Date Prepared: 09/30/88

Date Revised: 09/19/97

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HUMANE PROCEDURES FOR ANESTHETIZATION AND HANDLING OF FISH FOR SAMPLING PURPOSES

General:

During toxicity tests periodic sampling may be necessary to determine toxicant-induced effects on fish. The humane treatment of experimental fish in laboratory and field investigations insures quality test animals as well as complies with the guidelines and regulations in the Animal Welfare Act and ECRC's Animal Welfare Plan.

Procedure:

A. Disinfect nets and glass or polyethylene sampling containers with Sanaqua (see SOP B5.132). Label these containers for test chambers to be sampled. Remove fish from exposure concentration (sampling from low to high concentration) using a clean net and place into sampling container holding control exposure water. Handling should be accomplished as carefully and quickly as possible to avoid stress.

B. Water temperature in the sampling containers should be maintained within $\pm$ 1°C of the test water temperature while measurements are being taken. To maintain temperature, sample containers can be placed in a waterbath or larger container with ice chilled water, or warm water.

C. The accepted procedure for anesthetization of fishes at ECRC is with tricaine methanesulfonate (MS-222). This chemical agent is an acid, and if used in extremely soft water, (hardness less than 10 mg/L as $CaCO^3$) can reduce the pH of the solution to levels (pH of 5 or less) that will stress fish. Check the pH of the solution and add a buffering agent if needed. This anesthetic solution should be changed frequently to ensure that there is sufficient oxygen.

D. Place only one test fish into sample container that will be used to anesthetize the fish. Carefully dissolve enough MS-222 into this container filled with control water to cause the fish to lose equilibrium and turn on its side but still

ventilate.

Note: Many times fish in the higher concentrations are weak and under stress. Add MS-222 slowly and sparingly when anesthetizing these fish. What will anesthetize a control fish may kill a fish from the higher concentration exposure levels. When a safe and effective level of anesthetic has been determined, 3 to 5 fish may be anesthetized and sampled at the same time.

E. Remove the anesthetized fish by carefully grasping the caudal fin with clean forceps. Without causing injuries, quickly and carefully blot fish and place on the measuring board. The standard, fork, or total length of the fish is recorded to the nearest millimeter.

F. Place a large disposable weigh boat containing control water on the balance weighing pan. Tare out the weight of the boat and water. Carefully place the fish into the weigh boat and record the weight to the nearest milligram. Remove the weigh boat and gently pour the fish into the container of fresh control water. These procedures will need to be accomplished with speed and accuracy to prevent the fish from being exposed to the anesthetic any longer than necessary. Wait until all fish have recovered before returning them to the appropriate test chamber.

G. Carefully, gently, and quickly pour fish back into the test chambers. Check back periodically to make sure fish have recovered fully. If there are injuries or mortalities record them on the comment sheets or log book for the test diluter.

Prepared by:

Eugene Greer Date
Leader, Fish Culture Section

Approved by:

Christopher Ingersoll Date
Branch Chief, Toxicology

Linda Sappington Date
Quality Assurance Officer

References:

SOP B5.132 "Equipment Disinfection"

Date Prepared: 01/31/89

Date Revised:

REPORTING DEFICIENCIES IN ANIMAL CARE AND TREATMENT

The humane treatment of experimental animals in laboratory and field investigations is an utmost concern from both a scientific and ethical standpoint. NFCRC staff and members of the general public who observe deficiencies in care or treatment of fish and other vertebrate species or who question procedures in the various research activities have the right to report their concerns to the NFCRC Animal Care and Use Committee (ACUC). The ACUC is obligated to review these concerns and to address them in a timely manner.

If an individual has a question or alleges a deficiency, the concern can be expressed to any ACUC member. Preferably, concerns should be communicated in writing, but a verbal account will carry just as much weight. Include in the report the date, time, place, and the activity which warrants review. The ACUC will review the details and is obligated to file a written report within 15 working days. The ACUC will try to resolve any deficiencies noted through the research supervisory chain and final decisions will be made by the Director of NFCRC. Major deficiencies noted and confirmed will be reported to the Regional Director, Region 8, through the Director of NFCRC. Files of deficiencies reported and action taken to correct them will be maintained by the chairman of the Animal Care and Use Committee. These files may be examined by interested members of the staff or the general public during Center working hours.

Prepared by:

Eugene Greer
Fishery Biologist

Approved by:

Paul M. Mehrle
Chief Biologist

James W. Hogan
Quality Assurance Officer

Date Prepared: 3/8/89

Date Revised: 09/19/97

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ACCLIMATION OF FISH TO RESEARCH WATERS

When fish lots have passed the necessary quarantine period after acquisition, lots may be pulled from the culture tank and transferred to an acclimation reservoir. Any fish being transferred to a water quality different than the culture water in which they are normally maintained must be acclimated to the new water quality and new test temperature over time.

Fish should be acclimated to the research exposure water gradually by changing the water in the acclimation tank from 100% holding water to 100% dilution water over a period of 2 days or more; and they should be acclimated to the new water temperature by increasing/decreasing the temperature approximately 1°C per hour - no more than 8°C per 24 hours. Always aerate the fish acclimation tank to maintain suitable dissolved oxygen levels during the acclimation period. Alternate procedures should be delineated in the appropriate study protocol if deviations from methods stated in this procedure are planned. Standard Operating Procedure B5.13 Procedures for Keeping Fish Culture Records contain details on using the Fish Acclimation Record Form and this form should be used whenever appropriate.
Be sure that all nets and containers used to transfer the fish have to be disinfected with Sanaqua (see SOP B5.132). Handling should be accomplished as carefully and quickly as possible to avoid stress or injury.

Prepared by:

Eugene Greer — Date
Fishery Biologist

Approved by:

Christopher Ingersoll — Date
Branch Chief, Toxicology

Linda Sappington — Date
Quality Assurance Officer ECRC SOP

References:

SOP B5.13 "Procedures for Keeping Fish Culture Records"
SOP B5.132 "Equipment Disinfection

Date Prepared: 29 October 1991

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METHOD FOR MEASURING AVOIDANCE/ATTRACTANCE BEHAVIOR IN FISH

General

Certain chemical substances can be aversive or attractive to aquatic organisms. Attractance/avoidance responses often mediate exposure to contaminants and provide the only experimental means of assessing the risk of exposure in freely mobile organisms. The ability to detect and avoid a contaminant provides a means of mitigating hazardous exposure, even though avoidance can involve displacement from preferred habitats to areas that are less optimal in terms of shelter, food, or protection from predators. Loss of populations in the field could result from avoidance responses, therefore avoidance provides an important measure of ecosystem injury resulting from the release of hazardous substances. Avoidance/attractance tests measure spatial selection in relation to a gradient of contaminant applied in the water column. The responses to contaminant gradients include preference for uncontaminated or contaminated areas, or a change in spatial preference during contaminant application relative to preexposure preferences. The response measured may include movements into or away from contaminated areas, duration of time spent in contaminated space, frequency of entry into contaminated space, or differences in the speed and patterns of movement in contaminated and uncontaminated space.

Acclimation

Fish shall be acclimated to the temperatures of test control water at a rate of change not to exceed 2° C per day. Acclimation to test control hardness shall not exceed greater than two 50% dilutions in hardness per day over the hardness range of 50 to 300 mg/L hardness as $CaCO_3$.

Fish should be held under acclimation conditions for at least 72 hours prior to testing.

Fish shall be deprived of food for a minimum of 12 hours prior to test, but shall not be deprived of food for a period greater than 24 hours.

Fish selected for testing shall be in good apparent condition, free of obvious disease, injury, or distress. Fish shall not receive treatments for disease required, in accordance with standard fish culture practices, upon receipt of shipment of eggs or fish.

Apparatus

A cylindrical plexiglass counter-current chamber (approximately 11 cm diameter x 92 cm long) is used to create a contaminant gradient. Control and test solutions enter the chamber from each end, and drain from six drains in the center.

Head tanks receiving control and test solutions from a diluter or from a storage tank of premixed

SOP: B-5.232 Page 2 of 6

solutions provide constant flow to the chamber through gravity flow or low volume electric pump. Flow into the chambers is regulated by a pair of two-way solenoid valves which, in the rinse mode, will provide uncontaminanted water to the chamber, and in the test mode, will supply the experimental solution and control water.

A second pair of switching solenoids directs the flow of test solution to one end of the chamber and control solutions to the other. When activated, the switching solenoid valves will reverse the flow of control and test solutions to each end of the chamber. Activation of the switching valves is achieved through a toggle switch or programmable controller and provides randomization of the contaminant presentation in the chamber to control for position bias.

The bottom of the chamber is covered to provide a uniform, opaque background and overhead fluoresent lighting provides uniform illumination of the chamber to eliminate shadows.

The chambers are surrounded by a partition to prevent visual disturbance of the organism. If necessary, accoustical foam may be used to minimize background sounds.

As necessary, the chamber and/or water supply may be placed in shallow water bath or covered with insulation to maintain constant temperature.

An overhead video camera is used to view the chamber and all studies are video taped. The rinse/test status of the chamber and the position of the test solutions in the chamber are encoded by a 3-LED signal panel. An illuminated central LED indicates a rinsing/acclimation period, whereas LEDs illuminated on either side would indicate the end of the apparatus receiving the test solution. A time readout showing minutes and seconds will be recorded on video tape for later reference during playback of the video recording.

Calibrations

Test chamber calibrations include measuring the rate for flow into each end of the chamber during rinse and test conditions as well as before and after switching. Water flows should not be less than 750 ml per minute and should not vary by more than 10 % between rinse and test phases, or from one end of the chamber to the other.

Temperatures of acclimation water, and of rinse, control, and test solutions should be within 0.4°C and should not vary more than 0.4°C during an avoidance test.

Other water quality variables such as pH and hardness should be within 10% of the target range.

Water levels within the avoidance chamber should not vary more than 10 % during an avoidance test.

Fluorescein dye calibrations with a spectrofluorometer must be conducted when the chamber is modified, flow rates less than 750 ml/min are used, or average water level in the chamber is greater than 2 cm from the top drain.

Avoidance Test Procedures

Diluter is activated or experimental solutions are mixed in stock tanks, water temperatures are verified, then the control and test solution head boxes are filled.

Avoidance apparatus is filled with rinse water of appropriate temperature and water quality. Temperature is monitored in the head tanks at the beginning and end of the acclimation period and at the beginning and end of the test period. Water temperature is monitored in the avoidance chamber prior to the introduction of the fish and at the end of the test. Water levels within the chamber are monitored throughout the test.

Overhead lights and video camera are turned on. Test date, species, test solution and concentration are listed on a note card which is placed within the field of view of the camera, to identify the avoidance test.

Two juvenile fish are taken from acclimation tank and added to the avoidance chamber. Fish that appear physically or behaviorally abnormal are discarded. Fish that are chased in excess of 2 seconds prior to netting, or handled roughly are not used.

The testing area is enclosed with the partition and the fish are acclimated for 20 minutes, during which the fish arior, territorial behavior, or abnormal responses. Fish showing abnormal responses at this point should be removed and another group selected for test.

. . . . . .

ONCE FISH ARE PLACED IN THE CHAMBER AND THE SYSTEM IS ENCLOSED, PARTICULAR CARE MUST BE TAKEN TO AVOID NOISE,VIBRATION, OR VISUAL MOVEMENTS THAT MIGHT DISRUPT THE BEHAVIORAL ACTIVITY OF THE FISH. THE SYSTEM MUST REMAIN CLOSED FOR THE DURATION OF THE TEST.

A 5 minute video sample is recorded during the last five minutes of the acclimation period.

The position where the test solution shall enter is randomly selected, and the switching solenoids are activated as appropriate. The switches are similarly wired and plumbed so as to eliminate the possibility that both solutions could enter the same end of the chamber at once.

The Rinse/Test solenoids are switched 'off' to release the test solutions. The fish are video recorded and monitored in the chamber for 30-40 minutes. This period includes a duration of approximately 5 minutes required for the formation of the gradient and an additional 5 to 10 minute latency period of the fish's reaction

At the conclusion of the 30-40 minute exposure to test solutions in the avoidance chamber, the video recording ends, and the fish are removed from the chamber.

The Rinse/Test solenoids are turned 'on' to rinse the chamber. At flow rates of 750 ml/min, rinsing is completed by 20 minutes.
At the end of the rinse period, another group of fish are selected for the next test.

Data Collection

Data collection can be conducted during the test by viewing the monitor, or by reviewing the video tape at a later date. Even when the data are collected 'live', the session must also be video recorded for future reference.

The video tape is advanced to the beginning of the acclimation footage where the test identification card appears. Information on the card is entered on the transcription sheet and includes; date of test, species tested, nature of test solutions, control water quality, and test temperature. The time readout in the field of view is used to time the experimental sequence and provides an accurate identification of the intervals during which the data are recorded.

The video tape is advanced to the final five minutes of the acclimation period and the video footage is recorded on the transcription form. At intervals of at least 30 seconds, the number of fish in each side of the avoidance chamber is recorded on the transcription form, along with the time shown in the field of view. The acclimation data may be used for comparisons of pre-exposure and exposure periods, as well as for determining position bias in the chamber.

The video tape is advanced to the onset of the test period, when the center indicator LED is turned off and either of the end LEDs are lighted. The time, video footage, and end of chamber receiving the test solution, as indicated by the lighted LED, is recorded on the transcription form. At 30 second intervals thereafter the number of fish in each side of the chamber is recorded, along with the time of the observation.

Throughout the acclimation and test periods, the behavior of the fish will be monitored for aggressive activity, abnormal swimming patterns, or changes in water flow or water level that might interfere with the fish's spatial selection, and the test will be invalidated and data discarded should such complications arise.

Alternative measured variables

The spatial preference of the fish should be effectively measured at discrete 30 sec intervals, however, when the fish exhibit a high level of activity, with frequent side changes, it may be more effective to count the frequency of entrys into one side of the chamber or the amount of time spent on either side of the chamber. Depth of movement into each side of the chamber or relative

swimming speed during entry and exit may also be noted.

Test Replication

Each avoidance test in which the same toxicant quality is presented shall be the experimental unit. The number of test replications shall range from 5 to 20 based on the variance of response.

Data Analysis

The contaminant gradient requires approximately five minutes to reach the target test concentration; therefore, data analysis should not include data obtained during the initial five minutes of the test period. Data will be collected from time 20 minutes to 40 minutes of the test period. It may be desirable to note responses that occur during the initial 20 minutes, as an indication of sensitivity to lower than target concentrations. The data should be analyzed in five minute segments, with the potential to combine segments in subsequent analyses. The data will be defined as the number of observations in which the fish are present in the side of the chamber receiving the test solution, or the cumulative amount of time spent there. These responses shall be divided by the number of total observations, or total cumulative time of the test to calculate an avoidance ratio. This ratio can also be mutiplied by 100 and expressed as percent avoidance. Statistical analyses will be conducted on ratios or cumulative responses from replicate tests.

Rejection of Test Results

Test results will be deemed invalid when abnormal spatial distributions are found to result from social aggression, or abnormal behavior, or by disruption of normal behavior by noise, vibration, or visual stimuli. Malfunction of the apparatus including delivery of test solutions, drainage of the chamber, and failure to control temperatures to within 0.4° C between the ends of the chamber; to within 0.4° C between the test and control solutions; to within 0.4° C between acclimation and test temperatures; or to be in excess of 0.4° C of the target test temperature. Test results may also be deemed invalid if chemical analyses of water quality and test concentrations reveal contaminated control water, or if the pH and hardness vary by more than

10% of specified values.

Sample Collection for Analysis of Test Water

Once during each day of testing, for each concentration applied, water shall be sampled from the end of the chamber receiving the test solution. The samples will be collected at the end of the test period, from the partitioned end of the chamber where the test solution enters the chamber. The samples will be collected, filtered, and preserved as specified in SOP B-5.134. The sample containers will be marked for identification using appropriate study codes.

Disposal of Test Organisms

At the conclusion of the avoidance tests, the fish will be euthanized with 300 mg/L MS-222 for 5 minutes and will be disposed in the sanitary disposal following SOP B-5.148.

Prepared by: Edward E. Little
General Biologist

Approved by: Denny R. Buckler
CHIEF BIOLOGIST

Joseph B. Hunn
Quality Assurance Officer

DATE PREPARED 03/23/95

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CULTURE, ANESTHETIZATION, AND EUTHANASIA OF AMPHIBIANS USED IN TOXICITY TESTING

Introduction

This SOP is based on ASTM Guide E 729 - 88a, "Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians" and ASTM Guide E 1439 - 91 "Standard Guide for conducting the Frog Embryo Teretogenesis Assay - Xenpous (FETAX). Amphibians are used as model species in aquatic toxicity studies primarily because of their developmental stages, and their ease of handling, maintenance, and reproduction in the laboratory.

CULTURE

Adult Xenopus laevis are obtained from commercial sources and cultured in the laboratory in MSC well water (Hardness, 283 mg/L as CaCO3; alkalinity 255 mg/L as CaCO3). The adults are held in flow-through glass aquaria under a 12:12 (light:dark) photoperiod at 20 ± 3 C. The density in the culture tanks should not exceed 4 - 6 frogs/1800 cm2 of water surface area. The adult Xenopus are fed 3 - 4 times per week with live fish obtained from MSC cultures. Breeding of the adult Xenopus to obtain embryos is carried out in the following manner:

1. Adults are bref at 20 ± 3 C under a 12:12 (light:dark) Photoperiod,
2. Males and females are bred as single pairs,
3. Culture water described above is used for breeding,
4. Breeding is induced by injecting males with 250 to 500 IU of Human Chorionic Gonadotropin (HCG) and females with 500 to 1000 IU of HCG. The HCG is injected into the dorsal lymph sac with a 1-ml tuberculum syringe fitted with a ½ inch long 26 gauge needle. The HCG concentration is 1000 IU/ml in 0.9% sterile NaCl,
5. The eggs are dejellied by swirling them for 1-3 minutes in 2% v/v of L-cysteine prepared in culture water adjusted to ph 8.1 with 1N NaOH,
6. The "Atlas of Abnormalities" is used to select normally cleaving embryos for use in the tests, and
7. Midblastula (stage 8) to early gastrula (stage 11) is used to start the FETAX test.

Embryos of Bufo sp., Rana sp., and Hyla sp., are obtained from commercial sources or wild populations. The embryos are cultured in the laboratory under flow-through or static renewal conditions in the well water described above at 18 to 22 C and a 12:12 light:dark) photoperiod. The tadpole stages of these species used in testing are maintained in glass aquaria or other glass chambers. During culture and testing the tadpoles are fed daily with a mixture of ground rabbit chow and fish flakes.

Anesthetization

During the injection of Xenpous with HCG no anesthetization is required. The injections are performed or supervised by trained biologists. The adult Xenpous are restrained in large nylon nets or nylon mess gabs and the injections are done quickly with only minor discomfort to the animals.
Anesthetization to tadpoles (Bufo sp., Rana sp., and Hyla sp.) for growth determinations is accomplished by submerging the tadpoles in a 100 mg/l solution of MS-22 in cultured water. The tadpoles are quickly weighed and measured, placed in cluture water for resuscitation, and then returned to the exposure chambers.

Euthanasis

Larval Xenpous and tadpoles of Bufo sp., Rana sp., and Hyla sp., are euthanized for growth, contaminant tissue residue analyses, and analysis of biochemical endpoints. Euthanization is accomplished by submerging the animals in a 200 mg/l solution of MS-222.

Prepared by:

_____
Laverne Cleveland Date
Fishery Biologist (Research)

Approved by:

_____
Denny R. Buckler Date
Chief Biologist

_____
Linda Sappington Date
Quality Assurance

_____
Eugene Greer
Chairman, Animal Care and Use Committee

Date Prepared: 09/21/87

Date Revised: 09/09/97

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PROCEDURE FOR COLLECTING WATER SAMPLES FROM CHRONIC FLOW-THROUGH TOXICITY TESTS FOR RESIDUE ANALYSIS

I. General:

A. Chronic toxicity tests with fish are typically conducted in proportional flow-through diluters. Exposure water samples are usually taken for residue analysis after fish have been added to the exposure tanks and following a period of operation (at least 24 hours) to ensure that nominal test concentrations have been obtained (day 0 of test). Sampling frequency is dependent on the test duration and nature of the toxicant and is described in the Test Protocol or attachments to the Test Protocol. Parameters such as 1) sample volumes, 2) type of sample containers, 2) type of filters, 3) type and number of reagent blanks, 4) number of spiked and duplicate samples, and 5) sample preservation procedures depend on the chemical characteristics of the toxicant and are determined prior to initiating the test. These parameters are described in the Test Protocol or attachments to the Test Protocol.

II. Sampling Procedure for Non-Filtered Water Samples:

A. Water samples greater than 100 milliliters are siphoned from the center of the exposure chamber at mid-dept with a clean glass siphon tube. Three tube volumes are discarded to ensure adequate flushing of the tube and the appropriate sample volume is collected in a clean graduated cylinder. Water samples less than 100 milliliters are collected with a clean 100 milliliter glass pipet attached to a Drummond[R] portable pipet-aid. As in the collection of the larger samples, the tip of the pipet is placed in the center of the exposure chamber at mid-dept. All samples are collected in ascending order of exposure concentrations (i.e. controls first up through the highest concentration) to avoid having to wash the sampling tube or pipet between concentrations. The samples are then transferred to the sample containers and labeled with the date, toxicant name, a unique sample identification number, and the collector's initials. The sample is then preserved according to procedures that are described in the Test Protocol or attachments to the Test Protocol.

B. Filtered samples are collected in the same manner as described above for non-filtered samples. Following collection of the appropriate volumes, samples are transferred to the sample reservoir of a filtration apparatus and filtered under vacuum across an

appropriate filter. The filtered sample is then decanted into an appropriate container and preserved.

C. Residue Analysis Transmittal Sheets (Attachment A) are filled out completely for each set of samples.

Prepared by:

Laverne Cleveland Date
Research Fishery Biologist

Approved by:

Ed Little Date
Branch Chief, Population Ecology

Linda Sappington Date
Quality Assurance Officer

Attachments:

1. Chain of Custody Form

United States Department of the Interior

U. S. GEOLOGICAL SURVEY

Environmental and Contaminants Research Center
4200 New Haven Road
Columbia, Missouri 65201

Chain-of-Custody Record

Attachment 1

Study No	Study Name	Control No
Samplers (Signature)		Page ___ of ___

Sample Identification	Date	Time	Type *	Remarks and Observations

Relinquished by (Signature)	Date/Time	Received by (Signature)	Relinquished by (Signature)	Date/Time	Received by (Signature)
Relinquished by (Signature)	Date/Time	Received by (Signature)	Relinquished by (Signature)	Date/Time	Received by (Signature)
Relinquished by (Signature)	Date/Time	Received for Laboratory by (Signature)	Date/Time	Remarks	

* W=water, S=sediment, P=plant, F=fish, B=benthos, O=other, define in remarks

Date Prepared: 09/21/87

Date Revised: 09/09/97

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PROCEDURE FOR COLLECTING FISH FROM CHRONIC FLOW-THROUGH TOXICITY TESTS FOR TISSUE RESIDUE ANALYSIS

Samples of fish are collected during chronic flow-through toxicity tests to determine the amount of toxicant absorbed by the test organism and the rate at which the organism eliminates the toxicant. Sampling frequency, amount of tissue collected, and preservation techniques depend on the nature of the test and toxicant and are determined prior to initiating the test. These parameters are described in detail in the Test Protocol or attachments to the Test Protocol. Fish tissue samples are collected from chronic flow-through toxicity tests in the following manner.

1. During a chronic flow-through toxicity test, fish are exposed to a series of toxicant concentrations and a control which are replicated. Samples of fish are taken from each replicated exposure concentration for residue analysis.

2. A top-loading scale is used for quick and easy access. The scale is turned on and allowed to warm-up for at least 30 minutes. The appropriate calibration procedures are performed to ensure that the scale is functioning properly. The scale should also be checked periodically with standard weights to ensure its accuracy.

3. Live fish are randomly removed from the exposure chambers with a clean net and placed into clean rectangular glass or polyethylene sampling containers in control exposure water. The number of fish taken per replicate sample depend on the size of the fish and the amount of tissue required to perform the analysis.

4. The fish are dosed with a sufficient amount of tricaine methanesulfonate (MS-222) to cause immediate death.

5. Individual fish from the replicate samples are removed from the anesthetic solution, blotted, pooled, and a wet weight determined to the nearest hundredth gram. The fish are then transferred to an appropriate sample container. Labels containing the date, a unique sample identification number, test day, species, exposure concentration, weight of pooled sample, and the collector's initials are affixed to the sample containers. Residue Analysis Transmittal Sheets or Chain of Custody forms (Attachment A) are filled out completely for all samples. The samples are then preserved and stored until

analysis according to procedures described in the Test Protocol or attachments to the Test Protocol.

Prepared by:

Laverne Cleveland Date
Research Fishery Biologist

Approved by:

Ed Little Date
Branch Chief, Population Ecology

Linda Sappington Date
Quality Assurance Officer

Attachment:

1. Chain of Custody Record

United States Department of the Interior

U. S. GEOLOGICAL SURVEY

Environmental and Contaminants Research Center
4200 New Haven Road
Columbia, Missouri 65201

Chain-of-Custody Record

Attachment I

Study No	Study Name	Project No
Sampler (Signature)		Page ___ of ___

Sample Identification	Date	Time	Type *	Remarks and Observations

Relinquished by (Signature)	Date/Time	Received by (Signature)	Relinquished by (Signature)	Date/Time	Received by (Signature)
Relinquished by (Signature)	Date/Time	Received by (Signature)	Relinquished by (Signature)	Date/Time	Received by (Signature)
Relinquished by (Signature)	Date/Time	Received for Laboratory by (Signature)	Date/Time	Remarks	

* W=water, S=sediment, P=plant, F=fish, B=benthos, O=other, define in remarks

Date Prepared: 09/22/87

Date Revised: 09/19/97

For users other than ECRC staff, this document is for reference only. This is not a citable document.

ANESTHETIZATION OF FISHES FOR SAMPLING PURPOSES

I. General:

Tricaine methanesulfonate (TMS, MS-222) has been used for over 50 years to anesthetize fish. TMS has been intensively studied over the past 25 years as to efficacy, toxicity and residues in a variety of fishes. The water concentrations of TMS necessary to induce anesthesia varies with the species of fish used and the water chemistry of the anesthetizing solution.

II. Anesthetic Solution:

A. Because there are differences in the response to TMS, the following concentrations are given as guides with the actual levels to be determined by the species used and the response and time to that response being the final determinants.

Fish	Guidelines are for Rapid Anesthesia*
Salmonids	100-150 mg/l
Escids	100-200 mg/l
Cyprinids	150-200 mg/l
Ictalurids	150-250 mg/l
Centrarchids	200-300 mg/l
Percids	100-150 mg/l

*Loss of reflex activity in 2-5 minutes (see Schoettger and Julin, 1967).

B. TMS is an acid and if used in extremely soft water (hardness less than 10 mg/l as $CaCO_3$) can reduce the pH of the anesthetic solution to levels that will stress fish (pH of 5 or less). Check the pH of the anesthetic solution and if needed bring to pH 7 with bicarbonate.

C. The anesthetic solution should have sufficient oxygen so as not to stress the fish if the fish are to be returned live to the test system.

D. The length of time that fish are anesthetized should not exceed the time to medullary collapse or recovery of the fish from anesthesia will be difficult if not impossible. Over-anesthetization can be used as a method for euthanasia.

Prepared by:

Eugene Greer Date
Fishery Biologist

Approved by:

Christopher Ingersoll Date
Branch Chief, Toxicology

Linda Sappington Date
Quality Assurance Officer

References:

1. Schoettger, R.A. and A.M. Julin. 1967. Efficacy of MS-222 as an anesthetic on four salmonids. Investigations in Fish Control No. 13. 15 p. U.S. Department of Interior, Bureau of Sport Fisheries and Wildlife, Washington, DC.

2. Finquel brand of tricaine methane sulfonate. Technical Data Sheet. Ayerst Laboratories Incorporated, New York, NY.

DATE PREPARED 04/18/90
REVISED 05/21/96

For users other than MSC staff, this document is for reference only. This is not a citable document.

COLLECTION AND PRESERVATION OF WATER AND TISSUE TO BE ANALYZED FOR TRACE METALS

General: Samples from the environment can be highly variable in character. This variability, in addition to the numerous possibilities of analytical requirements for the sample, makes it difficult to provide a set of guidelines to cover all sample collection and preservation situations. Thus, the following collection and preservation information is offered as a general guideline and is subject to changes to allow adaptation to specific sample characteristics and/or analytical requirements.

WATER SAMPLES

1.**Field.** Collect two 500 mL samples at each site, one filtered and the other unfiltered, unless study protocol directs otherwise. Recommended materials and acid cleaning procedures are listed in a later section.

(a)non-filtered sample - collect by submersing an acid-cleaned conventional polyethylene bottle into the water using tongs or waterproof gloves; it is a good idea to rinse the bottle out with a small volume of the water to be collected prior to the actual collection; preserve with high purity HNO_3 or HCl (refer to study protocol) by adding enough acid to achieve an effective concentration of 1% v/v. Leave about a 1 inch head space below the cap and seal tightly. Sealed ampules of HNO_3 or HCl are available for convenient field use (see section entitled "Recommended materials, supplies, and other information").

(b)filtered sample - use Geotech filtration unit and follow attached procedures for use and operation. Perform filtration with a Nucleopore 0.40 m polycarbonate membrane. Use the first few mLs of filtrate to rinse out the sample bottle. After filtered sample is in a pre-cleaned polyethylene bottle, acidify to a 1% effective acid concentration with high purity HNO_3 or HCl (refer to study protocol). Collect enough water to fill the bottle, leaving about a 1 inch head space below the cap and seal tightly.

2.**Laboratory.** Collect two 100 mL samples from each tank, location, etc., one filtered and one non-filtered or follow study protocol.

(a) Collect a filtered sample as described in 1(a) above.

(b) Collect filtered sample as above. Smaller hand-held filtration units may be used in place of the Geotech unit i.e., the Nalgene polysulfone filtration unit (Cole-Parmer # N-06730-55). If the Nalgene unit is used, remove the membrane filter between samples and rinse the unit with at least 20 mL of dilute nitric acid (0.1 - 1.0% v/v), followed by an equal volume of high purity water. The unit may be rinsed by use of a squirt bottle to the upper chamber, allowing the rinse to drain through the filter support into the receiver, then swirling and discarding.

(c)Sample water is received from field unfiltered and unacidified. Shake 15 seconds and pour off sample if needed for head space and let acclimate to room temperature. Add 10mL of sub-boiled H2NO3 per 1L (1% effective) water. Let set for 24 hours. Shake sample. Filter as in section 2b. This will be the dilute acid extractable.

TISSUE SAMPLES

Collected samples may be placed in either a Whirl-Pak bag or small Zip Lock bag (for small samples), or in large clear polyethylene bags (or large Zip Locks) for the larger tissue samples. Pre-cleaned 4 oz wide-mouth bottles with Teflon®-lined closures (e.g., I-Chem) are recommended for invertebrates, eggs, etc. Larger, whole specimens for compositing (such as whole body fish) should be individually wrapped in plastic (Glad wrap or Saran wrap) before placing in larger bags. When transferring samples to storage containers, care should be exercised to minimize handling of specimens. Where applicable, polypropylene tongs, forceps, or spatulas, should be utilized for manipulation of samples. Specimens which appear to have surface contamination from sediment must be flushed with water from which they were collected. Samples should be put on dry ice or in a freezer immediately.

LABELING

On a dry surface of the water bottle, place a pressure- sensitive label and fill in with an indelible marking pen. Place clear scotch tape over the entire label to waterproof it. For tissue samples, write on the Whirl-Pak bag with an indelible marker; tie a cloth tag around the outer polyethylene bag for the larger samples. Write the sample ID on the cloth tag with an indelible marking pen. Cloth tags 2 5/8" x 5 1/4" are available from the GSA Supply Catalogue.

QUALITY CONTROL - WATER

The following should be included as additional samples with each collection unless the study protocol directs otherwise:

(1) Field reagent blank - distilled or deionized water poured into the sample container and then the appropriate preservative is added. This is a check on the purity of reagents and cleanliness of

container(s). If filtration is performed, deionized water is filtered and preserved.

(2) Duplicate samples - identical samples are taken assigned separate sample numbers, and sent back to the lab as blind samples. This is a check on the precision of analytical results.

(3) Spiked samples (optional) - a known quantity of analyte is added to one of a pair of duplicate samples. This is a check on accuracy of analytical results and possible loss of analyte during storage.

Numbers of the above samples to be taken - a minimum of three reagent blanks for each collection of samples and two duplicate sample pairs per collection of samples. If spikes are included: two spiked sample pairs, one member of a pair spiked with low concentration and one member spiked with a high concentration of the analyte. (Study protocol may include additional guidelines).

TISSUE QUALITY CONTROL

About the only form of quality control that can be exercised with collections of tissue samples is a replicate sample collection. For example, if a group of fish were captured in a net, select several fish of approximately the same species, length, weight, and age. Divide this group of similar fish into two groups. When these two groups are supplied to the lab as two composite fish samples, the contaminant profile should be similar for each sample. If desired, blind tissue control materials, such as NBS Bovine Liver, etc. can also be supplied, but unfortunately most of these materials can be identified on the basis of their appearance, color and odor.

RECOMMENDED MATERIALS, SUPPLIES AND OTHER INFORMATION

Acid Cleansing Procedure - follow MSC SOP C5.5.

0.40 um polycarbonate membrane Filters - Corning Costar, One Alewife Center, Cambridge, Massachusetts 2140 1-800-882-7711, 47 mm diameter, Costar stock #111107; 142mm diameter, Costar stock #112107 or 112207.

Nalgene polysulfone filter holder; Cole-Parmer N-06730-50.

Geotech filtration unit, available from Geotech Environmental Equipment, Inc.; 1441 West 46th Ave., Unit #17, Denver, Colorado 80211 1-800-833-7958.

Whirl-Pak sampling bags; available from Cole-Parmer Instrument Company, 7425 North Oak Park Avenue, Niles, IL 60714. 1-800-323-4340.

Polyethylene bags of all shapes and sizes, from Associated Bag Company, 400 West Boden Street, Milwaukee, Wisconsin 53207-7120 1-800-926-6100.

Pre - cleaned sample containers,(200 series, polyethylene; 300 series, glass) available from I-Chem, 2 Boulden Circle, Suite B, New Castle, DE 19720-2064 (302) 322-3808

Cloth tag labels of varying sizes, available from GSA Supply

JT Baker Ultrex-II Nitric or Hydrochloric Acid. Available from VWR Scientific.

Sealed ampules of reagent grade HNO_3 and HCl are available from Eagle Picher, 200 B.J. Tunnell Blvd., Miami, OK 74354-3300 1-800-331-7425.

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Date revised: 06/30/97

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PROCEDURE FOR ACID CLEANING PROPORTIONAL DILUTERS

GENERAL:

Organisms used in toxicity tests must be disease-free to limit the number of stress factors imposed on them during a toxicity test to only the toxicant(s) being tested. To prevent disease and residual chemical transmittal from one test to another conducted in the same diluter, a rigorous cleaning procedure must be accomplished on the various components of a diluter between tests. This SOP describes the procedure for acid cleaning diluters between toxicity tests at the Yankton Field Research Station.

APPARATUS:

1. Scouring pad.
2. Long-handled brush.
3. One liter beaker.

REAGENT:

1. Fish Pesticide Research Laboratory acid cleaning solution: a combination of HCl (38.2%) and a detergent, commercially prepared.

PROCEDURE:

1. At the completion of a diluter toxicity test, remove all remaining organisms present in the diluter aquaria and discard into the zero-discharge lagoon. Turn OFF the water supply to the diluter. Also, disconnect the power supply, interval
timer, cycle alarm timer, Micromedic(s), water circulators, and water chillers, if present.
2. Wearing the proper safety attire, e.g., rubber gloves, rubber boots, lab coat, and goggles, remove the aquaria plexiglass cover lids and clean with soap and soft water, rinse with deionized water, and allow to air dry. Remove the glass/stainless steel dividers and clean with cleaning acid, rinse well with deionized water and allow to air dry.
3. Remove the waterbath standpipe and drain the waterbath until empty. Remove the aquaria standpipes and allow the aquaria to drain until empty. Place all standpipes into a cleaning acid bath.
4. Carefully place an appropriately-sized rubber stopper into the open end of the

delivery line to each aquaria which delivers test water from the flow-splitting boxes (SOP 5.2) to the test system. Using the one liter beaker, fill the flow-splitting boxes on the diluter back with cleaning acid. This acid will drain into the delivery lines. Let acid set in the delivery lines while cleaning the aquaria (step 5).

5. Scrub the sides of each aquaria with a scouring pad and cleaning acid to remove any residual material. After cleaning all aquaria, scrub the flow-splitting boxes with a brush. Then remove the rubber stoppers from the delivery lines and allow the acid to drain into the aquaria.
6. Rinse the flow-splitting boxes and delivery lines thoroughly with soft or deionized water until all traces of soap are removed.
7. Place an appropriately-sized rubber stopper in aquaria drain and the delivery lines. (**NOTE**: Rubber stoppers are again inserted in the delivery lines to prevent any backsplash of soapy water into the cleaned lines.) Fill each aquaria with soft water and allow to overflow into the waterbath until all traces of soap are removed.
8. After all aquaria have been rinsed thoroughly and drained, vacuum dry with the shop vacuum.
9. Remove the aquaria standpipes from the cleaning acid bath, and brush with the scrub brush to remove any residual material. Rinse all standpipes several times with deionized water and allow to air dry.
10. Carefully remove all components of the diluter back (glass boxes, Y-shaped venturi tubes, delivery lines) and place into the cleaning acid bath. Using the long-handled brush, swab the inside of each glass box with cleaning acid. Then clean with the scouring pad to remove any residual materials, rinse several times with deionized water, and allow to air dry. Clean all glass tubing with a brush, rinse well with deionized water, and allow to air dry also.
11. Reinstall all diluter parts, including glass boxes, Y-shaped venturi tubes, and delivery lines on the diluter back, and all test aquaria parts such as standpipes, dividers, and cover lids. Replace latex tubing used to connect Y-shaped venturi tubes with delivery lines and vacuum manifold. The diluter is now ready for reuse. If any glass components of the diluter back such as water cells, chemical cells, mixing box, or flow-splitting cells were broken during the cleaning procedure, consult SOP 5.2 concerning the proper construction and recalibration of components.

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REFERENCES:

SOP F5.2 "Construction, Operation, Calibration, and Maintenance of the Proportional Diluter"

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PROCEDURE FOR PH DETERMINATION - ORION EA 940

A. Equipment and Standards

1. The Ph meter used is an Orion ionalyser model EA940 equipped with a temperature compensating probe Orion 917001 (See Jackson SOP: F20.E10).

2. The probe used is a Ross 81-02 combination glass body electrode 0-14 pH range. The probe is stored in a 200 ml pH 7 standard buffer solution with 1 g of KCl added. The internal filling solution for the probe is 3M KCl.

B. Two Buffer Calibration

1. Two buffers are selected that will best bracket the expected pH sample. Generally they are selected from: pH 4.01, 7.00 and 10.00. For example, if the expected pH is 5.0, then the two buffers selected would be pH 7.00 and pH 4.01.

2. The pH meter can either be calibrated automatically or manually.

3. The Orion EA 940 operates by asking a series of questions on a digital display board. The operator answers these questions by pressing yes or no keys and the meter moves on to the next step.

4. We have found that manual calibration fits our needs best. The following is the series of steps the operator must go through to calibrate the EA 940 and measure pH of sample.

--The operator presses "speed" key then numeric key "2." The meter then displays "operator menu?"

--The operator presses "no" key. Then the meter displays "calibrate 1 pH?"

--The operator presses "yes" key. Meter will display "enter number of buffers.

--The operator presses "2." Meter will display "automatic calibration?"

--The operator presses "no" key. The meter will display "1 pH electrode in buffer 1?"

--The operator then place the electrode and the temperature compensating probe in the pH 7.00 buffer and presses "yes" key. The meter will then read "buffer 1 = 7.06 cal. as 7.00?"

--Operator then enters the actual pH value of the buffer by pressing the proper numeric keys. (For example if the actual value of the buffer is pH 6.97, the operator enters 6.97). The meter then displays "buffer 1 = 7.06 cal. as 6.97?"

--The operator presses "yes" key. The meter will then display "1 pH electrode in buffer 2."

--The operator places the electrode in buffer number 2 and presses the "yes" key. The meter then displays "buffer 2= 4.04 cal. as 4.01?"

--The operator enters the actual pH value of the second buffer and presses "yes" key. The meter will then display "Slope 99.5% yes to continue" (Slope must be between 92 and 104%).

--The operator then presses "yes." The meter will display the pH value of the sample. After the meter is calibrated, electrodes can continuously be placed from sample to sample and the pH value can be read directly form the display board for each sample. Probes should be rinsed with distilled water and excess moisture blotted off between each buffer and each sample that is measured.

6. Name of operator, date, time, and the buffers used are entered in a log book that is kept by the pH meter after each calibration.

7. PH meter is calibrated a minimum of once each day.

C. pH Readings

1. The pH of exposure tanks are checked daily in each individual tank during the treatment period by taking approximately 200 ml samples in a beaker from the tank. The pH and temperature probes are placed directly into the sample and the reading is taken from the meter display board.

2. Results of the pH reading are then recorded by the operator on a special form attached to the diluter. The tolerance of the pH reading is held within $\pm$ 0.2 of the required pH.

D. Automatic Temperature Compensating Probe (ATC)

1. The ATC enables the operator of the EA 940 to samples and buffers at any temperature without correcting for the difference in temperature. The ATC is placed in the sample along with the pH probe and automatically makes correction for temperature differences.

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REFERENCES:

SOP F20.E10 "Operation, Calibration, and Maintenance of the Orion Model EA 940 Ionanalyzer"