

ORIGINAL

A research protocol to determine the:

ANALYTICAL VERIFICATION OF CHLORAMINE-T TO CONFIRM TARGET DOSAGE IN A BATH SOLUTION ADMINISTERED USING A FLOW-THROUGH TREATMENT METHOD

Study Protocol Number: BFTC-01-CHLT-FT

Study start date: July 19, 2001

Study end date: July 27, 2001

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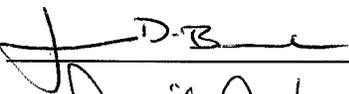
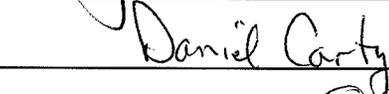
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1. TITLE:

Analytical verification of chloramine-T to confirm target dosage in a bath solution administered using a flow-through treatment method.

2. PROTOCOL NUMBER:

BFTC-01-CHLT-FT

3. SPONSOR:

3.1 Name and address:

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3.2 Telephone and fax numbers:

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4. PROTOCOL OBJECTIVE:

To determine whether a specified chloramine-T target dosage (i.e., 12 mg/L) can be achieved and maintained in a water bath over a specified duration (1 h) using a charged, flow-through treatment method.

5. STUDY OBJECTIVES:

5.1 Pivotal or non-pivotal:

The study will be a pivotal study.

5.2 Standards applied to the conduct of the study:

The study will be near-Good Laboratory Practice (GLP) compliant. Efforts will be made to comply with all sections of GLP regulations (21

CFR, Part 58), with the exception that there will be no quality assurance inspections of the (1) protocol; (2) standard operating procedures; (3) data collection; (4) raw data; or (5) the final study report.

6. INVESTIGATIONAL DRUG AND CONTROL:

6.1 Test substance:

6.1.1 Chemical name:

Chloramine-T

6.1.2 Trade name:

Halamid (CAS# 127-65-1)

6.1.3 Active/inactive ingredients:

Chloramine-T is a white, crystalline powder with a weak chlorine odor, and it is a pure compound with no inactive ingredients. The chemical name of chloramine-T is "benzene sulfonamide, N-chloro-4-methyl, sodium salt" (synonym, "sodium p-toluenesulphonchloramide"). The chemical formula of chloramine-T is $C_7H_7ClNNaO_2S \cdot 3H_2O$.

6.1.4 Dosage form:

Solution (i.e., chloramine-T powder dissolved in an appropriate volume of water; i.e., solubility of chloramine-T in water: 15 g/L water at 25°C).

6.1.5 Dose and duration to be tested:

12 mg/L for 1 h

6.1.6 Manufacturing site:

The chloramine-T to be used in this study was manufactured by Akzo-Nobel Chemical, Inc., (1600 Broadway, Suite 1450, Denver, CO 80202). Akzo-Nobel Chemical, Inc., recently sold

its rights to the manufacture of chloramine-T to Axcentive BV (Nijrerheidsplein 21 G, 3771 MR Barnveld N, The Netherlands).

6.1.7 Lot number:

2530015

6.1.8 Packaging:

The chloramine-T used in this study was kept in its original container (e.g., a large, plastic barrel with a screw-top plastic lid)

6.1.9 Drug storage during study:

Chloramine-T used during the study will be stored in the hazmat building at the Bozeman Fish Technology Center (BFTC).

6.1.10 Material Safety Data Sheet(s):

Material Safety Data Sheet(s) will be on file at several locations at the BFTC. At least one copy will be on file in the Aquaculture Drug Research Lab at the BFTC. In addition, all study participants will be familiar with the hazards of handling chloramine-T and will use appropriate safety precautions when using chloramine-T.

6.2 Control:

No control will be used in this study.

7. STUDY SCHEDULE:

7.1 Proposed date of initiation:

The study will be initiated on July 19, 2001.

7.2 Schedule of events:

Study days and a description of significant events are listed in Table 1.

7.3 Proposed date of completion:

The study will be completed on July 27, 2001.

8. STUDY DESIGN:

8.1 Treatment group(s):

A single treatment group will be used in which each of two raceways will be dosed at 12 mg/L chloramine-T. Testing to determine whether a specified chloramine-T dosage can be achieved and maintained will be duplicated in each raceway.

8.2 Experimental design:

The experimental design used in this study is best described as systematic, both in selecting the days that each raceway will be dosed and selecting sampling sites within the raceway from which water samples will be collected.

8.2.1 When test article will be administered:

Two raceways will be used in this study (Raceway 5 and Raceway 6, Figure 1). Raceway 5 will be dosed with chloramine-T on two different occasions and water samples will be collected and measured to determine chloramine-T concentrations throughout the dosage period. Raceway 6 will be dosed with chloramine-T on two different occasions and water samples will be collected and measured to determine chloramine-T concentrations throughout the dosage period. Note: Figure 1 shows the dimensions of one raceway; dimensions of both raceways used are nearly identical to each other.

8.2.2 Selection of water sample collection sites:

A systematic sampling approach, in which water samples will be collected over the area of the raceway, will be used. Gilbert (1987) describes a "central aligned square grid" systematic sampling scheme for sampling over space. We will apply this to three dimensions and collect water samples in three strata: (1)

head, middle, and tail-end of the raceway; (2) surface, mid-depth, and bottom of the raceway; and (3) along the right-hand side, in the middle, and along the left-hand side of the raceway (Figures 2a and 2b).

8.3. Blocking factors:

No blocking factors will be used because only two raceways will be used as the experimental units.

8.4 Randomization procedures:

No randomization procedures will be used.

9. STUDY PROCEDURES:

9.1 Test animals: Note: test animals may or may not be used in the study. Treatment or exposure effect of chloramine-T on test animals will not be a measured variable.

9.1.1 Description (if test animals are used):

9.1.1.1 Age:

Test animals used in the study will be characterized as "juvenile" or larger, and this characterization will be based on length (i.e., fish will be 5 inches or longer in total length).

9.1.1.2 Sex:

Sex of fish will not be determined or considered; however, it will be assumed that males and females will be present in roughly equal proportions.

9.1.1.3 Species:

The fish species to be used will be rainbow trout (Order: Salmoniformes; Family: Salmonidae; Genus and species: *Oncorhynchus mykiss*).

9.1.1.4 Physiological state:

The physiological state of test fish will not be determined nor considered; however, fish will be healthy-appearing and show no signs or symptoms of stress.

9.1.2 Number of fish:

A sufficient number of fish will be held in the test unit (i.e., raceway) to approximate near-production loading. Density Index (DI) and Flow Index (FI) values, which are indicators of whether or not hatchery-reared fish are being maintained within the carrying capacity of a given rearing unit, will not exceed those recommended by Piper et al. (1982) for rainbow trout.

9.1.3 Source of fish:

Test fish will be obtained as eggs from the U.S. Fish and Wildlife Service, Ennis National Fish Hatchery, Ennis, Montana. Eggs will be hatched and reared at the BFTC.

9.1.4 Identification method:

Individual fish will not be tagged for identification. The group of fish used will be identified by lot number. Lot number designation will be defined on the egg shipment paperwork by the staff at the Ennis National Fish Hatchery. The egg shipment paperwork will also identify the fish species and strain and will accompany the egg shipment to the BFTC.

9.2 Inclusion criteria:

Healthy-appearing fish, showing no obvious signs or symptoms of bacterial or parasitic infections, will be used in the study. The study will be initiated during a period of calm weather when no rain is forecast.

9.3 Exclusion criteria:

If a "fatal" situation occurs, such as an (1) epizootic among the fish, (2) dewatering of the raceway, (3) interruption of water flow to the

raceway, or (4) other situation that might profoundly affect the outcome of the study, then the test unit treatment will be considered a "non-treatment" and will be excluded from the study. In the event that a treatment is excluded from the study, the treatment will be repeated on the next available day.

9.4 Acclimation of fish:

9.4.1 Duration:

If used, fish will be acclimated in the raceway for at least one week prior to initiation of the study.

9.4.2 Medication during acclimation period:

Fish will not be treated with any medicinal therapeutant during the acclimation period.

9.4.3 Baseline data collected prior to initiating the study:

Daily mortality will be collected prior to initiating the study.

9.5 Blinding:

9.5.1 Extent of blinding:

Because only a single treatment group will be used in the study, all study personnel will know the chloramine-T target dosage used in the study.

9.5.2 Blinding methods:

No blinding methods will be used.

9.5.3 List of personnel with access to treatment codes and rationale:

All study personnel will have access to treatment codes.

9.6 Drug administration:

9.6.1 Dosing regimen:

The dosing regimen of the test unit will be 12 mg/L chloramine-T for 1 h using a charged, flow-through treatment method. The raceway will be initially dosed with an appropriate amount chloramine-T in a standing bath (i.e., no water flowing into the raceway) to achieve the desired target dose (i.e., charged) using the following equation from Piper et al. (1982):

Amount of chloramine-T (g) = target dose (mg/L) x volume of water in the test unit (gal) x 0.00378.

After chloramine-T has been administered and adequately distributed throughout the raceway (i.e., by physically mixing and possible by fish swimming in the raceway), water flow to the test unit will be resumed at a predetermined flow rate, and an appropriate amount of chloramine-T dissolved in water will be metered into the head-end of the raceway (i.e., the end where water flows into the raceway) to maintain the desired target dose for the treatment duration using the following equation from Piper et al. (1982):

Amount of chloramine-T (g) = target dose (mg/L) x water flow rate into the test unit (gal/min) x treatment duration (min) x 0.00378.

9.6.2 Route of administration:

Chloramine-T will be administered to the raceway as a fully-dissolved solution. A commercially available chicken-waterer will be used to hold the stock chloramine-T solution. A pipette, known to deliver a specific volume of water per unit of time, will be used to administer the stock chloramine-T solution to the raceway during the flow-through portion of the study.

9.6.3 Investigational withdrawal period:

45 days

9.6.4 Proposed withdrawal period:

45 days

9.7 Removal of fish from the study:

9.7.1 Criteria for removal of fish from the study:

Dead or moribund fish will be removed daily from the study.

9.7.2 Procedures for removal of fish from the study:

Dead or moribund fish will be dipnetted from the raceway and removed from the study.

9.7.3 Fate of removed study fish:

Fish removed from the study will be disposed of in the local land-fill.

9.8 Concurrent/concomitant medications/therapies:

No concurrent or concomitant medications or therapies will be administered to test fish during the course of the study.

9.9 General management practices:

Fish will be reared and maintained according to standard hatchery procedures described by Piper et al. (1982). At least one person involved in the study will be at the testing facility each day during the acclimation, pre-treatment, treatment, and post-treatment periods (i.e., the study). Raceway water temperature and dissolved oxygen concentration will be measured twice daily during the study. If fish are used in the study, the raceway will be cleaned twice daily, and the fish will be fed twice daily by hand during the study.

9.10 Provisions for necropsy and disposal of expired test subjects:

Because of the nature of the study, it is not anticipated that fish necropsy will be necessary. The chloramine-T dosage used in this study has been demonstrated to safe and effective, and no deleterious

effects due to exposure are anticipated. In the event a fish necropsy is considered necessary, the necropsy procedure will be conducted at the BFTC in the Aquaculture Drug Research Lab. Fish used for necropsy will be disposed of in the local landfill.

10. SPECIFICATION OF VARIABLES:

10.1 Variable to be measured for evaluating labeled claim:

10.1.1 When variable will be measured:

The concentration of chloramine-T in water samples collected at various times and locations during the treatment period will be the only variable measured to address the protocol objective. Water samples will be collected at a sufficient number of locations (i.e., up to 27 locations) within the test unit to adequately profile chloramine-T concentrations in the test unit during the course of treatment. Water samples for chloramine-T dose verification will be collected (1) at the beginning of the treatment period at time = 0 min (i.e., after chloramine has been added to the test unit to achieve the target dosage in a standing bath, but before (a) water flow has been resumed and (b) chloramine-T has been administered into the test unit); (2) during the middle of the treatment period (time = 30 min); and (3) at the end of the treatment period (time = 60 min) while chloramine-T is still being metered into the test unit and prior to pulling the test unit standpipe (which will facilitate flushing chloramine-T from the test unit). Water samples will be collected using (1) equipment designed to collect samples from various depths in the raceway (60 mg/L plastic syringe with tubing of appropriate length), and (2) techniques that will not further mix the contents of the raceway.

10.1.2 Procedures for addressing variable:

Chloramine-T will be measured following general instructions for analyzing chloramine-T using the HACH Pocket Colorimeter (HACH Company, Loveland, CO) DPD Test, according to procedures described in SOP No. INST 101

10.1.3 Equipment used to assess variable:

A HACH Chlorine Pocket Colorimeter will be used to verify chloramine-T doses.

10.1.4 Calculation of derived data:

The DPD method used will measure mg/L of free and total chlorine in the water sample. The resultant "bound" chlorine (i.e., total chlorine minus free chlorine) will be multiplied by 3.97 to determine the concentration of chloramine-T (mg/L) in the water sample.

10.1.5 Forms for retention of source data:

Appropriate forms will be used to clearly and adequately record source data.

10.2 Other variables to be recorded during the study:

10.2.1 When variables will be assessed:

Water temperature and dissolved oxygen (DO) concentration will be measured twice daily - once in the morning and once in the afternoon. On days when chloramine-T is to be administered, water temperature and DO will be measured in the raceway during static bath conditions, before chloramine-T is added, at two times during the 1 h treatment period, and once again after the treatment period has ended. Water temperature and DO concentrations will be measured at two locations. Water hardness, alkalinity, and pH will be measured twice during the study - once prior to the treatment period and once after the treatment period.

10.2.2 Procedures, equipment, and calculations of derived data for assessing variables:

The following standard operating procedures, which describe the procedures, equipment, and calculations of derived data, will be used to measure the other variables during the study:

Water temperature	SOP No. INST 120
Dissolved oxygen	SOP No. INST 120
Water hardness	SOP No. INST 105
Alkalinity	SOP No. INST 104
pH	SOP No. INST 125

10.3 Study facilities:

10.3.1 Containment equipment:

Two cement raceways, with dimensions described in Figure 1, will serve as the test units. Raceways will be fitted with a tail screen to help prevent fish escapement and an outflow standpipe to regulate water depth.

10.3.2 Lighting, heating, cooling, watering, and ventilation equipment:

Raceways are located outdoors, and no specialized lighting, heating, cooling, watering, or ventilation equipment will be used.

10.3.3 Feeding equipment:

Fish will be fed by hand and with appropriate type, size, and amount of feed as described by Piper et al. (1982).

10.3.4 Space allocation per animal:

Fish loading, described by Flow Index and Density Index values, will be sufficient to adequately rear healthy salmonids (Piper et al. 1982).

Flow index = total weight of fish in the test unit (lbs) ÷ (fish length (in) x water flow into test unit (gal/m)).

Density index = total weight of fish in the test unit (lbs) ÷ (fish length (in) x rearing space within test unit (gal)).

10.4 Experimental diets:

Not applicable.

11. DATA ANALYSIS:

11.1 Define the experimental unit:

The raceway will be the experimental unit.

11.2 Define the number of replicates per treatment:

There will be one treatment group consisting of two raceways. Treatment will be administered to the raceways two times (i.e., duplicate) on two different days.

11.3 Define statistical methods and hypotheses to be tested:

The null hypothesis to be tested is $H_0: \mu_{\text{chloramine-T concentration at } t=0 \text{ min, 30 min, and 60 min}} = 12 \text{ mg/L chloramine-T } (\pm 25\%; \text{ i.e., } 9 - 15 \text{ mg/L chloramine-T})$. A one-sided, one-sample t-test will be used to determine whether (1) $H_0: \mu_{\text{chloramine-T concentration at } t=0 \text{ min, 30 min, and 60 min}} < 9 \text{ mg/L chloramine-T}$; or (2) $H_0: \mu_{\text{chloramine-T concentration at } t=0 \text{ min, 30 min, and 60 min}} > 15 \text{ mg/L chloramine-T}$.

At each sampling time, water samples will be collected from each sample location. The chloramine-T concentration of each sample will be measured once. Ideally, the mean chloramine-T concentration for each time period will be based on measurements from all samples. If it becomes apparent that combining data in such a manner would be inappropriate, then select data will be combined, and the justification for combining data in such a manner will be described in the final study report.

11.4 Define how the statistical results will be used to draw conclusions about the study's objective:

Results from one-sided, one-sample t-tests conducted for data collected at $t = 0, 30, \text{ and } 60 \text{ min}$ will be used to determine if the mean measured chloramine-T concentration in the test unit differs significantly from the proposed chloramine-T treatment regimen by more or less than 25%.

12 ANALYTICAL METHODS:

12.1 Describe the analytical measurement to be made and the relevance to the protocol objective:

Water samples collected at various locations within the test unit at various times during the treatment period will be measured to analytically verify chloramine-T concentrations using the HACH Pocket Colorimeter DPD Test. The analytical methods used conform to FDA-guidelines. Measurement procedures used will be those described in SOP No. INST 101 (Appendix A). Mean measured chloramine-T concentrations will be used to determine whether the proposed treatment regimen can be achieved and maintained.

12.2 Specify the analytical plan to be used for the protocol measurement:

12.2.1 An abstract of the method:

A written draft of the analytical procedure (Schmidt 1997; Appendix B) describes the methodology.

12.2.2 Describe the procedures for sample selection, preparation, and storage:

Sample selection, defined as the (1) sample collection location within the test unit; and (2) the time at which the sample was collected during the treatment period, will be designed to adequately profile the chloramine-T treatment regimen (Figures 2a and 2b). Water samples will be collected from up to 27 locations within the raceway. However, the number of sample locations may vary based on results of a pilot study to be conducted before initiating this study. If the number of sample locations is modified due to pilot study findings, then a protocol change or deviation will be prepared and submitted with the study protocol or final study report.

Samples will be collected at three time periods during the treatment period: (1) at time = 0 min (after water to the raceway has been turned off and chloramine-T has been added to achieve the target dosage in a static bath); (2) 30 min after the water to the raceway has been turned back on and

chloramine-T has been metered into the raceway; and (3) 60 min after the water to the raceway has been turned back on and chloramine-T has been metered into the raceway. After the final sample has been collected, metering of chloramine-T into the raceway will be terminated, and the chemical will be flushed from the raceway.

Samples will be collected using 60-mL plastic syringes (pre-rinsed with spring water) and transferred to plastic bottles. Following sample collection, bottles will be capped. No special sample preparations will be required. Samples will be measured the day they are collected and will not be stored for a prolonged period. In the event samples are to be stored for a brief period (i.e., ≤ 8 h), then samples will be stored in a cool, dry place.

12.2.3 Evidence of methods validation:

A letter confirming FDA-validation of the analytical method is in Appendix C.

12.2.4 Quality control procedures for the method:

For quality control purposes, one sample will be collected in triplicate. Chloramine-T concentration in each sample will be analytically verified, and the precision and accuracy of the method will be described by the relative difference between the chloramine-T concentration of the replicate samples.

12.2.5 The criteria and procedures used to assess analytical results:

The instrumentation and methodology used to analytically verify chloramine-T concentration in water samples is relatively simple. If the measured chloramine-T dose differs from the target dose by $\pm \geq 15\%$, then the sample may be re-analyzed. In addition, one or more water samples will be collected from the test unit prior to administering chloramine-T to verify that no chloramine-T or other chlorine-based product is present in the source water.

12.3 Relevant scientific literature supporting the use of the analytical method for the intended measurements:

Relevant scientific literature citations are listed in Appendix D.

13. STUDY LOCATION:

The study will be conducted at the U.S. Fish and Wildlife Service Bozeman Fish Technology Center, 4050 Bridger Canyon Road, Bozeman, Montana 59715.

14. PERSONNEL:

14.1 Study director:

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(See Appendix E for curriculum vitae)

15 COLLECTION AND RETENTION OF SOURCE DATA:

Original source data (i.e., raw data) will be collected using appropriate quality control procedures and retained in archived files at the Bozeman Fish Technology Center in compliance with Good Laboratory Practice procedures.

16 ADDENDUMS AND DEVIATIONS TO THE PROTOCOL:

Addendums (i.e., protocol changes) and deviations will be made if necessary. Protocol changes will be made if there are changes in the general study procedures that do not negatively affect the study. Deviations to the study protocol will be documented, including a statement describing whether the deviation negatively affected the outcome of the study. All protocol changes and deviations will be discussed between the Study Director and Investigator.

17 DRUG DISPOSITION, ANIMAL ACCOUNTABILITY, FEED DISPOSITION, AND FEED ACCOUNTABILITY:

All unused chloramine-T will be (1) retained on site (2) shipped to an aquaculture facility to be used under INAD # 4000 or INAD # 9321 or (3) disposed of in the local landfill.

All fish used in the study will be (1) retained on site (2) harvested at some time after the 45-d withdrawal period or (3) disposed of in the local landfill.

Feed used in the study is neither experimental nor medicated. Therefore, the disposition and accountability of feed will not be considered.

18 PROTOCOL APPROVAL SIGNATURE:

18.1 Sponsor (David A. Erdahl) _____

18.2 Study Director (James D. Bowker) _____

18.3 Investigator (Daniel Carty) _____

19 APPENDICES:

19.1 Appendix A, Standard operating procedures

19.2 Appendix B, Chloramine-T analytical verification method draft report.

19.3 Appendix C, Letter confirming FDA-validation of the chloramine-T analytical verification method.

19.4 Appendix D, Relevant scientific literature citations

19.5 Appendix E, study personnel curriculum vitae's.

Table 1. Schedule and description of significant events for study protocol number BFTC-01-CHLT-FT

Study day and description of significant event	
Acclimation phase	
^a 1-week prior to first treatment of Raceway 5	If fish are used: transfer fish into raceway(s) ; estimate fish size and number/raceway
Pre-exposure phase	
1-d prior to first treatment of Raceway 5	Measure strength and purity of test article (chloramine-T). Measure hardness, alkalinity, and pH of source water. Begin collecting water quality data.
Treatment phase	
First treatment in Raceway 5	Weigh out chloramine-T. Administer chloramine-T to achieve target dosage; administer chloramine-T in a flow through system to maintain target dosage. Archive test article. Verify chloramine-T doses. Collect water quality data during exposure.
Second treatment in Raceway 5	Weigh out chloramine-T. Administer chloramine-T to achieve target dosage; administer chloramine-T in a flow through system to maintain target dosage. Verify chloramine-T doses. Collect water quality data during exposure.
First treatment in Raceway 6	Weigh out chloramine-T. Administer chloramine-T to achieve target dosage; administer chloramine-T in a flow through system to maintain target dosage. Verify chloramine-T doses. Collect water quality data during exposure.
Second treatment in Raceway 6	Weigh out chloramine-T. Administer chloramine-T to achieve target dosage; administer chloramine-T in a flow through system to maintain target dosage. Verify chloramine-T doses. Collect water quality data during exposure.
Post-treatment phase	
1-d after the second treatment to Raceway 6	Measure strength and purity of test article (chloramine-T). Measure hardness, alkalinity, and pH of source water. Terminate study

^aThis event (i.e., transfer of fish to raceways) will occur if fish are to be used in the study. If no fish are used in the study, then fish will not be transferred to raceways.

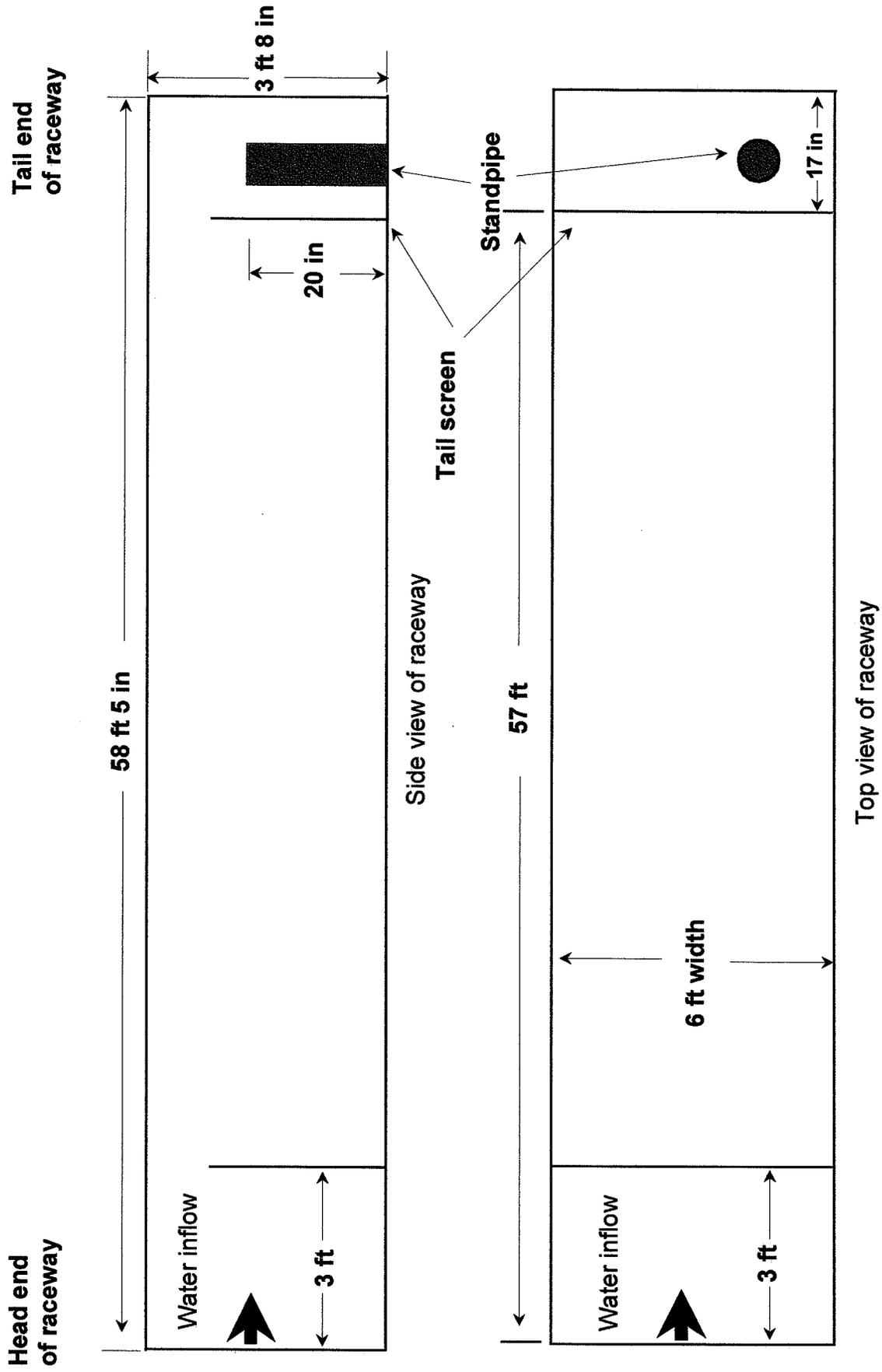


Figure 1. Diagram of raceway used in study (BFTC-01-CHLT-FT) showing dimensions, tail screen, and standpipe location.

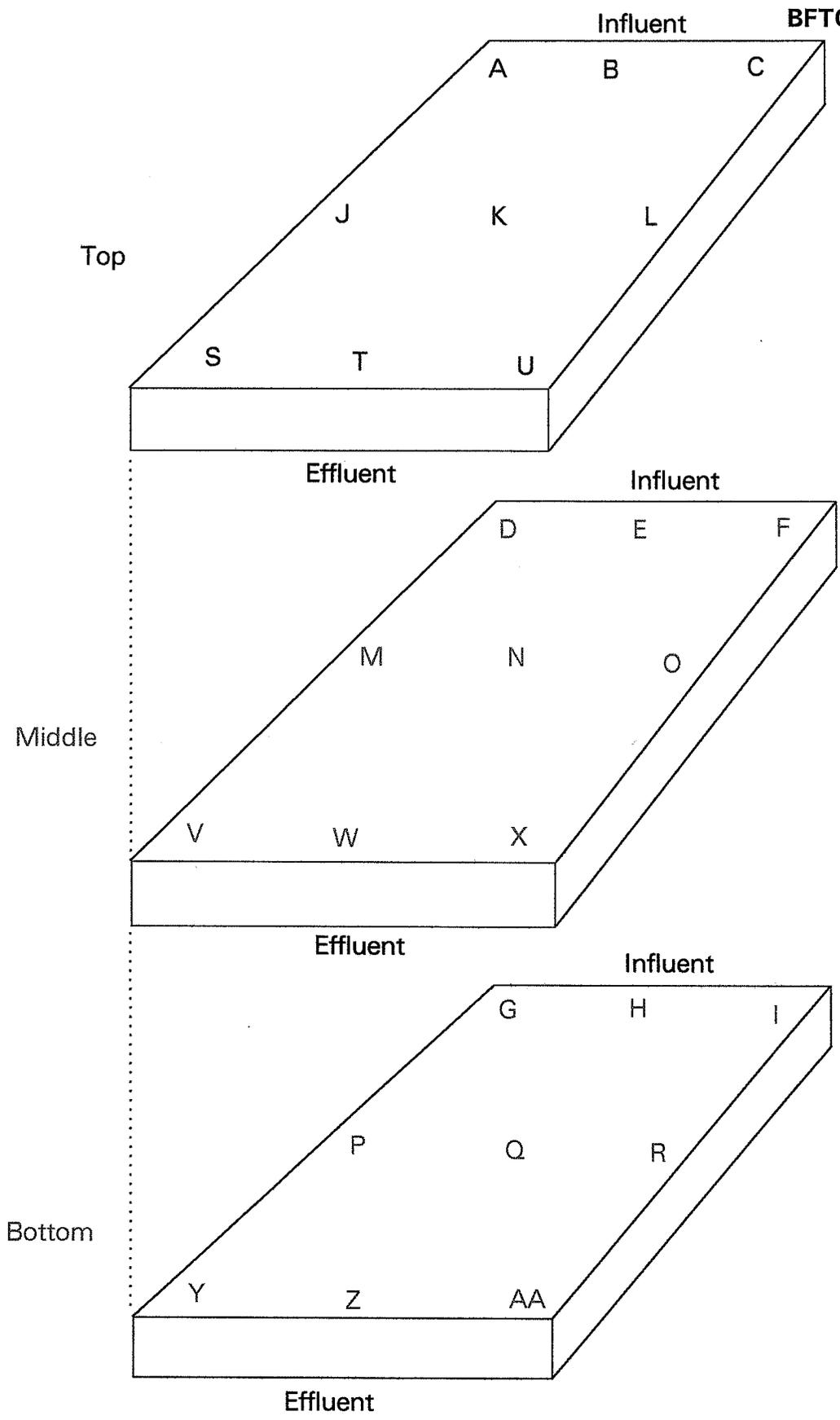


Figure 2a. Sample identification for water samples collected for chloramine-T dose-verification.

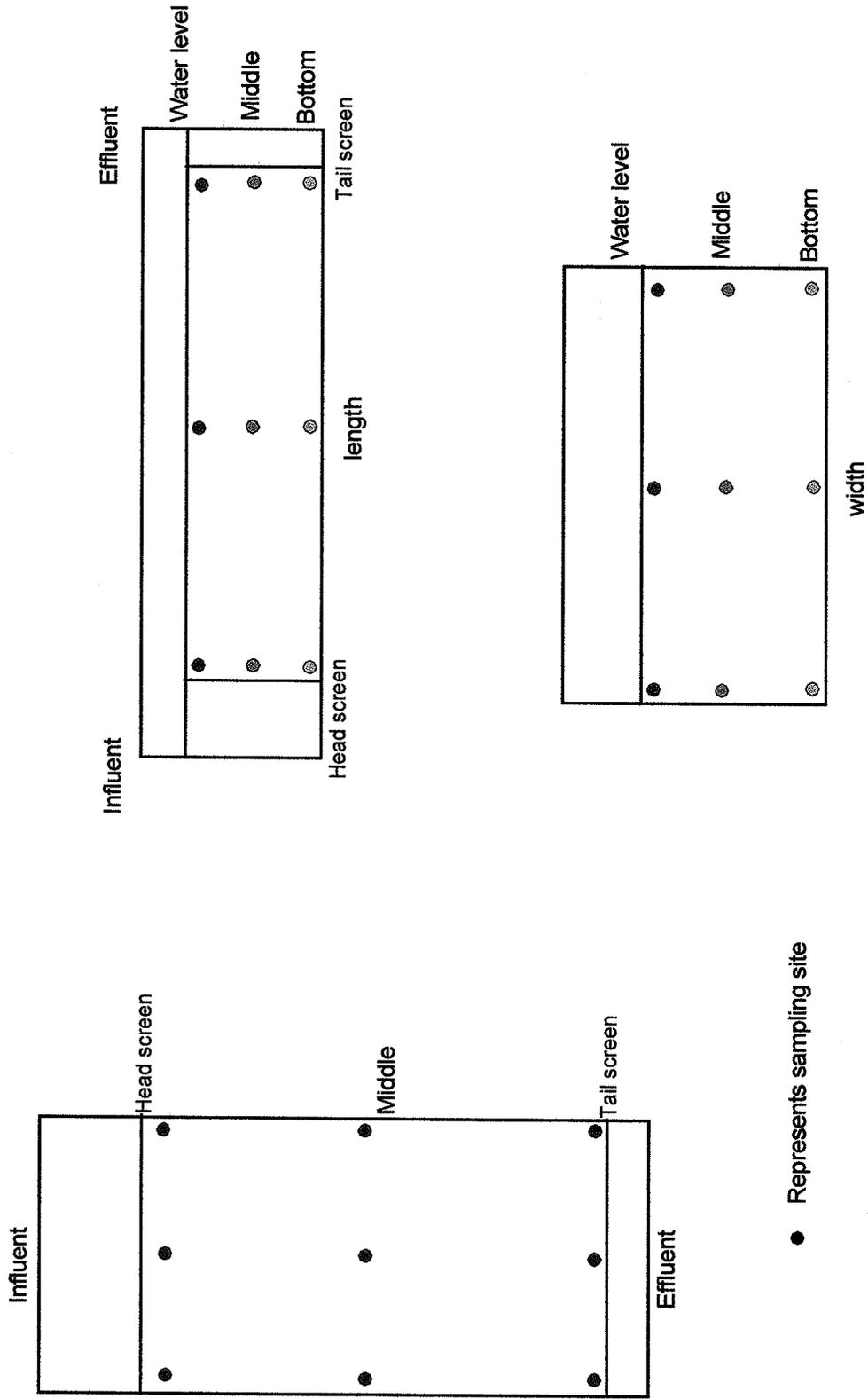


Figure 2b. Sites from which water samples will be collected for chloramine-T dose-verification.