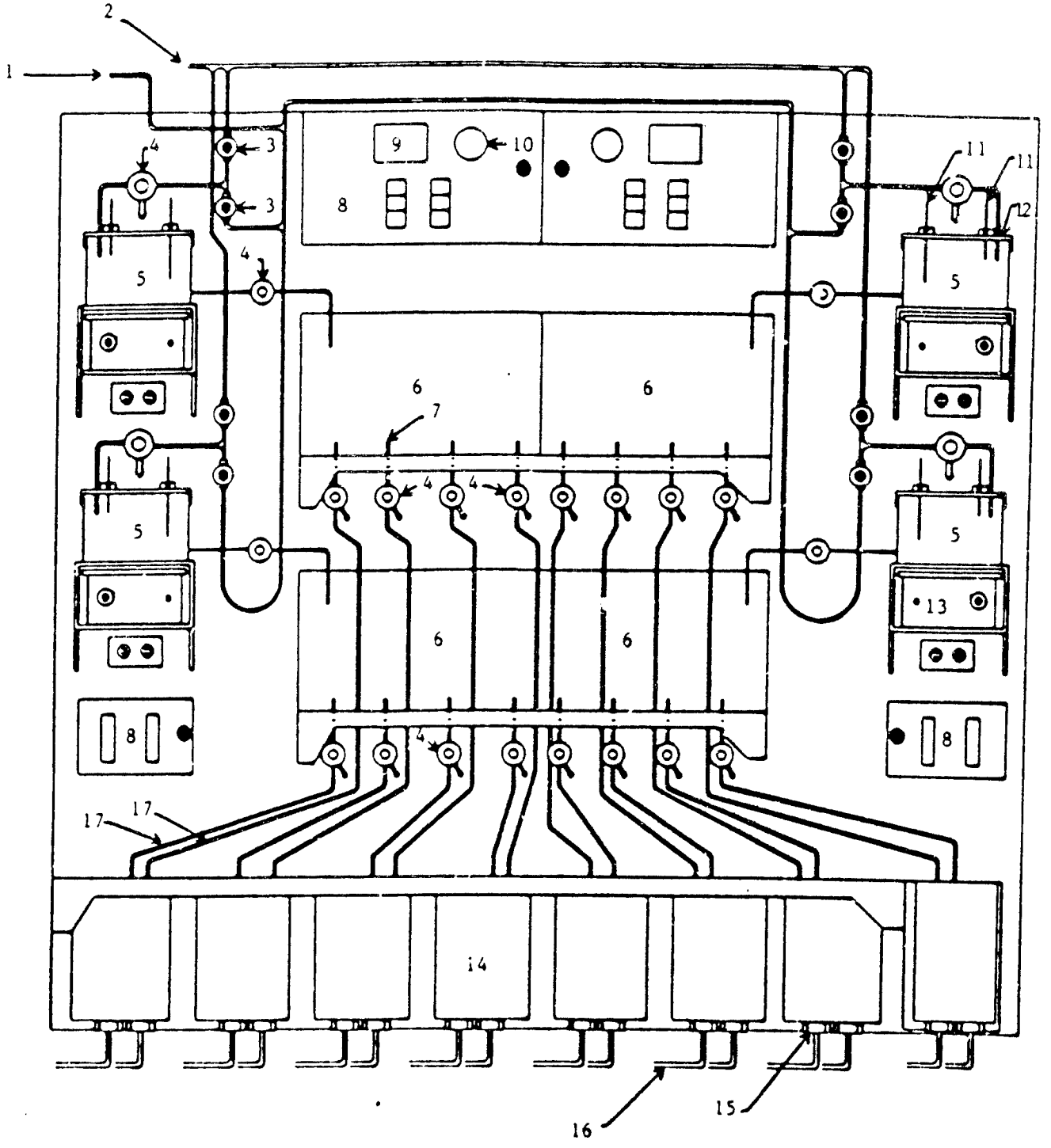

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Figure 8. Ventilatory chamber holding box.

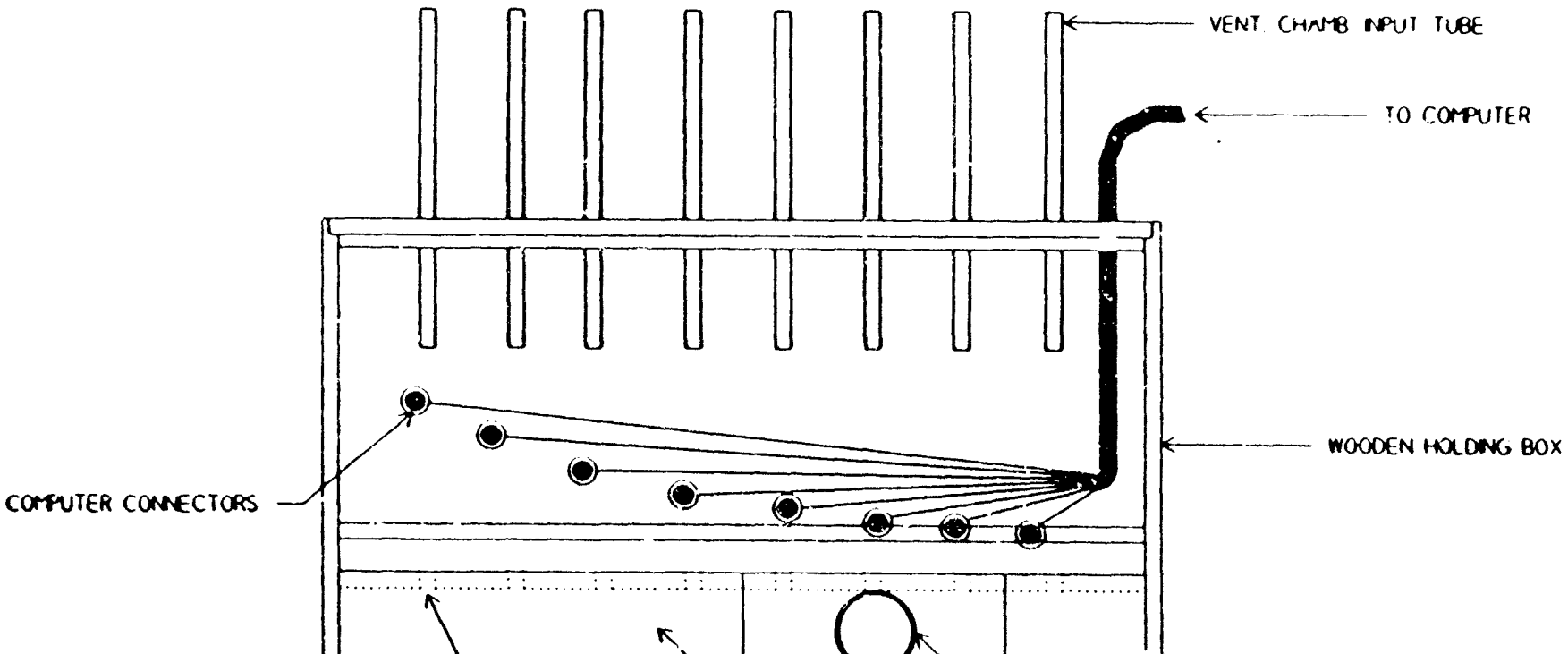
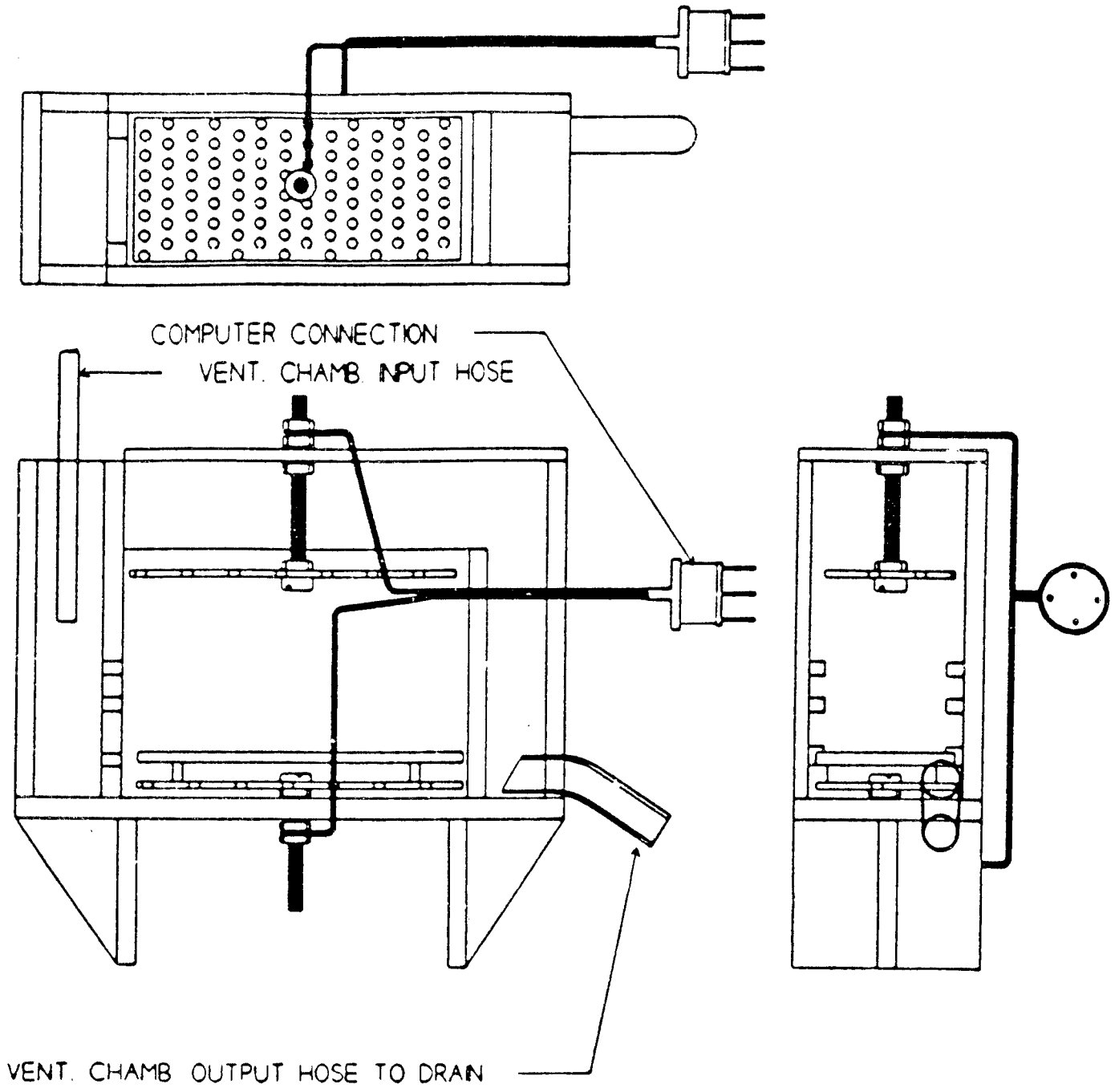

VENT CHAMB INPUT TUBE
TO COMPUTER
WOODEN HOLDING BOX
COMPUTER CONNECTORS
PVC DRAIN
"T" JOINT IN DRAIN
DRAIN LINE INSERT HOLES

Figure 9. Ventilatory chamber.


COMPUTER CONNECTION
VENT. CHAMB. INPUT HOSE
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SECTION 9

AUTOMATED WATER QUALITY TESTING/SAMPLING EQUIPMENT

This section describes the operation and maintenance of the automated water quality testing and sampling equipment utilized in the trailer. Included are the Hach® Surface Scatter 5 turbidimeter, Hydrolab® Scout®, and Isco® Model 2700 refrigerated sampler.

9.1 Hach® Surface Scatter 5 Turbidimeter

9.1.1 Purpose

This describes the procedures followed when using the Hach® turbidimeter during a 21-day ventilatory test to measure the turbidity of the diluent water and test material systems.

9.1.2 Turbidimeter Operation

All relevant information needed to operate the Hach® turbidimeter may be found in the Surface Scatter 5 Turbidimeter Manual, Cat. No. 15625, 1984, Hach® Co., Loveland, CO.

9.1.3 Regulation of Flow into the Turbidimeter

1) Two in-line solenoid valves determine which system will flow to the turbidimeter. These valves are programmed to open and close every 30 minutes so that water quality data may be obtained for both the diluent water and test material systems. For example, water quality data for the diluent water system is collected for 30 minutes while the solenoid valve for the test material system is closed. At the end of the 30 minute data collection period, the diluent water system's solenoid closes and the test material system's solenoid opens. Thus, collecting test material water quality data for 30 minutes. This cycle runs continuous throughout a 21-day ventilatory test.

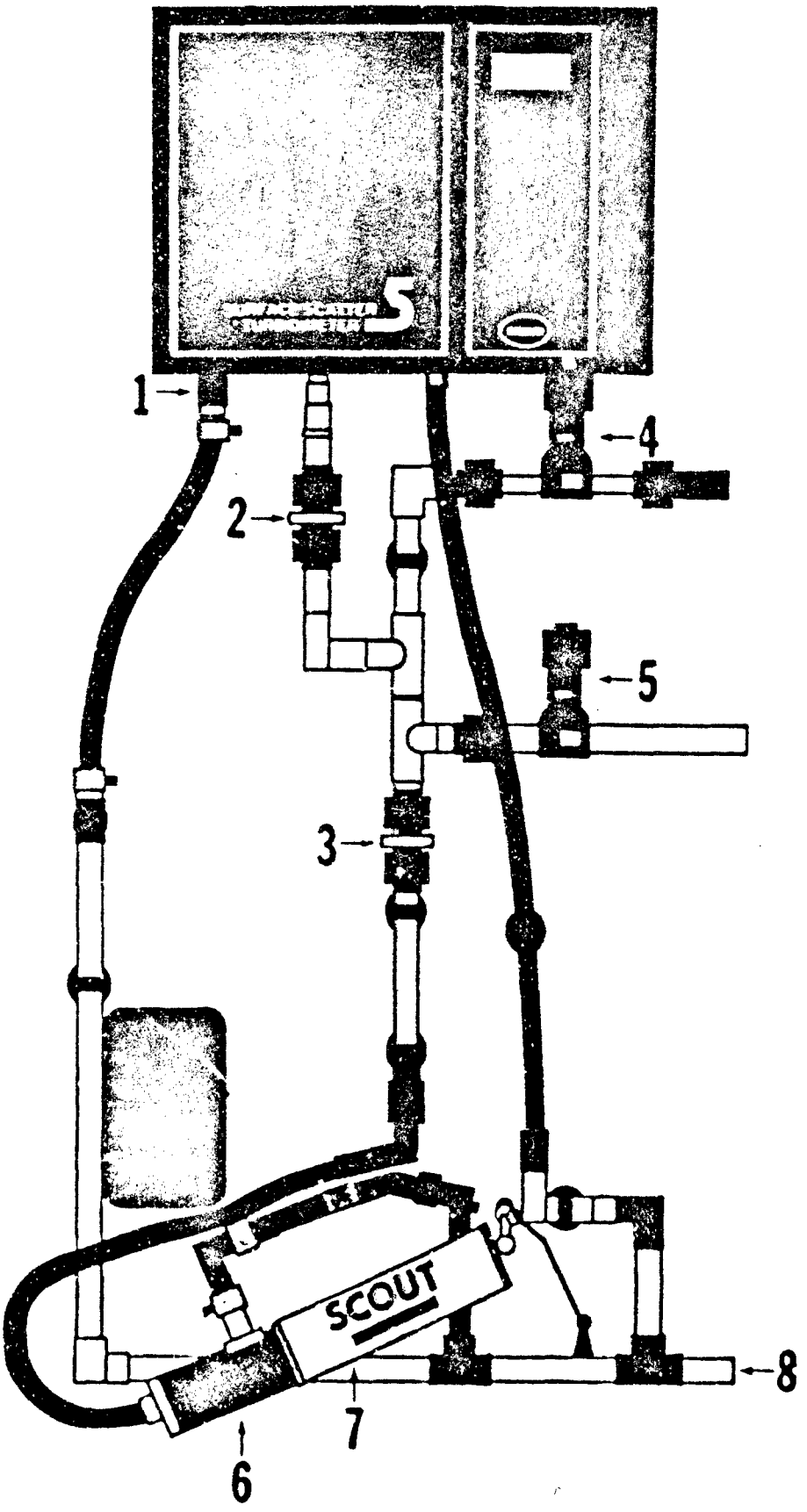
2) Flow of diluent water or test material is regulated by adjusting the PVC valves located directly below the unit. See Figure 10 - Hach® turbidimeter and Hydrolab® Scout® systems.

9.1.4 Recording Test Results

The data from the turbidimeter is automatically logged into the ventilatory computer through the ventilatory data acquisition system.

Figure 10. Hach® turbidimeter and Hydrolab® Scout® systems

1. Hach® Turbidimeter drain valve.
2. Hach® Turbidimeter in-flow valve.
3. Hydrolab® Scout® in-flow valve.
4. Diluent water solenoid valve.
5. Test material solenoid valve.
6. Flow through cup attachment for Hydrolab® Scout®.
7. Hydrolab® Scout®.
8. Main drain line from both the Hach® Turbidimeter and Hydrolab® Scout® systems.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SCOUT

9.2 Hydrolab® Scout®

9.2.1 Purpose

This describes the procedures followed when using the Hydrolab® Scout® during a 21-day ventilatory test to collect conductivity, DO, pH, and temperature water quality data on the diluent water and test material water systems.

9.2.2 Scout® Operation

All relevant information needed to operate the Scout® may be found in the Scout® Operating Manual (And Performance Manual), 1988, Hydrolab® Corporation, Austin, TX.

9.2.3 Connection to the Diluent Water and Test Material Systems Inside the Trailer

1) The Scout® should be connected to a flow through cup, located beneath the wall-mounted Hach® Turbidimeter, by screwing the cup into the sensor end of the unit. See Figure 10 - Hach® turbidimeter and Hydrolab® Scout® systems. This type of apparatus allows diluent water or test material to flow into the cup, over the sensors, and out to drain.

2) Two in-line solenoid valves determine which system will flow to the Scout®. These valves are programmed to open and close every 30 minutes so that water quality data may be obtained for both the diluent water and test material systems. For example, water quality data for the diluent water system is collected for 30 minutes while the solenoid valve for the test material system is closed. At the end of the 30 minute data collection period, the diluent water system's solenoid closes and the test material system's solenoid opens. Thus, collecting test material water quality data for 30 minutes. This cycle runs continuous throughout a 21-day ventilatory test.

3) The flow rate of diluent water and test material into the Scout® may be controlled by opening or closing the PVC valve located below the solenoid valves.

9.2.4 Recording Test Results

The data from the Hydrolab® Scout® is automatically logged into the ventilatory computer through the ventilatory data acquisition system.

9.3 Isco® Model 2700R Refrigerated Sampler

9.3.1 Purpose

This describes the procedures followed when using the ISCO® Model 2700R refrigerated sampler to obtain samples of diluent water or test material for use in Microtox® and off-site mutagenicity testing and chemical analyses.

9.3.2 Isco® Operation

All relevant information needed to operate the ISCO® sampler may be found in the Instruction Manual, Model 2700R/2740 Refrigerated Sampler, Part # 60-2743-027, 1988, ISCO Inc., Lincoln, NE.

SECTION 10

FINAL TRAILER SHUTDOWN FOR SITE RELOCATION

This section outlines the procedures followed prior to trailer removal for study site relocation.

10.1 Dilutors

10.1.1 Purpose

This describes the procedures followed when cleaning up the proportional and ventilatory biological monitoring dilutors for final trailer shutdown and site relocation.

10.1.2 Methods

Refer to Sections 8.1 and 8.2.

10.2 Cleaning Glassware

10.2.1 Purpose

This describes the procedures followed when cleaning all glassware prior to final trailer shutdown for site relocation.

10.2.2 Methods

1) Place glassware in a mild detergent solution.

2) Scrub with a brush and rinse with diluent water.

3) Rinse with a 50% nitric acid (HNO_3) solution.

4) Rinse with diluent water and then distilled or deionized water.

5) Rinse with acetone.

6) Rinse with diluent water and then distilled or deionized water and allow to air dry.

10.3 Cleaning Aquaria

10.3.1 Purpose

This describes the procedures followed when cleaning all aquaria (5 & 10 gallon) prior to final trailer shutdown for site relocation.

10.3.2 Methods

1) Remove the drains from the 5 gallon and 10 gallon aquaria.

2) Prepare a mild detergent solution, with which the aquaria are initially cleaned. Scrub the tanks with an abrasive pad or aquarium brush. Rinse with diluent water.

3) Rinse both the 5 gallon and 10 gallon aquaria with a 20% hydrochloric acid solution (HCl) contained in a squirt bottle. Scrub the tanks with an abrasive pad or aquarium brush.

4) Rinse the aquaria thoroughly with diluent water to remove any acid residue or loose debris and allow to air dry.

10.4 Cleaning the Water Bath and Associated Equipment

10.4.1 Purpose

This describes the procedures followed when cleaning the water bath and its associated equipment prior to final trailer shutdown for site relocation.

10.4.2 Little Giant® Circulation Pumps

1) Unplug the pumps from their power source.

2) Remove the plastic screen from the bottom of each pump.

3) Clean the screen and pump body with an abrasive pad.

4) Store for use at next study location.

10.4.3 Heater/Circulator Pumps

1) Unplug the heater/circulators from their power source.

2) Clean the bottom (heater element and circulator) portion of the heater/circulator with a brush. Rinse off with diluent water.

3) Store for use at next study location.

10.4.4 Strip Chart Temperature Recorders

1) Unplug recorder from power source.

2) Wipe temperature probe clean with a sponge.

3) Store for use at next study location.

10.4.5 Water Bath and Water Bath/Aquaria Drain Pipe

1) Remove drain from threaded drain hole located in the left rear section of the water bath.

2) Allow water contained in the water bath to drain completely.

3) Place a plug in the drain hole and add a mild detergent to the water bath. Scrub the water bath with an abrasive pad to remove all debris.

4) Disassemble the drain, scrub the outside with an abrasive pad, and the inside with a bottle brush.

5) Remove the plug from the drain and rinse the water bath and drain thoroughly with diluent water.

6) Reassemble the drain and place it back into the water bath.

10.5 Cleaning the Automated Water Quality Testing/Sampling Equipment

10.5.1 Purpose

This describes the procedures followed when cleaning the Hydrolab® Scout®, Hach® turbidimeter, and ISCO® refrigerated sampler prior to final trailer shutdown for site relocation.

10.5.2 Cleaning Procedures

See manuals referenced in Sections 9.1, 9.2, and 9.3.

10.6 Disassembly of the Test Material Water System

10.6.1 Purpose

This describes the procedures followed when disassembling the test material water system, which delivers test material to the trailer, prior to final trailer shutdown for site relocation.

10.6.2 Test Material Submersible Pump

1) Disconnect the pump from its power source.

2) Pull the pump from its point of sampling and disconnect it from its sampling line.

3) Clean the pump thoroughly by scrubbing it with a stiff, bristled brush and then rinse it off with clean water.

4) Store pump for use at the next study location.

10.6.3 Stainless Steel "Countercurrent Heat Exchanger"

1) Disconnect the tubing apparatus from the test material water line and remove it from the secondary 150-gallon diluent water aeration/equilibration tank.

2) Clean the tubing apparatus by "blowing" it out with a hose. Next, using a small hand operated kerosene or bilge pump, pump a 10% nitric acid (HNO_3) solution through the tubing.

3) After the acid wash, thoroughly rinse the tubing apparatus with diluent water.

4) Store tubing apparatus for possible use at the next study location.

10.6.4 PVC Test Material Water Line

1) Disconnect the test material water line from its point of attachment to the trailer.

2) Cut the line into small sections and dispose of properly (the test material water line should never be used more than one time because of the contaminants that may accumulate within it over the course of a study).

10.7 Disassembly of the Diluent Water System

10.7.1 Purpose

This describes the procedures followed when disassembling the diluent water system, which delivers diluent water to the trailer, prior to final trailer shutdown for site relocation.

10.7.2 Diluent Water Centrifugal Pump

1) Stop the diluent water supply prior to Culligan® water system filtration unit.

2) Unplug the pump from its power source.

3) Drain the 150-gallon aeration/equilibration tanks (See Section 10.8.4) and disconnect the diluent water lines from the pump.

4) Unscrew the lag bolts which secure the pump to the floor of the building housing the diluent water system.

5) Rinse pump out with diluent water and store for use at the next study site.

10.7.3 Aeration System

1) Unplug the air compressor from its power source.

2) Disconnect the compressor's air lines from the PVC pipe which delivers air to the 150-gallon aeration/equilibration tanks.

3) Remove the PVC air lines with the air stones from the 150-gallon aeration/equilibration tanks.

4) Unscrew the lag bolts which secure the compressor to the floor of the building housing the diluent water system.

5) Store the compressor and PVC air lines with the air stones for use at the next study site.

10.7.4 Primary and Secondary 150-gallon Tanks

1) Open the drain valves and allow the diluent water contained in each tank to flow to drain.

2) Once the tanks have drained completely, disconnect them from the diluent water lines.

3) Rinse the tanks out with a hose, scrub if necessary (do not use any cleaning compounds), and allow to dry.

4) Store the tanks for use at the next study location.

10.7.5 PVC Diluent Water Line

When disassembling the diluent water line, save as much of the PVC pipe and associated parts (i.e. elbows, t's, ball valves, etc.) as possible. The diluent water line may be reused at the next study site.

10.8 Final Procedures for Trailer Shutdown

10.8.1 Purpose

This describes the final procedures followed to finalize trailer shutdown for site relocation.

10.8.2 Drain Lines

Both the main trailer drain line (large) and the Hach® Turbidimeter and Hydrolab® drain line (small) should be disconnected from the trailer and disposed of properly.

10.8.3 Phone Line

The cable supplying the phone service to the trailer should be disconnected from the box fastened to the front trailer hitch.

10.8.4 Non-Secured Items

All non-secured items (i.e., equipment and materials) should be placed either in the water bath or in the cabinets in order to prevent damage in transit.

10.8.5 Trailer Leveling Jacks

1) Place the trailer leveling jacks key into the key slot, See Figure 1 - Internal layout of the aquatic biomonitoring trailer.

2) Turn the trailer leveling system on.

3) Raise the trailer leveling jacks.

4) Turn off the trailer leveling system.

5) Store the blocks of wood which the trailer jacks rest on in the small room of the trailer.

10.8.6 Steps and Handrails

The steps and handrails which provide entry to the trailer at the side and rear doors should be stored beneath the side door and strapped in with rope or elastic cables.

10.8.7 Trailer Power Cable and Ground Wire

1) Switch the breaker to the main power supply off.

2) Unplug the trailer power cable from the main power box.

3) Unplug the power cable from its point of attachment to the trailer, coil, and store inside the small room of the trailer.

4) Remove the ground wire from its point of attachment to the trailer, coil, and store inside the small room of the trailer.

END