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Letter of Transmittal

To: Mr. Michael Abbruzzi
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Town of Warren
514 Main Street
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Project No: 20071213.D20 Task No.: 2
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Signed: M. James Riordan, AICP
Project Manager

Warren Town Beach Investigation Follow-Up Study

Town of Warren
Rhode Island

June 2008



Fuss & O'Neill
275 Promenade Street
Suite 350
Providence, RI 02908



WARREN TOWN BEACH INVESTIGATION
FOLLOW-UP STUDY
Town of Warren

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WARREN TOWN BEACH INVESTIGATION
FOLLOW-UP STUDY
Town of Warren

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- A Calibration Standard Manual



1.0 INTRODUCTION

1.1 Purpose

On behalf of the Town of Warren (Town), Fuss & O'Neill recently conducted an investigation of the Warren Town Beach and analyzed probable sources of bacteria and resulting beach closures. As a follow-up, the work discussed in this report was conducted to further investigate the sources identified in the previous study with the intent of better understanding their contribution to contamination of the beach. In particular, this study focuses on the source(s) of groundwater contamination to the beach.

1.2 General Conditions at the Beach

Warren Town Beach is located off of Water Street along the west bank of the Warren River (as depicted in [Figure 1](#)). There is persistent pathogen impairment at Warren Town Beach that causes a number of beach closures following wet weather events each year. Infrastructure improvements have been made in an attempt to mitigate the pathogen problem, including relining a segment of the 12-inch sanitary sewer line that runs beneath Water Street along the beach.

After the sanitary sewer was relined in 2003 beach closures became very infrequent over the next two beach seasons. In 2006, bacterial monitoring at the beach again showed significant pathogen impairment resulting in 44 beach closures over the past two summers.

During a previous study, which we conducted on behalf of the Town, very high levels of bacteria (approximately 10^3 cfu) were identified in the groundwater. Three plausible sources of bacterial contamination to groundwater exist in the vicinity of the beach. These are:

- Blount Boats pumpout tank and sewer line
- 12-inch sewer line on Water Street
- 24/27-inch sewer line on Water Street

This report focuses on our investigation of these sources.

1.3 Report Structure

The remainder of this report discusses:

- Investigation of Blount Boats Sewer Line including review of existing design documents, televised inspection of the Blount Boats line, hydraulic leak testing, and dye testing.
- Investigation of the 12-inch sewer line along Water Street using dye testing and visual observations.
- Investigation of the 24-four-inch sewer line along Water Street using dye testing and visual observations.
- Recommended next steps for remediation.



2.0 BLOUNT BOATS SEWER LINE AND 27-INCH LINE

This section of our report discusses:

- Visual inspection of the Blount Boats tank.
- Televised inspection of the Blount Boats sewer line.
- Dye-testing of the Blount Boats sewer line and 27-inch sewer line on Water Street.

2.1 Design Plan Review

No designs plans were available for review and we proceeded to field inspection.

2.2 Televised Inspection of the Blount Boats Line

On January 24, 2008, we conducted an inspection of the Blount Boats tank and sewer line using televised and visual inspection. Our observations during this inspection are provided in Appendix A of this report. The follow is a summary of our observations:

- The Blount Boats pumpout tank was observed to have a crack on its bottom.
- No groundwater was observed to infiltrate into the tank through the crack.
- The Blount Boats line was observed to have two joint separations (i.e., disconnections at the joints in the pipe).
- The Blount Boats line connects to the 27-inch sewer line on Water Street not the 12-inch line as initially thought. (Due to sags in the pipe, the camera remained underwater during much of the inspection near Water Street. The connection of the Blount line to the 24-inch pipe was identified at a later date with dye testing performed by the Warren Sewer Department.)

2.3 Visual Inspection of Blount Boats Pumpout Tank

A visual inspection of the Blount Boats pumpout tank was performed during the televised inspection of the Blount Boats sewer line.

2.3.1 Methods

Wastewater was pumped out of the Blount holding tank and the tank was rinsed using a high-pressure hose. The tank remained empty for two hours during high tide.

2.3.2 Visual Inspection Results

During the high-pressure rinsing, several cracks were observed in the concrete on the bottom of the tank. The depth of the cracks could not be determined during this time, however, during the two hours the tank was left empty during high tide, no water infiltrated into the tank.



2.4 Dye Testing and Infiltration/Exfiltration Observations

2.4.1 Methods

The dye testing of the Blount Boats tank involved the steps listed below. This approach was agreed to during our project kickoff meeting with the Town and project partners on January 18, 2008. Similar procedures were used in dye testing the 12-inch and 24-inch pipes. Dye testing of the Blount Boats lines and 27-inch pipe was performed on April 15, 2008.

1. The inlets and outlets to the structures to be tested were blocked to isolate the tank and subject lines during the testing. For testing the Blount Boats line, we coordinated with the Warren Sewer Department to block the 27-inch line at the manhole across from the wastewater treatment plant and Campbell Street.
2. Approximately 1,600 ml of dye and 10,600 gallons of combined water and wastewater were added to the Blount Boats tank and 27-inch line. This was anticipated to generate an approximate average dye concentration of 8 ppm (parts per million). Bacteria concentrations found in the monitoring wells during our previous study indicated 3 – 4 log reduction of bacteria concentrations from levels typically found in raw sewage. Given soils between the sewers and the beach are saturated with groundwater during tidal influx, we anticipated that bacterial concentration decrease would occur primarily as a result of dilution as opposed to soil filtration. Therefore, we anticipated a similar drop in dye concentration and if exfiltrate was flowing from the Blount Boats line to the monitoring wells that it would be measureable in approximately the 1 – 100 parts per billion (i.e., 10^{-9} – 10^{-7} ppb) range.
3. The plugs were left in place to hold dye and water mixture in the system for 4-8 hours.
4. The fluid level in the tank was observed intermittently (approximately hourly). If the system is tight fluid levels should remain essentially static. If the fluid level falls significantly then the system is leaking. This was done in coordination with the Warren Sewer Commission.
5. Dye concentrations were observed at the monitoring wells and the beach by Fuss & O'Neill. (Beach and monitoring well sampling locations are shown in [Figure 2](#).) Dye detected in the monitoring wells or the beach can be attributed to dye is leaving the system and being carried via groundwater to the monitoring wells and beach. (Note: Dye may reach the monitoring wells and not reach the beach as it may take more than 48 hours for fluid to flow from the tank to the beach, also, dye may reach the beach and not the monitoring wells if it flows in a narrow path of groundwater).

2.4.2 Results

Samples were collected at four locations (see below) on the schedule shown in [Table 1](#):

- Monitoring Well 1
- Monitoring Well 2

- Monitoring Well 3
- Beach

TABLE 1
DYE SAMPLING SCHEDULE
DURING TESTING AT THE BLOUNT BOATS AND 27-INCH LINES

Location	Parameters	Frequency	Duration
○ Beach and monitoring well samples	<u>Samples</u> 1 VOA	One (1) set of samples at hours 1, 12, 24, 36, and 48 (anticipated times)	48 hours

All dye samples were stored in a closed cooler while in the field.

The samples were analyzed for the presence of Rhodamine dye using a flourometer. The flourometer was calibrated before each group of samples was analyzed. Dye samples were analyzed in Fuss & O'Neill's Providence offices. [Table 2](#) (below) indicates the readings we observed: Calibration standard methods are included as [Appendix A](#).

TABLE 2
RHODAMINE DYE CONCENTRATION
OBSERVED AT BEACH AND MONITORING WELLS
DURING TESTING OF THE BLOUNT BOATS AND 27-INCH LINES

Time Since Dye Injection	Beach (Dye in ppb)	MW1 (Dye in ppb)	MW2 (Dye in ppb)	MW3 (Dye in ppb)
Hour 0	0.64	2.56	-0.61	0.56
Hour 13	0.44	3.75	0.55	0.40
Hour 25	0.21	1.08	0.17	0.99
Hour 37	0.15	-0.61	1.14	0.51
Hour 49	0.00	-2.42	0.65	0.95
Minimum	0.00	-2.42	-0.61	0.4
Maximum	0.64	3.75	1.14	0.99
Change During Event	0.64	6.17	1.75	0.59
Change from Hour 0	0.00	1.19	1.75	0.43

The key to interpreting these results is examining the change in concentrations. The flourometer measures fluorescence, which may occur at background levels. Calibration at the beginning of the flourometer analysis adjusts for this; however, background may vary



somewhat during the event. This accounts for negative readings. Generally, the most important change in concentration is positive change during the event from Hour 0.

During this sampling very little change occurred over the reading at Hour 0. The most significant changes occurred in the monitoring wells furthest away from Blount Boats where the dye was injected. Increased dye concentrations in MW1 and MW2 are highly unlikely to be the result of dye injection at Blount Boats as very little change occurred at MW3. Therefore, this data implies that the leakage from the Blount Boats line and 27-inch line does not significantly affect water quality at the beach. There may be some longer term impacts that were not observed during this testing. However a leaking hold tank and sewers are issues that impact the environment and should be corrected.

2.4.3 Field Observations

In addition to the sampling, an inspection of the Blount tank on the 8th hour of testing revealed a drop in the fluid level of the tank of approximately six inches below the invert of the effluent pipe. This indicates that the tank and/or its influent line leak.

3.0 12-INCH SEWER LINE

3.1 Methods

Dye testing of the 12-inch pipe was performed on March 14, 2008 and involved the following procedure.

1. Block the inlets and outlets to the structures to be tested to prevent their influence during the testing. For testing the 12-inch line, we coordinated with the Warren Sewer Department to block the 12-inch line at the manhole across from the wastewater treatment plant and the north side of the manhole Cambell Street (to allow wastewater from Cambell Street to flow through the overflow in the Cambell Street manhole to the 24-inch pipe). The south side of the 12-inch was then plugged and the pipe at Bridge Street was plugged.
2. Approximately 500 ml of dye and 15,000 gals of water and wastewater were added to the 12-inch line. This was anticipated to generate an approximate average dye concentration of 8 ppm. Given soils between the sewers and the beach are saturated with groundwater during tidal influx, we anticipated that bacterial concentration decrease would occur primarily as a result of dilution as opposed to soil filtration. Therefore, as with the Blount Boats and 27-inch lines, we anticipated that a leaky line would probably result in measureable levels of dye in approximately the 1 – 100 parts per billion (i.e., 10^{-9} – 10^{-7} ppb) range.
3. Leave the plugs in place to hold dye and water mixture in the system for 4-8 hours.
4. Observe the fluid level in the pipes. If the system is tight fluid levels should remain essentially static. If the fluid level falls significantly then the system is leaking. This was done in coordination with the Warren Sewer Commission.



5. Observe dye concentrations at the monitoring wells and the beach. (Beach and monitoring well sampling locations are shown in [Figure 2](#).) If dye can be detected in the monitoring wells or the beach then dye is leaving the system and being transported. (Note: Dye may reach the monitoring wells and not reach the beach as it may take more than 48 hours for fluid to flow from the tank to the beach, also, dye may reach the beach and not the monitoring wells if it flows in a narrow path of groundwater).

3.2 Results

Samples were collected at four locations on the schedule shown in [Table 1](#):

- Monitoring Well 1
- Monitoring Well 2
- Monitoring Well 3
- Beach

[Table 3](#) (below) summarizes the readings we observed:

TABLE 3
RHODAMINE DYE CONCENTRATION
OBSERVED AT BEACH AND MONITORING WELLS
DURING TESTING OF THE 12-INCH SEWER LINE

Time Since Dye Injection	Beach (Dye in ppb)	MW1 (Dye in ppb)	MW2 (Dye in ppb)	MW3 (Dye in ppb)
Hour 0	0.82	4.67	0.84	1.07
Hour 13	0.97	3.58	0.38	1.73
Hour 25	0.83	4.48	0.35	1.54
Hour 39	0.44	4.33	0.51	25.87
Hour 51	0.57	4.56	0.42	2.21
Minimum	0.44	3.58	0.35	1.07
Maximum	0.97	4.67	0.84	25.87
Change During Event	0.53	1.09	0.49	24.8
Change from Hour 0	0.15	0	0	24.8

As discussed in [Section 2.4.2](#), the key to interpreting these results is examining the relative change in concentrations. The flourometer measures fluorescence, which may occur at background levels. Calibration at the beginning of the flourometer analysis adjusts for this; however, background may vary somewhat during the event. Generally, the most telling change in concentration is positive change during the event from Hour 0 to the maximum level.

During this sampling event significant change occurred over the readings at MW3 between Hour 25 and Hour 39. Although levels of fluorescence at MW1 register above what we would expect to find in background it is difficult to say whether the meter is picking up the dye we injected or something in background. We, therefore, conclude that groundwater transports



leakage from the 12-inch line to MW 3 and may or may not influence MW1 close to the upstream terminus of the lining (i.e., Bridge Street). We know from previous investigations that tides influence groundwater at the monitoring wells including MW3. Therefore, we anticipate that groundwater and tides are likely to carry leakage from the 12-inch line to the beach although this affect was not apparent over the course of our testing.

3.3 Field Observations

No remarkable visual observations were made during testing of the 12-inch line; however, visual observations made during the dye-testing of the 24-inch bolster our conclusion that the 12-inch line leaks (see [Section 4.1.2](#)).

4.0 24-INCH SEWER LINE

4.1 Methods

The dye testing procedure involved the steps listed below. Dye testing of the 24-inch pipe was performed on May 7, 2008.

1. Block the inlets and outlets to the structures to be tested to prevent their influence during the testing. For testing the 24-inch line, we coordinated with the Warren Sewer Department to block the 24-inch line at the manhole at Cambell Street and the 24-inch line at Bridge Street.
2. Approximately 2,500 ml of dye and 49,000 gallons of water were added to the 24-inch line. This was anticipated to generate an approximate average dye concentration of 4 ppm. Bacteria concentrations found in the monitoring wells during our previous study indicated 3 – 4 log reduction of bacteria concentrations in raw sewage. Given soils between the sewers and the beach are saturated with groundwater during tidal influx, we anticipated that bacterial concentration decrease would occur primarily as a result of dilution as opposed to soil filtration. Therefore, as with the other lines, we anticipated that a leaky line would probably result in measureable levels of dye in approximately the 1 – 100 parts per billion (i.e., 10^{-9} – 10^{-7} ppb) range
3. Leave the plugs in place to hold dye and water mixture in the system for 4-8 hours.
4. Observe the fluid level in the pipes. If the system is tight fluid levels should remain essentially static. If the fluid level falls significantly then the system is leaking. This was done in coordination with the Warren Sewer Commission.
5. Observe dye concentrations at the monitoring wells and the beach. (Beach and monitoring well sampling locations are shown in [Figure 2](#).) If dye can be detected in the monitoring wells or the beach then dye is leaving the system and being transported. (Note: Dye may reach the monitoring wells and not reach the beach as it may take more than 48 hours for fluid to flow from the tank to the beach, also, dye may reach the beach and not the monitoring wells if it flows in a narrow path of groundwater).

4.2 Results



Samples were collected at four locations on the schedule shown in Table 1:

- Monitoring Well 1
- Monitoring Well 2
- Monitoring Well 3
- Beach

Table 4 (below) summarizes the readings we observed:

TABLE 4
RHODAMINE DYE CONCENTRATION
OBSERVED AT BEACH AND MONITORING WELLS
DURING TESTING OF THE 24-INCH LINE

Time Since Dye Injection	Beach (Dye in ppb)	MW1 (Dye in ppb)	MW2 (Dye in ppb)	MW3 (Dye in ppb)
Hour 0	-1.46	3.96	-4.52	0.01
Hour 12	-1.02	-6.22	-4.51	0.00
Hour 24	0.17	-5.69	-4.33	2.16
Hour 36	0.22	8.26	1.04	0.11
Hour 48	0.17	-3.06	0.41	-0.73
Minimum	-1.46	-6.22	-4.52	-0.73
Maximum	0.22	8.26	1.04	2.16
Change During Event	1.68	14.48	5.56	2.89
Change from Hour 0	1.68	4.3	5.56	2.15

As discussed in Section 2.4.2 and 3.1.1, the key to interpreting these results is examining the relative change in concentrations. The flourometer measures fluorescence, which may occur at background levels and may represent residue from previous dye testing. Calibration at the beginning of the flourometer analysis adjusts for this; however, background may vary somewhat during the event. Generally, the most telling change in concentration is positive change during the event from Hour 0 to the maximum level.

During this sampling event significant change occurred over the readings at all four locations. The most pronounced change occurred at MW2 and MW1 between Hour 24 and Hour 36. Significant change also occurred at MW3 and the beach between Hour 12 and Hour 24. We, therefore, conclude that groundwater transports leakage from the 24-inch line to all three monitoring wells and the beach.

4.3 Field Observations

We made the following field observations during dye-testing of the 24-inch line:

- When the plugs were being installed, a significant trickle of groundwater was observed infiltrating into the pipe on the downstream side of the plug.



- Two hours after the dye was added to the pipe, the pipe was checked the groundwater infiltrating into the pipe was observed to be pink (Rhodamine dye is red in color).
- Five hours after the dye was added to the pipe, pink water was observed to be flowing in the 12-inch pipe which runs parallel to the 24-inch pipe.
- Eight hours after the dye was added to the pipe, and just before the plugs were pulled, the water had dropped to approximately ½ the height it was at during the first hour of the test.

These observations support our conclusion that the 24-inch line leaks.

5.0 RECOMMENDATIONS

The following text provides our recommendations regarding the Blount Boats line, the 12- and 24/27-inch lines on Water Street, and stormwater discharges to the beach.

5.1 Blount Boats Line

Based on our observations during the televised inspection and leak testing (see [Section 2.4.3](#)), the Blount Boats line leaks and needs to be repaired. However, based on dye testing (see [Section 2.4.2](#)), the leakage from these lines does *not* appear to significantly affect water quality at the beach or groundwater at the monitoring wells but still adds a significant bacteria load to groundwater that still has the potential to impact the beach.

We recommend that Blount Boats repairs the joint separations in the line near the connection with the 24-inch pipe on Water Street.

Given that water level in the tank dropped below the invert of the outlet pipe, some leakage must have occurred in the tank or the lines hydraulically below the tank. This must be remedied by repairing, replacing or abandoning the tank. In our opinion, the tank may not be a necessary part of the system. We, therefore, recommend that Blount Boats consider options to abandon it.

5.2 Sewer Lines on Water Street

Based on our observations and dye-testing results, both the 12-inch and 24/27-inch lines leak and contribute significantly to contamination at the beach (see [sections 3.1.1, 3.1.2, 4.1.1, and 4.1.2](#)). Four basic options exist for remediating these lines:

1. *Fully lining the sewers*— The Town of Warren has previously lined a part of the 12-inch pipe; however, it may not be functioning properly as evidenced by our observations during this study as well as bacterial pollution at the beach during the last two years. If the Town determines that lining is the preferred alternative, it may well be most effective to fully line both the 12-inch and 24/27-inch pipes.
2. *Fully line the sewers, but attempt to reuse the existing lining in the 12-inch pipe*— The partial lining in the 12-inch pipe may be functional and reusing it could save money. The 12-inch pipe's current condition could be examined through field testing. While this approach



may reduce the initial cost of repair, it presents the greatest risk of short-term failure. A previous attempt to partially line the 12-inch sewer appeared to abate beach contamination temporarily, but contamination problems returned after approximately two years.

3. *Replace the sewer lines*— A previous attempt to line the 12-inch sewer resulted in an apparent abatement of bacterial pollution at the beach, but the abatement did not endure. Replacing the sewers presents much greater long-term reliability, but will be expensive and disrupt the Water Street area during construction. This approach will require a greater level of up-front effort and will take longer to implement than lining the pipes. If the Town pursues this option, it should also consider simultaneous opportunities to address stormwater and traffic issues as working on these issues together will yield an overall economy of scale.
4. *Line the sewers now (using either option 1 or 2) and then replace them at a later date*— Warren may wish to combined the long-term reliability of sewer line replacement and the immediate benefit of reopening of the beach. Similarly, the Town may wish to line the pipes and take a watchful-waiting approach regarding the need for sewer replacement. In either case, the Town could line the pipes using either option 1 or 2 and plan for replacement at some time in the foreseeable future (e.g., 2 –5 years). If the Town is willing to accept the financial risk of failure, lining and watchful waiting could be an appropriate approach as Phase 1. Although, partial lining did not work as a long-term fix, full lining may work more effectively.

We recommend conducting an alternatives analysis to determine the most practicable approach to addressing the problems associated with the Water Street sewers.

The Town should also note that while the Water Street sewer lines clearly affect water quality at the beach, they may not be the only sources of dry-weather discharge and given their magnitude may mask other sources. We recommend that the Town address the problems associated with the Water Street sewers and then reexamine water quality to determine whether or not bacterial pollution has been abated to an acceptable level.

5.3 Stormwater

Two stormwater pipes outlet to the beach. The results of our previous study show that wet-weather discharge (i.e., stormwater) is a contributor to bacterial pollution at the beach. Therefore, the Town should consider options to abate stormwater drainage issues at the beach. Stormwater could be addressed through end-of-pipe management, upland attenuation, or low-impact development retrofit.

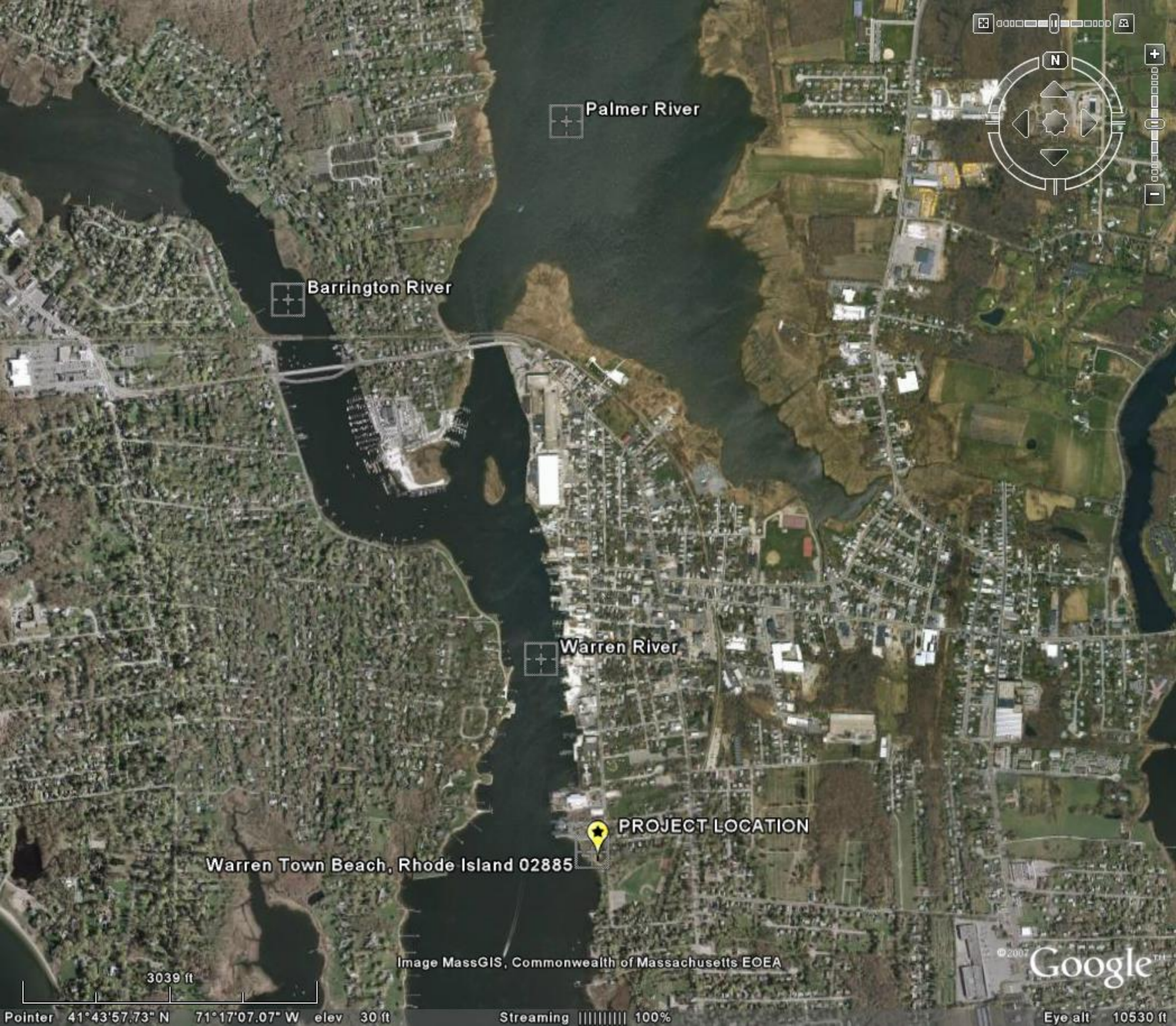
The Town has been awarded a nonpoint source abatement grant from DEM and intends to use this to examine upland attenuation and low-impact-development retrofits along Water Street. This approach may allow the Town to abandon or significantly reduce flow to the stormwater outlets at the beach.



FIGURES



FIGURE 1
WARREN TOWN BEACH LOCATION



Palmer River

Barrington River

Warren River

PROJECT LOCATION

Warren Town Beach, Rhode Island 02885

Image MassGIS, Commonwealth of Massachusetts EOE

© 2007 Google

3039 ft

Pointer 41°43'57.73" N 71°17'07.07" W elev 30 ft

Streaming ||||| 100%

Eye alt 10530 ft



FIGURE 2
BEACH AND MONITORING WELL SAMPLING
LOCATIONS



MW3



Beach



MW2



MW1



Warren Town Beach Sampling Locations for Dye Testing

Image MassGIS, Commonwealth of Massachusetts EOE

© 2008 Tele Atlas

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APPENDIX A
CALIBRATION STANDARDS MANUAL

Preparation of Standards for Dye Studies Using Rhodamine WT

CONTENTS

- ❖ WHAT IS A STANDARD?
- ❖ UNITS OF MEASUREMENT
- ❖ LINEARITY
- ❖ ACTIVE INGREDIENT OR 20% SOLUTION?
- ❖ MEASURING BY WEIGHT OR VOLUME
- ❖ THE VOLUMETRIC FLASK
- ❖ THE PIPETTE
- ❖ MAKING STANDARD DILUTIONS
- ❖ MAKING MORE THAN ONE CONCENTRATION
- ❖ pH, TURBIDITY, AND CHLORINE
- ❖ STORING SAMPLES AND STANDARDS

WHAT IS A STANDARD?

A standard is a known concentration of the dye you are injecting. It is used to calibrate the Turner Designs Model 10 Fluorometer to the desired sensitivity. The fluorometer reading of the standard will be compared with the readings of unknown samples to obtain their concentrations. A known concentration is made by weighing or measuring a sample of tracer and precisely diluting it.

In many cases, the standard will be a known dilution of dye, not a known concentration. For example, in flow rate measurements you are only interested in how much a stream dilutes the dye, not what the actual concentration is. Thus, you do not need to know the concentration of the dye (in parts per billion or other units) when you calibrate your fluorometer. You need only know the dilution factor for the standard and the fluorometer reading, which you will then compare with the fluorometer readings for your unknown samples collected after dye has been injected into the stream. This will allow you to calculate the extent to which the stream dilutes the dye.

Note: For more information on dye studies and whether a known concentration or dilution is required, see the monographs "A Practical Guide to Flow Measurement" and "Flow Measurements in Sanitary Sewers by Dye Dilution," available from Turner Designs.

UNITS OF MEASUREMENT

Use whatever units suit your purpose best. The EPA guidelines on allowable levels will typically be in micrograms per liter (1). Other studies are described in milligrams per cubic meter (mg/m^3) or parts per billion (ppb). The simplest to use when making dilutions is grams per gram, which, for all practical purposes, is the same as grams per milliliter.

The easiest way to record and to think of these units is in exponential notation. If you are not used to this, and are not comfortable with it, move your decimal point around. It is very easy, however, to think in exponential terms:

1.3 grams dissolved in 100 ml, makes a 0.013 g/g solution. In exponential notation this is 1.3×10^{-2} . If 1 ml is diluted to 100 ml (100 ml is 10^2 ml), then the new concentration is 1.3×10^{-4} .

Some conversion factors:

1 gram/liter 1 part per thousand (ppt) 10^{-3}

1 milliliter/liter 1 part per thousand (ppt) 10^{-3}

1 milligram/liter 1 part per million (ppm) 10^{-6}

1 microgram/liter 1 part per billion (ppb) 10^{-9}

1 milligram/cubic
meter 1 part per billion (ppb) 10^{-9}

LINEARITY

Note: Rhodamine WT comes as a 20% solution in water (meaning it is 20% active ingredient). The EPA guidelines are in terms of active ingredient. Thus, one microgram of the 20% solution in one liter is 0.2 ppb active ingredient (or 20% of 1 ppb).

Instrument readings for fluorescent dyes are proportional to concentration (linear) from the lowest detectable level up to a certain concentration. Above this concentration, a multipoint calibration curve may be used to obtain concentrations. Then, at a certain concentration (somewhere at about five to ten times the upper limit for the linear range) the curve flattens out and eventually takes a nose-dive. This critical concentration is a function of the compound and of the path length of the flow cell or cuvette.

For practical purposes (using the Turner Designs Model 10 Fluorometer equipped with the 25-mm flow cell or cuvette holder), Rhodamine WT is linear to 0.5 ppm (500 ppb, 5×10^{-7} , or 500 micrograms/liter). In terms of active ingredient (the 20% solution of Rhodamine WT), it is linear to 0.1 ppm (or 100 ppb, 1×10^{-7} , or 100 micrograms/liter).

ACTIVE INGREDIENT OR 20% SOLUTION?

Since Rhodamine WT comes as a 20% solution, a decision should be made at the outset about whether to make the standard as 100% tracer or in terms of active ingredient. The first impulse is to do all calculations on the basis of the active ingredient. However, even to meet the EPA guidelines, you need only keep in mind that 20% of the original solution is active ingredient. For other purposes, it is immaterial whether you take the 20% into account. If you discharge 20 pounds of Rhodamine WT solution, or inject a 10-fold dilution, it doesn't matter that the original material was only 20% pure. If you consider the Rhodamine WT solution to be pure tracer, then all dilutions are relative. For example, if you have made a 100 parts per billion dilution based on 100% tracer, your final dilution will be 20 parts per billion active ingredient. Whatever method you choose, be sure to clearly mark your dilutions as to 100% tracer or active ingredient.

MEASURING BY WEIGHT OR VOLUME

For flow measurements and other studies where dye is to be added to a body of water, if you plan to add dye in pounds, grams, or other weight measure, then your standard must be made by weighing. If you are adding by volume, then the standard must be made by volume measurements. The important thing is to make your standard the same way you make the dye concentration to be injected.

Note, however, that most of the literature cites the specific gravity of Rhodamine WT as 1.2 (sometimes 1.19). Recent literature accompanying the dye says 1.15. Thus, one gram equals 1/(sp.gr.) milliliter. If you add by volume, you could be adding as much as 20% more dye than if you add by weight. Therefore, if you are concerned with absolute concentrations (as with an EPA study), you should make an initial 100-fold dilution by weight (or compensate for the specific gravity). A 100-fold dilution by weight has a specific gravity of 1.002. For the vast majority of studies, errors of 0.2% in doing further dilutions, whether by weight or volume, are acceptable.

If you weigh, it is best to weigh directly into a volumetric flask. (See discussion of flasks below.) This avoids the problem of having to rinse whatever vessel is used for weighing. All of the material must wind up in the flask. Since it will be diluted with water, the rinsing can be done with water.

If you measure by volume, an accurate method is to use a large-tip pipette of at least 10-ml capacity and measure dye into a 1-liter volumetric flask (a 100-fold dilution). Even more accurate is to measure dye with a 20-ml pipette and dilute to 2 liters. The larger the pipette, the smaller the surface area with respect to the volume, hence the smaller the error due to incomplete drainage. The error from a 10-ml pipette will probably be negligible for most work. It certainly should be less than 1%.

The most accurate way to measure the tracer by volume is to fill a 10-ml volumetric flask to the mark, then rinse the tracer into a 1-liter volumetric flask. You can't rinse a pipette, as it is made to deliver the stated volume. Volumetric flasks contain the stated volume, so if you want the stated volume out of the flask, you must rinse.

Note, however, that there is not much point in preparing your standards to an accuracy greater than the means by which the tracer will be added to the system.

THE VOLUMETRIC FLASK

Put your measured sample into the flask, then add water up to the line. The correct level is when the bottom of the meniscus touches the calibration line.

The more precise Class A flasks will be accurate to 0.03% for a 1-liter flask, and to 0.16% for a 100-ml one. Less accurate flasks have a tolerance of twice this.

After adding the dye, mix thoroughly. The proper way is to invert the flask (hold the cap on!). When the air bubble has risen, swirl it for a few seconds. Right it; let the bubble rise; then invert and swirl again. Do this ten times.

To care for a volumetric flask, rinse it thoroughly with distilled water. Occasionally, wash it with a mild detergent. To make sure it is clean, put a few milliliters of water in it, swirl, and pour into a cuvette. Then, compare the fluorometer reading with clean water. Rinse until they read the same. The flask does not have to be dry to use it.

THE PIPETTE

There are three choices: a pipettor, a measuring pipette, or a volumetric pipette.

Pipettor. Advantageous for most users. It is not necessary to learn to control the leakage while you adjust to a line, and disposable plastic tips are used. Accuracy is generally about 1%. There are many brands on the market. They can be purchased in fixed volumes, for example, 100 microliters (0.1 ml), 1 ml; or adjustable volumes, 10-100 microliters, 0.1-1 ml.

Pipettors are relatively inexpensive and easy to use: 1) Push a button until you feel a stop; 2) Insert tip in solution; 3) Release slowly to draw up the set volume; 4) Push past first stop to a second one to eject and blow out the last drop. Some pipettors have a further position that ejects the tip.

Measuring Pipette. Available in disposable form. The accuracy of the disposable ones, +/- 2%, is no worse than the nondisposable, and a clean one is always available. When using, let it drain, then blow the last portion out.

Volumetric Pipette. Like the volumetric flask, this is very accurate. First, fill the volumetric flask about 1/2 full with water. Then, fill the pipette to the line, and, holding the pipette vertical, allow it to drain. When it stops dripping, hold it for about 10 seconds longer, then touch the drop on the end to the surface of the water (just once, and only briefly). Remove the pipette.

Problems: A pipette must be dry when you use it, or it must be rinsed with the material you intend to pipette. Volumetric pipettes are difficult to clean and rinse. If you need the accuracy, buy

quantities of them, so you can use them, then clean them at leisure (preferably with access to a laboratory pipette washer).

MAKING STANDARD DILUTIONS

For a standard, you need any concentration no greater than the linear range of your tracer (for the rhodamine dyes, approximately 100-ppb (0.1-ppm; 100-micrograms/liter) active ingredient).

To obtain this concentration, you will make serial dilutions. By this, we mean you take your concentrated solution and make a dilution of it. You mix it thoroughly, then make a dilution of that, and so on, until the desired concentration is obtained.

We recommend preparing a higher concentration, i.e., around 0.1-ppm (100-ppb or 10^{-7}) active ingredient. At these high levels (high for fluorescence), contamination will be less of a problem. Contamination from dirt or other things is not a problem, but spurious tracer could be. In preparing the standards, you are handling the pure material and high concentrations, and it is safer to use the highest standard that is convenient.

Note that the dilutions you are after can be achieved in a variety of ways. The easiest way is with 1- and 10-ml pipettors, and a choice of 100-ml and 1-liter flasks. If intermediate concentrations are desired, use an adjustable pipettor, or pipette several shots into one flask, or use intermediate-size flasks (they are available in 200-, 250-, and 500-ml sizes). Generally, all you are after is some concentration not greater than 500 ppb (0.5 ppm)--or 100 ppb (0.1 ppm) if dealing in active ingredient. Since readings are proportional to concentration at or below this point, it is simply a question of convenience.

Don't use all clean water. Your last dilution should always be done twice, once in distilled water, and once in the water in which the measurement will be made. This is because sometimes there are substances in the test water that interfere with the reading. This doesn't happen often, but it can invalidate your readings if you don't recognize it.

Your standard will be the dilution in the system water, but first you need to see that it reads the same as the dilution in distilled water -- or make sure you understand any difference.

A. To prepare a 100-ppb (active ingredient) standard of rhodamine WT (20% solution):

1. First, prepare a 100-fold dilution by weight. (See section MEASURING BY WEIGHT OR VOLUME for an explanation.) Using an accurate laboratory scale, weigh 1 gram of dye directly into a 100-ml volumetric flask. The dye may be dripped into the flask with a

pipette until 1 gram is obtained. Then dilute to the mark with distilled water. You now have a 10-g/liter (10 ppt, 10^{-2}) concentration of your tracer.

Note 1: You could obtain the same concentration by weighing 10 g into a 1-liter flask, or 20 g into a 2-liter flask.

Note 2: If you intend to inject dye by volume, then pipette 1 ml of dye into a 100-ml volumetric flask and dilute to the mark with distilled water. Or measure 10 ml of dye into a 10-ml volumetric flask and rinse into a 1-liter flask. Then, dilute to the mark with distilled water. This will yield a 10-ml/liter (10-ppt, 10^{-2}) dilution. (Keep in mind the specific gravity factor. See MEASURING BY WEIGHT OR VOLUME, above.)

2. Next, pipette 1 ml (or weigh 1 gram) of the dilution in #1 (10^{-2} or 10 ppt) into a clean 100-ml volumetric flask and dilute to the mark with distilled water. Mix thoroughly. You now have a 10^{-4} , or 100-ppm, dilution.
3. Now, pipette 5 ml (or weigh 5 grams) of the dilution in #2 (10^{-4} or 100 ppm) into a clean 1-liter volumetric flask and fill to the mark with system water. Mix thoroughly. You now have a 10^{-7} (or 100-ppb; 0.1-ppm) active ingredient standard.

Note: We measured 5 ml because rhodamine WT comes as a 20% solution (meaning 20% active ingredient). If you are not concerned with active ingredient, then diluting 1 ml 1000-fold yields a 100-ppb dilution of tracer (or 20-ppb active ingredient).

4. Repeat step 3, using distilled water. Compare fluorometer readings of this dilution with that of #3.

B. To prepare a 100-ppb standard of rhodamine B or other dye in powder form:

1. First, prepare a 100-fold dilution by weight. (See MEASURING BY WEIGHT OR VOLUME above, for an explanation.) Using an accurate laboratory scale, weigh 1 gram of dye directly into a 100-ml volumetric flask. Then dilute to the mark with distilled water. (Be sure to mix thoroughly; the powders can be difficult to mix.) You now have a 10-g/liter (10-ppt, 10^{-2}) - concentration of your tracer.

Note: You could obtain the same concentration by weighing 10 g into a 1-liter flask, or 20 g into a 2-liter flask.

- Next, pipette 1 ml (or weigh 1 gram) of the dilution in #1 (10^{-2} or 10 ppt) into a clean 100-ml volumetric flask and dilute to the mark with distilled water. Mix thoroughly. You now have a 10^{-4} , or 100-ppm, dilution.
- Now, pipette 1 ml (or weigh 1 gram) of the dilution in #2 (10^{-4} or 100 ppm) into a clean 1-liter volumetric flask and fill to the mark with system water. Mix thoroughly. You now have a 10^{-7} (or 100-ppb; 0.1-ppm) standard.
Note: Rhodamine B is also available in 30% or 40% active ingredient solutions. If you are working with one of these dilutions, then what you actually have is a 30-ppb (30%) or 40-ppb (40%) standard, based on active ingredient.
- Repeat step 3, using distilled water. Compare fluorometer readings of this dilution with that of #3.

Thus, the necessary concentration for your standard can be achieved in a variety of ways. For example, a 5×10^{-7} or 500-ppb dilution could be achieved in the following cases:

- You are doing a flow measurement, and have a dye concentration of 5×10^{-3} (5 ppt). To achieve a 5×10^{-7} (500-ppb) dilution, make two 100-fold dilutions of the 5 ppt.
- You have a 5% solution (5×10^{-2}) of dye; make one 1000-fold dilution and one 100-fold dilution; or a 10-fold, followed by two 100-fold dilutions.

MAKING MORE THAN ONE CONCENTRATION

Whether or not you make more than one concentration depends on such considerations as the confidence you have in your dilution, and how important it is that your standard be exactly right. If this is your first time, it would be important to prepare a range of concentrations and plot a standard curve. This will test your proficiency and make you more confident. The readings, however, should be linear with concentration. You really need only one concentration (and a blank).

The most important thing to do is to be sure there were no errors, i.e., a lapse in counting measurements, an accidental contamination, an air bubble in the pipetted sample, etc.

It is not so much, therefore, the need for more than one concentration as it is a need to duplicate your preparation. This means from the beginning. If you choose to make several concentrations at the final dilution, fine.

pH, TURBIDITY, AND CHLORINE

pH. When you are taking measurements, the most serious, nonvisible problem in test waters is pH. Any pH between 4.5 and 10.5 is fine. Most systems should fall in this range, but if the pH does not, the fluorescence will drop off rapidly. It is, however, reversible. If the dilution in the system water reads very low and there is no obvious reason (intense color, very high turbidity, etc.), check the pH. If you can't check the pH, get some vinegar and some baking soda. Try adding a pinch of the soda to one test tube, and a drop or two of vinegar to another. Neither is capable of taking the pH too far in the other direction. If this causes the reading to increase, add a bit more to see if you have enough, then plan on adding the same amount to all your samples.

If pH is not the problem, then the study probably cannot be done with a fluorescent tracer. This is extremely rare, and it is likely that someone is dumping a high concentration of a very strong oxidizing agent. Investigate.

Turbidity and Color. These are covered in the monograph "A Practical Guide to Flow Measurement." Generally, it takes considerable turbidity or color to interfere with the readings. The interference is a percentage reduction in reading. For example, say 100 ppb reads 900 in clean water. A 10-ppb solution would read 90.0, and a 1-ppb solution, 9.00. Your 100-ppb solution in the system water reads 810 (a 10% reduction). The 10-ppb solution will read 81.0 and the 1-ppb solution, 8.10. In other words, if you use the dilution in the system water as the standard, there will be no error, and no correction needs to be made.

Should you calibrate this way if there is a 90% loss of reading? Theoretically you could, if you were absolutely positive that the turbidity or color would be constant during the study. In practice, it would be much better to increase the dye concentration by a factor of 10, then dilute all samples, the blank, and the standard 10-fold with clean water.

pH and Chlorine. In potable water, we found in lab tests that chlorine appears to destroy rhodamine WT within a few minutes at all pH levels, even with very low levels of chlorine (.1 part per million). There is at least one very thorough published study that showed little effect of chlorine on rhodamine WT in wastewater (2). We speculate that the suspended solids in wastewater have a prophylactic effect.

STORING SAMPLES AND STANDARDS

The tracers will not degrade, and if stored in the dark, are stable for years. After you have completed your study, you may find that something doesn't fit. If you have a sample of the tracer you

