88-e11

Segment No.: 05-10-03

WA-10-1050

PUYALLUP WASTEWATER TREATMENT PLANT
CLASS II INSPECTION
January 19-20, 1988

by
Patrick Hallinan

Washington State Department of Ecology
Compliance Inspections Section
Olympia WA 98504-6811

May 1988

ABSTRACT

A Class II inspection was conducted at the Puyallup Wastewater Treatment Plant (WTP) on January 19 and 20, 1988. The WTP is a rotating biological contactor facility serving the city of Puyallup. High total suspended solids (TSS) and BOD_5 removals were achieved by the plant during the inspection. The WTP laboratory has had difficulties meeting EPA quality assurance tests in the past. Lab procedures as reviewed by Ecology indicated protocols at the WTP lab have been improved. Bioassay test results indicated the effluent was not toxic.

INTRODUCTION

A Class II inspection was conducted on January 19 and 20, 1988, at the Puyallup Wastewater Treatment Plant (WTP). Conducting the inspection were Pat Hallinan and Marc Heffner from the Ecology Compliance Inspection Section. Ralph Stevenson, chief operator at the WTP, and Martha Spear, WTP laboratory analyst, provided assistance.

The WTP utilizes rotating biological contactors (RBCs) to provide secondary treatment of wastewater. Treated effluent is discharged into the Puyallup River in accordance with NPDES permit #WA-003716-8. National Semiconductor of Puyallup shares the outfall line with the WTP. Sludge from the process is gravity thickened and then anaerobically digested. Dried digested sludge is mixed with soil and used as a soil conditioner.

Objectives of this survey included:

1. Verify effluent compliance with NPDES permit limits.
2. Use a priority pollutant scan to identify possible chemical pollutants in plant effluent.
3. Use bioassays to determine the toxicity of plant effluent and combined discharge (consisting of WTP effluent and National Semiconductor effluent).
4. Determine plant loading and efficiency by collecting samples and verifying flows.
5. Review laboratory procedures for conformance with approved standard laboratory techniques--also, split samples with the plant to compare the accuracy of results.

PROCEDURES

WTP influent and effluent composite samples were collected by Ecology. ISCO automatic samplers were set to collect approximately 200 mLs of sample every 30 minutes for 24 hours. The influent sample composite was collected from a wet well where influent flow and digester supernatant are mixed. Sampling ahead of return

supernatant flow would have been impossible, given the present plant configuration. The plant inflow pipe was buried underground and only the end of the pipe was exposed in the wet well. The effluent composite sample was collected from the chlorine contact chamber.

The permittee also collected composite samples of influent and effluent using automatic time-proportional samplers. The WTP influent compositor was located at a point after the digester supernatant return flow, but ahead of the flow de-gritting units. The WTP effluent compositor was in the same location as the Ecology sampler. Samples were split for analysis by Ecology and the permittee. Grab samples were collected for field and lab analysis by Ecology. WTP effluent and combined discharge grab samples were collected for bioassay tests. Combined discharge included WTP effluent and National Semiconductor effluent. Sampling times and parameters are listed in Table 1.

Instantaneous flow measurements of influent and effluent were made by Ecology. Influent plant flow was measured at the 36-inch Palmer-Bowles flume. Effluent plant flow was measured at the chlorine contact basin effluent weir.

RESULTS AND DISCUSSION

Data collected during the inspection are summarized in Table 2 (flow measurements) and Table 3 (data analysis).

Plant Operation

A schematic diagram of plant flow is given in Figure 1. Wastewater enters the plant to a wet well where it is mixed with digester supernatant return flow. Three screw pumps lift the wastewater to the plant headworks. Wastewater flow is first de-gritted by two pistigrit units and then routed through two comminutors. Influent flow is measured following the comminutors by a 36-inch Palmer Bowles flume. Four primary clarifiers are located after the flume. During the inspection three of the four were in operation--the other was down for routine maintenance. Effluent from the primary clarifiers is fed to 20 RBC units operating as four parallel trains of five units. Four secondary clarifiers follow the RBCs. Wastewater is then chlorinated and fed to two chlorine contact basins. Because of low flow to the WTP, one secondary clarifier and one chlorine contact basin were not in use during the inspection. Effluent plant flow is measured prior to chlorination by an in-line ultrasonic flow meter which automatically adjusts the chlorine dosage rate. Secondary clarifier settled sludge is fed back to the primary clarifiers. Co-settled sludge from the primary clarifier is sent to the anaerobic digesters.

Instantaneous effluent flow measured at the chlorine basin effluent weir by Ecology agreed closely with the permittee's recorded effluent flow. Instantaneous influent flow measured at the 36-inch Palmer-Bowles flume did not match plant recorded flow.

Table 1. Sampling schedule and parameters analyzed - Puyallup, 1/88.

					Field Analyses					Laboratory Analyses																								
								Chl. Resid. (mg/)							Solids (mg/L)					Nutr. (mg/L)							Priority Pollutants						Bioassays		
Station	Date	Time	Sampler	Laboratory	Temp. (°C)	pH (S.U.)	Cond. (umhos/cm)	Free	Total	F.C. (#/100 mL)	BOD_5 (mg/L)	Inhib. BOD_5 (mg/L)	COD (mg/L)	TS	TNVS	TSS	TNVSS	Turbidity (NTU)	NH_3-N	NO_2+NO_3-N	Total-P	pH (S.U.)	Cond. (umhos/cm)	Hardness (mg/L as $CaCO_3$)	Alk. (mg/L as $CaCO_3$)	BNA	Volatile Org.	Pest./PCBs	Metals	Sludge Metals	Percent Solids	Trout	Microtox	Ceriodaphnia
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	18	20	21	22	23	24	25	26	27	28	29	30	31	32	33
Influent	1/19	1000			X	X	X																											
		1550			X	X	X																											
	1/20	1124			X	X	X																											
		Comp.	Ecology	Ecology							X	X	X	X	X	X	X	X	X	X	X	X	X		X									
		(1100-		Puyallup							X																							
		1100)	Puyallup	Ecology							X	X	X	X	X	X	X	X	X	X	X	X	X		X									
				Puyallup							X					X																		
Effluent	1/19	1145			X	X	X			X			X			X																			
		1540			X	X	X	X	X	X			X			X																		
		**																														X	X	X
	1/20	1000			X	X	X	X	X	X			X			X																		
		1000	Puyallup	Puyallup				X	X	X																								
		Comp.	Ecology	Ecology							X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X					
		(1100-		Puyallup							X																							
		1100)	Puyallup	Ecology							X	X	X	X	X	X	X	X	X	X	X	X	X		X									
				Puyallup																														
Dischg+	1/19	1205			X	X	X																											
		1605			X	X	X																											
	1/20	1040			X	X	X																											
		++																															X	X	X
Digester Sludge	1/20	0930																													X	X			

* = grab samples are sampled and analyzed by Ecology unless otherwise specified
** = 1/3 collected on 1/19 at 1145; 1/3 collected on 1/19 at 1540; 1/3 collected on 1/20 at 1000
\+ = Consisted of WTP effluent and National Semiconductor effluent
++ = 1/3 collected on 1/19 at 1205; 1/3 collected on 1/19 at 1605; 1/3 collected on 1/20 at 1040

Table 2. Ecology analytical results - Puyallup, 1/88.

				Field Analyses						Laboratory Analyses														
							Chlorine Residual (mg/L)						Solids (mg/L)					Nutrients (mg/L)						
Station	Date	Time	Sampler	Temperature (°C)	pH (S.U.)	Cond. (umhos/cm)	Free	Total	Fecal Coliform (#/100 mL)	BOD_5 (mg/L)	Inhib. BOD_5 (mg/L)	COD (mg/L)	TS	TNVS	TSS	TNVSS	Turbidity (NTU)	NH_3-N	NO_2+NO_3-N	Total-P	pH (S.U.)	Cond. (umhos/cm)	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	18	20	21	22	23	24
Influent	1/19	1000		12.6	6.8	390																		
		1550		12.2	7.0	410																		
	1/20	1124		12.0	7.3	400																		
		Comp. (1100-1100)	Ecology							150	>99	320	380	150	110	27	43	13.4	0.3	1.1	7.3	430		160
			Puyallup							130	>98	260	430	210	130	38	35	16.3	1.4	1.2	7.3	448		160
Effluent	1/19	1145		12.0	6.9	360			1			48			14									
		1540		12.2	6.8	365	0.3	1.0	2			50			20									
		*																						
	1/20	1000		12.2	6.9	375	0.5	1.0	1			50			19									
		1000	Puyallup				0.4	0.0	3															
		Comp. (1100-1100)	Ecology							12	10	53	290	170	6	<1	10	5.1	10.1	0.8	7.1	391	80	86
			Puyallup							12	10	50	280	160	2	2	9	4.9	10.2	1.1	7.2	389		82
Discharge+	1/19	1205		11.8	6.8	385																		
		1605		12.0	7.0	385																		
	1/20	1040		12.0	7.1	390																		
		++																						
Digester Sludge	1/20	0930																						

* = 1/3 collected on 1/19 at 1145; 1/3 collected on 1/19 at 1540; 1/3 collected on 1/20 at 1000
\+ = Consisted of WTP effluent and National Semiconductor effluent
++ = 1/3 collected on 1/19 at 1205; 1/3 collected on 1/19 at 1605; 1/3 collected on 1/20 at 1040

Table 3. Flow measurements - Puyallup, 1/88.

	Date	Time	Instantaneous Flow (MGD)	Totalizer Reading	Flow for Time Increment (MGD)
Influent					
	1/19	1155	4.00	6055056	
					4.34
	1/19	1515	3.60	6055659	
					3.96
	1/20	1043	4.25	6058886	
					4.90
	1/20	1212	4.50	6059172	
	1/20	1330	4.80*	--	
		Average flow during inspection =			4.07
Effluent					
	1/19	1155	4.50	3458343	
					4.03
	1/19	1515	4.50	3458903	
					3.62
	1/20	1048	4.50	3461850	
					4.48
	1/20	1212	5.00	3462111	
	1/20	1330	4.50+	--	
		Average flow during inspection =			3.72

* = Ecology instantaneous measurement = 4.25 MGD
+ = Ecology instantaneous measurement = 4.46 MGD

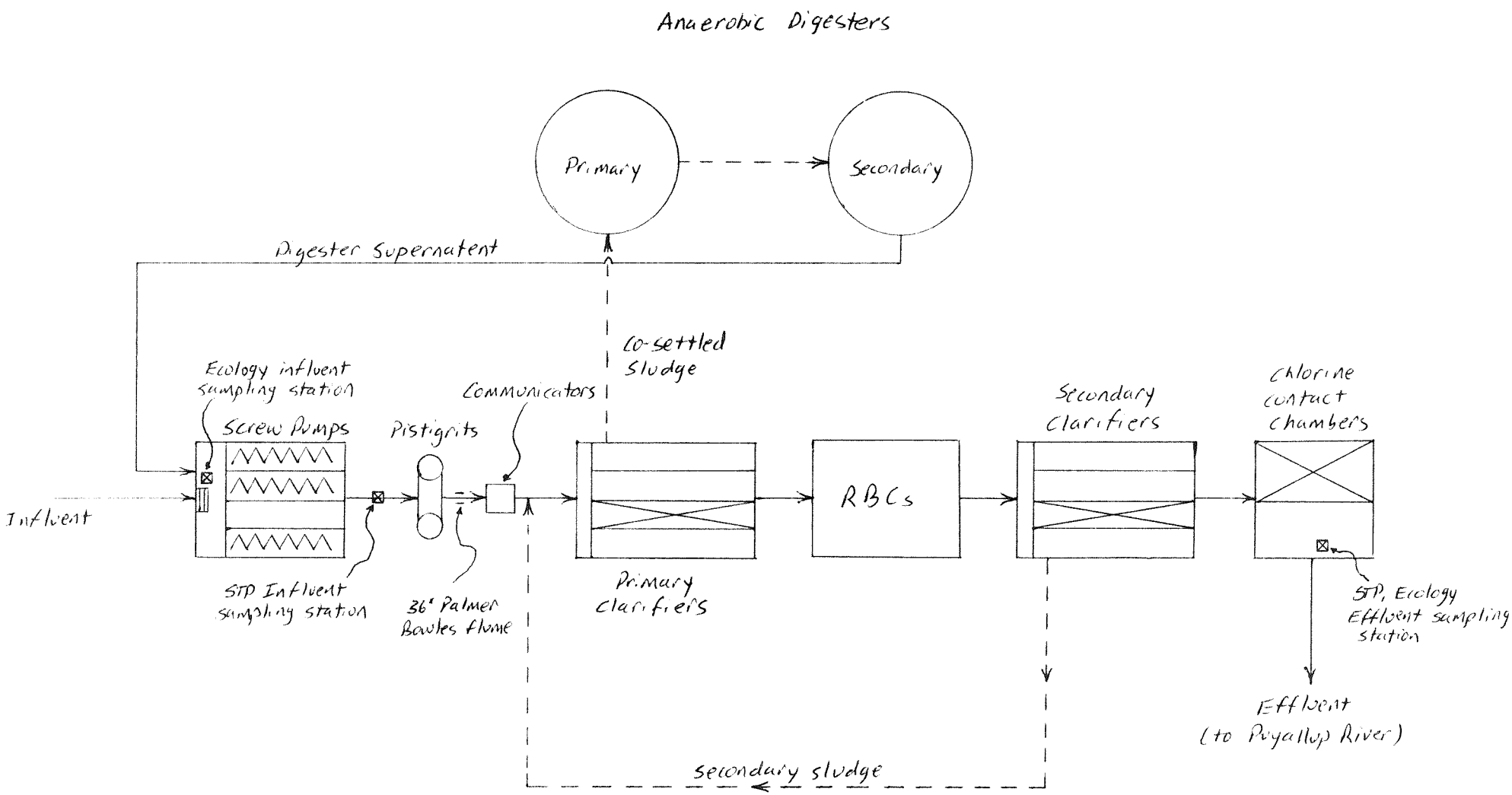

Figure 1. Flow scheme - Puyallup, January 1988.

Ecology measured a flow of 4.25 MGD when the instantaneous plant inflow was 4.8 MGD. However, the Ecology measurement was considered inaccurate. The flume calibration curve used to obtain a flow was very steep; any variation in a flow depth reading resulted in a large flow variation. Therefore, despite the disagreement between the flow measurements, the flume is believed to be accurately calibrated.

Data Analysis

Comparison of plant effluent parameters to NPDES permit limits is given in Table 4. BOD_5, TSS, fecal coliform, and pH values were all well within permit limits. The plant was providing BOD_5 and TSS removal efficiencies in excess of 90 percent. Plant flow was also well within design capacity. The plant was designed for a daily dry weather flow of 4.79 MGD; a daily wet weather flow of 10.72 MGD and a peak design flow of 19.0 MGD (Gray & Osborne, 1976). Average influent flow during the inspection was 4.1 MGD.

Results of metal analysis on digested sludge from the plant are given in Table 5. Metals found in the sludge were within ranges found at other RBC or trickling filter plants during previous Class II inspections in Washington State (Hallinan, 1988).

Results of a priority pollutant scan for pesticides, polychlorinatedbiphenols (PCBs), base-neutral-acid organics (BNA's), volatile organics (VOA's), and metals in WTP effluent are given in the appendix of this report. There were no pesticides or PCBs identified in the effluent from the plant. A phthalate was found in the BNA scan. Phthalates are used in the manufacture of plastics and are commonly found in wastewaters.

The VOA scan identified a number of organics in the WTP effluent. Table 6 lists the chemicals found at detectable limits in the sample analyzed. Methylene chloride was found in the blank sample and the effluent sample, indicating a contaminated blank. Levels of chloroform in the effluent are most likely the result of the wastewater chlorination process (EPA, 1980a). Tetrachloroethylene is used primarily as a dry-cleaning solvent (EPA, 1980b). Trichloroethylene is also used as a dry-cleaning solvent as well as a metal degreasing solvent, extractive food solvent, and inhalation anesthetic (EPA, 1980c). Acetone, carbon tetrachloride, and toluene are all used as chemical solvents (EPA, 1980d,e). All chemicals were detected at levels far below EPA freshwater acute or chronic criteria.

Table 7 lists the priority pollutant metals detected in the WTP effluent. Nickel and zinc were detected at levels below the EPA four day and one hour criteria for fresh water. Mercury was found at a level slightly higher than the EPA four-day criteria. Copper was detected at a level higher than the EPA one-hour and four-day criteria. However given the dilution of the effluent, the metals would not be expected to have any toxic effect in the receiving water.

Table 4. Comparison of inspection data to NPDES permit effluent limitations - Puyallup 1/88.

Parameter	NPDES Permit Limits		Inspection Data		
	Monthly Average	Weekly Average	Ecology Composite	WTP Composite	Grab Samples
Influent BOD_5 (mg/L)			150	130	
BOD_5					
(mg/L)	30	45	12	12	
(lbs/D)	1390	2085	372	372	
(% removal)			92	91	
Influent TSS (mg/L)			110	130	
TSS					
(mg/L)	30	45	6	2	
(lbs/D)	1390	2085	186	62	
(% removal)			95	98	
Fecal Coliform (#/100 mL)	200	400			1, 1, 2
pH (S.U.)	6.0-9.0				6.8, 6.9, 6.9
Flow (MGD)	10.72		3.72	3.72	

Table 5. Sludge metals data - Puyallup, 1/88.

Metal	WTP Sample** (mg/kg dry wt.)	Data from Previous Inspections* Range (mg/kg dry wt.)	Geometric Mean (mg/kg dry wt.)	Number of Samples
Cadmium	3	0.01 - 16	5.5	17
Chromium	20	0.4 - 313	40.9	17
Copper	475	28 - 3100	532	17
Lead	106	100 - 1140	284	17
Nickel	18	12 - 46	28.6	15
Zinc	968	680 - 2500	1620	17
Mercury	2	---	---	--

* = data collected during previous Class II inspections at trickling filter or RBC plants

** = percent solids = 1.40

Table 6. VOA chemicals identified in effluent - Puyallup 1/88.

Organic Compound	Analytical Result (ug/L)	EPA Water Quality Criteria Freshwater Acute (ug/L)	EPA Water Quality Criteria Freshwater Chronic (ug/L)
Acetone	21.0	--	--
Chloroform	3.3	28,900	1,240
Carbon Tetrachloride	1.0	35,200	--
Trichloroethene	0.8	45,000	21,900
Tetrachloroethene	5.3	5,280	840
Toluene	2.9	17,500	--

Table 7. Metals (total) detected in WTP effluent - Puyallup, 1/88.

Metal	Effluent Sample* (ug/L)	EPA Freshwater Criteria One-hour (ug/L)	EPA Freshwater Criteria Four-day (ug/L)
Mercury	0.12	2.4	0.012
Copper	18	14.4	9.8
Nickel	16	130	1175
Zinc	24	88	97

*hardness = 80 mg/L as $CaCO_3$

Rainbow trout (*Salmo gairdneri*), Ceriodaphnia (*Ceriodaphnia dubia*) and microtox bioassays were used to test the toxicity of WTP effluent and combined discharge (consisting of WTP and National Semiconductor effluent). Results of these tests are given in Table 8. No mortalities were noted in the rainbow trout bioassay. The microtox test results indicate the WTP effluent was considerably more toxic than the combined discharge. Residual chlorine levels in the plant effluent could have been toxic to the microtox test (M. Stinson, personal communication). The National Semiconductor effluent appears to dilute the toxicity of the WTP effluent.

An acute toxic effect was observed for both effluents in the 100 percent concentration Ceriodaphnia test. The combined discharge appeared to be more lethal to the organism than the WTP effluent. Chronic effluent toxicity measured by Ceriodaphnia total reproduction was difficult to interpret. For both samples, reproduction was about 30 to 50 percent lower than the control at lower test concentrations (3 and 6 percent). However, reproduction was approximately equal to or slightly higher than the control at higher test concentrations (25 and 50 percent). Reproduction by Ceriodaphnia is sometimes stimulated by exposure to nutrient-enriched wastewaters (Bernhardt, 1988). The results of these bioassay tests indicate the effluents show little or no acute toxicity and further biomonitoring is not recommended at this time.

Laboratory review

Laboratory procedures at the WTP were good. The laboratory was clean and appeared to be well organized. A laboratory review sheet is included in the Appendix of this report. Important comments to keep lab procedures in conformance with standard methods include:

BOD_5

1. The BOD of the seed material should be determined as for any sample. This is the seed control (APHA, 1985, #5d, p. 529). When calculating BOD for a sample, the seed correction should be made with the seed control D.O. depletion (APHA, 1985, #6, p. 531).

TSS

1. For a quality assurance check, the drying cycle should be repeated to assure constant filter weight has been obtained (APHA, 1985, #3c, p. 97). This should be done once every two months.

Fecal Coliform

1. Samples should be dechlorinated by adding 1 mL of 1 percent sodium thiosulfate solution per 120 mLs (4 ounces) of sample collected (APHA, 1985, #2, p. 856.).

Table 8. Effluent Bioassay Results* - Puyallup, 1/88.

96-hour Rainbow trout (*Salmo gairdneri*) bioassay - 100 percent concentration

Sample	Number of Live Test Organisms Initial	After 96 hours	Percent Mortality
Control	30	30	0
WTP effluent	30	30	0
Combined discharge	30	30	0

7-day ceriodaphnia (*Ceriodaphnia dubia*) bioassay

Sample	Percent Mortality	Total Reproduction
WTP Effluent		
Control	0	218
1.5% effluent	0	200
3.0% effluent	0	115
6.0% effluent	0	105
13.0% effluent	0	131
25.0% effluent	0	204
50.0% effluent	0	239
100.0% effluent	30	176
Combined Discharge		
Control	0	204
1.5% effluent	0	148
3.0% effluent	0	126
6.0% effluent	0	143
13.0% effluent	0	246
25.0% effluent	0	277
50.0% effluent	0	232
100.0% effluent	90	22

Microtox Bioassay

Sample	EC50** (5 minutes)	EC50** (15 minutes)
WTP effluent	23.5%	11.4%
Combined discharge	66.4%	32.1%

*Salmo gairdneri test performed by E.V.S. Consultants
Ceriodaphnia dubia test performed by E.V.S. Consultants
Microtox test performed by Ecology laboratory
**Estimated effluent concentration that adversely affects 50 percent of the test population

A comparison of Ecology and WTP laboratory results is given in Table 9. BOD_5, TSS, and fecal coliform results agreed closely. However, total residual chlorine (TRC) levels did not compare favorably. Ecology measured a TRC of 1.0 mg/L for WTP effluent while the permittee's result was 0.0 mg/L. The WTP laboratory uses the amperometric titration method to measure chlorine residuals. A brief review of the method by Ecology showed that no procedural errors were being made by the WTP analyst. Furthermore, the free available chlorine concentration measured by the method of 0.4 mg/L agreed closely with the Ecology result of 0.5 mg/L. It is chemically impossible for free available chlorine to be greater than total residual chlorine. A possible explanation was the chemical reagents used in the TRC titration were not fresh. Fresh reagents should be made and used. In addition, another method of chlorine measurement should be used by the plant to check the accuracy of the titration method. A chlorine DPD colorimetric kit is recommended. Also, a TRC of 1 mg/L is probably more than enough to provide adequate disinfection of the effluent (a 0.5 mg/L TRC level is usually recommended). The plant should consider lowering the chlorine dosage rate, while still maintaining low fecal coliform counts.

RECOMMENDATIONS AND CONCLUSIONS

The Puyallup WTP was performing very well during the inspection. Recommendations and conclusions include:

1. Plant effluent parameters (BOD_5, TSS, fecal coliform, and pH) were all well within permit limits.
2. Results of VOA, BNA, and PCB priority pollutant scans of WTP effluent indicated no major pollutants at elevated levels. The city of Puyallup might consider identifying the source or sources of the VOA chemicals being discharged to their sewer system. The priority pollutant metal scan detected copper and mercury in amounts greater than EPA fresh water criteria. However, the bioassay test results suggest that the metals do not cause any severe acute effluent toxicity.
3. WTP and combined discharge effluents were not toxic to the rainbow trout bioassay. However, both showed acute effects in the 100 percent Ceriodaphnia test. Microtox test results indicate that the National Semiconductor effluent dilutes the WTP effluent toxicity. No further biomonitoring is suggested at this time.
4. The plant performed very well during the inspection, providing BOD_5 and TSS removal efficiencies in excess of 90 percent. Plant flow was well below design capacity.
5. Laboratory procedures were good. Minor recommendations are made in the laboratory review section of this report.

Table 9. Comparison of laboratory results - Puyallup 1/88.

Station	Date	Time	Sampler	Laboratory	Chlorine Residual (mg/L) Free	Total	Fecal Coliform (#/100 mL)	BOD_5 (mg/L)	TSS (mg/L)
Influent	1/20	Comp.	Ecology	Ecology				150	110
				Puyallup				117	
		Comp.	Puyallup	Ecology				130	130
				Puyallup				112	118
Effluent	1/20	1000	Ecology	Ecology	0.5	1.0	1		
		1000	Puyallup	Puyallup	0.4	0.0	3		
		Comp.	Ecology	Ecology				12	6
				Puyallup				9	
		Comp.	Puyallup	Ecology				12	2
				Puyallup				9	9

REFERENCES

APHA-AWWA-WPCF, 1985. Standard Methods for the Examination of Water and Wastewater, 16th ed.

Bernhardt, J., 1988. "Summary of findings--effluent biomonitoring surveys performed by the Water Quality Investigations Section 1986-1988," memorandum to N. Glenn, Dept. of Ecology, Olympia, WA. February 24, 1988

EPA, 1980a. Ambient Water Quality Criteria for Chloroform, EPA 440/5-80-033, October 1980.

EPA, 1980b. Ambient Water Quality Criteria for Tetrachloroethylene, EPA 440/5-80-073, October 1980.

EPA, 1980c. Ambient Water Quality Criteria for Trichloroethylene, EPA 440/5-80-077, October 1980.

EPA, 1980d. Ambient Water Quality Criteria for Carbon Tetrachloride, EPA 440/5-80-026, October 1980.

EPA, 1980e. Ambient Water Quality Criteria for Toluene, EPA 440/5-80-075, October 1980.

EPA, 1986. Quality Criteria for Water 1986, EPA 440/5-86-001, May 1986.

Gray & Osborne, Inc., City of Puyallup, Pierce County, WA, Engineering and Facilities Plan, Vol. 1, November 1976.

Hallinan, P., 1988. "Metals concentrations found during Ecology inspections of municipal wastewater treatment plant," memorandum to J. Bernhardt, Dept. of Ecology, Olympia, WA. April 11, 1988

Stinson, M., personal communication.

APPENDIX A. EFFLUENT PRIORITY POLLUTANT SCAN

Appendix A. Results of VOA, BNA, Pest/PCB and metal priority pollutant scan - Puyallup 1/88.

Priority Pollutant	Analytical Result (ug/L)	Priority Pollutant	Analytical Result (ug/L)
VOA Compound		VOA Compound	
Chloromethane	3.8 U	1,2-Dichloropropane	0.7 U
Bromomethane	3.1 U	Trans-1,3-Dichloropropene	1.8 U
Vinyl Chloride	2.0 U	Trichloroethene	0.8
Chloroethane	3.3 U	Dibromochloromethane	0.7 U
Methylene Chloride	0.8 JB	1,1,2-Trichloroethane	0.7 U
Acetone	21.0	Benzene	1.0 U
Carbon Disulfide	1.2 U	cis-1,3-Dichloropropene	1.9 U
1,1-Dichloroethene	0.7 U	2-Chloroethylvinylether	2.7 U
1,1-Dichloroethane	0.6 U	Bromoform	2.5 U
1,2-Dichloroethene (total)	0.4 M	4-Methyl-2-Pentanone	3.5 U
Chloroform	3.3	2-Hexanone	3.2 U
1,2-Dichloroethane	0.5 U	Tetrachloroethene	5.3
2-Butanone	6.2 U	1,1,2,2-Tetrachloroethane	2.7 U
1,1,1-Trichloroethane	0.6 U	Toluene	2.9
Carbon Tetrachloride	1.0	Chlorobenzene	0.9 U
Vinyl Acetate	3.1 U	Ethylbenzene	0.8 U
Bromodichloromethane	0.3 M	Styrene	1.1 U
		Total Xylenes	1.8 U
Pest/PCB Compound		Priority Pollutant Metal (total)	
Alpha-BHC	0.1 U	Arsenic	1 U
Beta-BHC	0.1 U	Lead	5 U
Delta-BHC	0.1 U	Thallium	1 U
Gamma-BHC (Lindane)	0.1 U	Silver	0.2 U
Heptachlor	0.1 U	Antimony	10 U
Aldrin	0.1 U	Selenium	1 U
Heptachlor Epoxide	0.1 U	Mercury	0.12
Endosulfan I	0.1 U	Beryllium	1 U
Dieldrin	0.2 U	Cadmium	5 U
4,4'-DDE	0.2 U	Chromium	10 U
Endrin	0.2 U	Copper	18
Endosulfan II	0.2 U	Nickel	16
4,4'-DDD	0.2 U	Zinc	24
Endosulfan Sulfate	0.2 U		
4,4'-DDT	0.2 U		
Methoxychlor	0.2 U		
Endrin Ketone	0.2 U		
Chlordane	0.4 U		
Toxaphene	20.0 U		
Aroclor-1016	2.0 U		
Aroclor-1242	2.0 U		
Aroclor-1248	2.0 U		
Aroclor-1254	2.0 U		
Aroclor-1260	2.0 U		

Appendix A - continued.

Priority Pollutant	Analytical Result (ug/L)	Priority Pollutant	Analytical Result (ug/L)
BNA Compound		BNA Compound	
Phenol	0.6 U	Acenaphthene	0.6 U
bis(2-Chloroethyl)Ether	0.4 U	2,4-Dinitrophenol	3.2 U
2-Chlorophenol	0.5 U	4-Nitrophenol	1.0 U
1,3-Dichlorobenzene	0.2 U	Dibenzofuran	0.8 U
1,4-Dichlorobenzene	0.4 U	2,4-Dinitrotoluene	0.5 U
Benzyl Alcohol	0.5 U	2,6-Dinitrotoluene	1.3 U
1,2-Dichlorobenzene	0.1 U	Diethylphthalate	0.4 U
2-Methylphenol	0.6 U	4-Chlorophenyl-phenylether	0.7 U
bis(2-chloroisopropyl)ether	1.3 U	Fluorene	0.6 U
4-Methylphenol	0.3 U	4-Nitroaniline	1.8 U
N-Nitroso-Di-n-Propylamine	0.8 U	4,6-Dinitro-2-Methylphenol	3.3 U
Hexachloroethane	0.8 U	N-Nitrosodiphenylamine	1.6 U
Nitrobenzene	0.5 U	4-Bromophenyl-phenylether	0.6 U
Isophorone	1.2 U	Hexachlorobenzene	0.9 U
2-Nitrophenol	1.6 U	Pentachlorophenol	0.6 U
2,4-Dimethylphenol	1.4 U	Phenanthrene	0.8 U
Benzoic Acid	1.7 U	Anthracene	0.5 U
bis(2-Chloroethoxy)Methane	1.2 U	Di-n-Butylphthalate	0.8 U
2,4-Dichlorophenol	1.7 U	Fluoranthene	1.8 U
1,2,4-Trichlorobenzene	0.9 U	Pyrene	1.6 U
Naphthalene	1.6 U	Butylbenxylphthalate	2.0 U
4-Chloroaniline	0.9 U	3,3'-Dichlorobenzidine	0.8 U
Hexachlorobutadiene	0.9 U	Benzo(a)Anthracene	1.3 U
4-Chloro-3-Methylphenol	0.9 U	bis(2-Ethylhexyl)Phthalate	3.3
2-Methylnaphthalene	0.9 U	Chrysene	0.3 U
Hexachlorocyclopentadiene	0.8 U	Di-n-Octyl Phthalate	1.6 U
2,4,6-Trichlorophenol	0.3 U	Benzo(b)Fluoranthene	0.5 U
2,4,5-Trichlorophenol	0.4 U	Benzo(k)Fluoranthene	2.1 U
2-Chloronaphthalene	0.1 U	Benzo(a)Pyrene	0.2 U
2-Nitroaniline	1.6 U	Indeno(1,2,3-cd)Pyrene	0.9 U
Dimethyl Phthalate	0.5 U	Dibenz(a,h)Anthracene	1.0 U
Acenaphthylene	0.2 U	Benzo(ghi)Perylene	0.9 U
3-Nitroaniline	0.9 U		

U = compound was analyzed for but not detected at the given detection limit.

B = Used when the analyte is found in the blank as well as the sample. Indicates possible/probable blank contamination.

J = An estimated value when result is less specified detection limit.

M = An estimated value of analyte found and confirmed by analyst but with low spectral match parameters

APPENDIX B. BIOASSAY RESULTS

ANDREA BEATTY RINIKER
Director

STATE OF WASHINGTON

DEPARTMENT OF ECOLOGY

Post Office Box 346 • Manchester, Washington 98353-0346 • (206) 895-4740

M E M O R A N D U M

March 22, 1988

To: Pat Hallinan

From: Margaret Stinson MDS

Re: Puyallup STP
Microtox Bioassay of Effluent #04-8084 and #04-8088

Introduction

Bioassays were conducted to assess toxicity of effluent from the Puyallup Sewage Treatment Plant, part of a regularly scheduled Class II inspection. Toxicity was measured using Microtox.

Methods

The effluent samples were collected January 19, 1988, and held on ice until delivery at Manchester Laboratory January 21, 1988. Sample 04-8084 was tested on January 22, 1988. Sample 04-8088 was tested on January 25, 1988.

The test was conducted following the method described in the Beckman Microtox System Operating Manual. Reagents for the test were obtained from Microbics Corporation.

Samples were diluted (after osmotic adjustment) in three 2:1 serial dilutions from 100%. This resulted in test concentrations of 90.9%, 45.5%, 22.7%, and 11.4%.

Results

EC50 values were calculated using the Microbics "Microtox Calculation Program for IMB-PC." EC50 estimates for the Puyallup STP effluent samples were as follows:

Sample 04-8084 (WTP Effluent)	EC50 (5 minutes):	23.5%
	EC50 (15 minutes):	11.4%
Sample 04-8088 (Combined discharge)	EC50 (5 minutes):	66.4%
	EC50 (15 minutes):	32.1%

E.V.S. CONSULTANTS
ACUTE LETHALITY BIOASSAY RECORD

Client- WDOE

E.V.S. Project #- 21192-06

Work Order #- 880029

E.V.S. Analyst(s)- BY

SAMPLE

Identification- 048088 (Combined Discharge)

Amount Received- 2 x 20 g.

Date Collected- Jan 19-20

Date Received- Jan 22, 1988

pH- 6.9

Dissolved Oxygen (mg/l)- 10.4

Conductivity (umhos/cm)-

Other-

Bioassay Type- LETT

Test Initiation Date- Jan 25, 1988

DILUTION AND CONTROL MEDIUM

Fresh Water (dechlorinated)- ✓

Salt Water (Burrard Inlet)- -

pH 5.9

Dissolved Oxygen (mg/l)- 10.4

Conductivity (umhos/cm)- 10

Hardness (mg/l as $CaCO_3$)- 4.5

Alkalinity (mg/l as $CaCO_3$)- 6.5

Salinity ($^o/_{oo}$)- n/a

Other-

TEST SPECIES

Rainbow Trout- ✓

Threespine Stickleback-

Daphnia (D. magna)-

Amphipod (R. abronius)-

Other-

TEST CONDITIONS

Temperature (^oC)- 14

pH Range- 5.9-8.0

Dissolved Oxygen Range- 8.2-10.6

Conductivity Range-

Aeration (7.5 cc/min./l)- ✓

Photoperiod (L:D-in hours)- 14:10

No. Fish/Test Volume- 10/15L

Fish Loading Density (g/l)- 0.66

Other-

Bioassay Results- 0/30 mortalities = pass

Certified By- Cathy McPherson E.V.S. Consultants Ltd.

Puyallup STP

E.V.S. CONSULTANTS
ACUTE LETHALITY BIOASSAY RECORD

Client- WDOE
E.V.S. Project #- 21192-06
Work Order #- 880029

E.V.S. Analyst(s)- BJ

SAMPLE

Identification- 04-8084 (WTP Effluent)
Amount Received- 2x 20L
Date Collected- Jan 19-20
Date Received- Jan 22, 1988
pH- 7.2
Dissolved Oxygen (mg/l)- 9.6
Conductivity (umhos/cm)-
Other-

Bioassay Type- LETT
Test Initiation Date- Jan 25

DILUTION AND CONTROL MEDIUM

Fresh Water (dechlorinated)- ✓
Salt Water (Burrard Inlet)- –
pH 5.9
Dissolved Oxygen (mg/l)- 10.4
Conductivity (umhos/cm)- 10
Hardness (mg/l as $CaCO_3$)- 4.5
Alkalinity (mg/l as $CaCO_3$)- 6.5
Salinity ($^o/_{oo}$)- n/a
Other-

TEST SPECIES

Rainbow Trout- ✓
Threespine Stickleback-
Daphnia (D. magna)-
Amphipod (R. abronius)-
Other-

TEST CONDITIONS

Temperature (^oC)- 14-15
pH Range- 5.8-8.0
Dissolved Oxygen Range- 8.5-10.6
Conductivity Range-
Aeration (7.5 cc/min./l)- ✓
Photoperiod (L:D-in hours)- 14:10
No. Fish/Test Volume- 10/15L
Fish Loading Density (g/l)- 0.66
Other-

Bioassay Results- 0/30 mortalities = pass

Certified By- Cathy McPherson E.V.S. Consultants Ltd.

CERIODAPHNIA LIFE-CYCLE TESTS

Test results were as follows:

Sample	Concentration (%v/v)	Survival (%)	Total Reproduction
048088	Control	100	204
(combined	1.5	100	148
Discharge)	3.0	100	126
	6.0	100	143
	13.0	100	246
	25.0	100	277
	50.0	100	232
	100.0	10	22
048084	Control	100	218
(WTP Effluent)	1.5	100	200
	3.0	100	115
	6.0	100	105
	13.0	100	131
	25.0	100	204
	50.0	100	239
	100.0	70	176

Analysis of survival data using Fisher's Exact test indicated that for sample 048088, survival at 100% concentration was significantly different from the control. For sample 048084, survival at all concentrations was not significantly different from the control.

Reproduction data were analyzed using Dunnett's procedure (one-sided analysis of variance and one-sided comparison of t-value). For sample 048088, reproduction was significantly lower than the control at 3.0 and 6.0% concentrations, and significantly higher than the control at 25.0% concentration. For sample 048084, reproduction was significantly lower than the control at 3.0 and 6.0% concentrations. Statistical analysis was conducted at the 95% confidence level.

The above results were summarized as NOEC and LOEC, as follows:

Sample	NOEC (%v/v)	LOEC (% v/v)
048088	1.5	3.0
048084	1.5	3.0

No Observed Effect Concentration (NOEC) is the highest concentration of sample to which organisms are exposed which causes no statistically significant adverse effects on survival and reproduction.

Lowest Observed Effect Concentration (LOEC) is the lowest concentration of sample to which organisms are exposed which causes statistically significant adverse effects on survival and reproduction.

APPENDIX C. LABORATORY REVIEW SHEET

Laboratory Procedure Review Sheet

Discharger: Puyallup WTP

Date: 1/20/88

Discharger representative: Martha Spears

Ecology reviewer: Heffner/Hallinan

Instructions

Questionnaire for use reviewing laboratory procedures. Circled numbers indicate work is needed in that area to bring procedures into compliance with approved techniques. References are sited to help give guidance for making improvements. References sited include:

Ecology = Department of Ecology Laboratory User's Manual, December 8, 1986.

SM = APHA-AWWA-WPCF, Standard Methods for the Examination of Water and Wastewater, 16th ed., 1985.

SSM = WPCF, Simplified Laboratory Procedures for Wastewater Examination, 3rd ed., 1985.

Sample Collection Review

1. Are grab, hand composite, or automatic composite samples collected for influent and effluent BOD and TSS analysis? Inf, Ef

2. If automatic compositor, what type of compositor is used? Manning
The compositor should have pre and post purge cycles unless it is a flow through type. Check if you are unfamiliar with the type being used.

3. Are composite samples collected based on time or flow? time

4. What is the usual day(s) of sample collection? MWF

5. What time does sample collection usually begin? ≈ 9:00

6. How long does sample collection last? 24 hrs

7. How often are subsamples that make up the composite collected? hourly

8. What volume is each subsample? 150 mls

9. What is the final volume of sample collected? ~1 gal

10. Is the composite cooled during collection? yes

1. To what temperature? 36-39°F
The sample should be maintained at approximately 4 degrees C (SM p41, 5b: SSM p2).

2. How is the sample cooled? Mechanical
Mechanical refrigeration or ice are acceptable. Blue ice or similar roducts are often inadequate.

3. How often is the temperature measured? ~3 months
The temperature should be checked at least monthly to assure adequate ooling.

4. Are the sampling locations representative? Influent after supernatant flow

5. Are any return lines located upstream of the influent sampling ocation?
This should be avoided whenever possible.

6. How is the sample mixed prior to withdrawal of a subsample for inalysis?
The sample should be thoroughly mixed. yes

17. How is the subsample stored prior to analysis?
The sample should be refrigerated (4 degrees C) until about 1 hour before analysis, at which time it is allowed to warm to room temperature.
2-3 hours at room temperature - try to shorten

18. What is the cleaning frequency of the collection jugs?
The jugs should be thoroughly rinsed after each sample is complete and occasionally be washed with a non-phospate detergent. ✓

19. How often are the sampler lines cleaned?
Rinsing lines with a chlorine solution every three months or more often where necessary is suggested. ✓

pH Test Review

1. How is the pH measured? Corning meter
A meter should be used. Use of paper or a colorimetric test is inadequate and those procedures are not listed in Standard Methods (SM p429).

2. How often is the meter calibrated? daily
The meter should be calibrated every day it is used.

3. What buffers are used for calibration? pH 7
Two buffers bracketing the pH of the sample being tested should be used.

If the meter can only be calibrated with one buffer, the buffer closest in pH to the sample should be used. A second buffer, which brackets the pH of the sample should be used as a check. If the meter cannot accurately determine the pH of the second buffer, the meter should be repaired.

BOD Test Review

1. What reference is used for the BOD test?
Standard Methods or the Ecology handout should be used.
1980 primary

2. How often are BODs run? 3x's a week
The minimum frequency is specified in the permit.

3. How long after sample collection is the test begun? 2-3 hrs
The test should begin within 24 hours of composite sample completion (Ecology Lab Users Manual p42). Starting the test as soon after samples are complete is desirable.

4. Is distilled or deionized water used for preparing dilution water?

5. Is the distilled water made with a copper free still? yes - glass
Copper stills can leave a copper residual in the water which can be toxic to the test (SSM p36).

6. Are any nitrification inhibitors used in the test? No What?
2-chloro-6(trichloro methyl) pyridine or Hach Nitrification Inhibitor 2533 may be used only if carbonaceous BODs are being determined (SM p 527, #4g: SSM p 37).

6. Are the 4 nutrient buffers of powder pillows used to make dilution water?
If the nutrients are used, how much buffer per liter of dilution water are added? ✓
1 mL per liter should be added (SM p527, #5a: SSM p37).

7. How often is the dilution water prepared? fresh for test
Dilution water should be made for each set of BODs run.

8. Is the dilution water aged prior to use? added morning of test
Dilution water with nitrification inhibitor can be aged for a week before use (SM p528, #5b).
Dilution water without inhibitor should not be aged.

9. Have any of the samples been frozen? no
If yes, are they seeded?
Samples that have been frozen should be seeded (SSM p38).

10. Is the pH of all samples between 6.5 and 7.5? 6.9 - 7.1 should check
If no, is the sample pH adjusted?
The sample pH should be adjusted to between 6.5 and 7.5 with 1N NaOE or 1N H_2SO_4 if 6.5 > pH >7.5 if caustic alkalinity or acidity is present (SM p529, #5e1: SSM p37).
High pH from lagoons is usually not caustic. Place the sample in the dark to warm up, then check the pH to see if adjustment is necessary.

If the sample pH is adjusted, is the sample seeded? N/A
The sample should be seeded to assure adequate microbial activity if the pH is adjusted (SM p528, #5d).

11. Have any of the samples been chlorinated or ozonated?
If chlorinated are they checked for chlorine residual and dechlorinated as necesssary? checked TRC usually zero
How are they dechlorinated?
Samples should be dechlorinated with sodium sulfate (SM p529, #5e2: SSM p38), but dechlorination with sodium thiosulfate is common practice. Sodium thiosufate dechlorination is probably acceptable if the chlorine residual is < 1-2 mg/L.
If chlorinated or ozonated, is the sample seeded?
The sample should be seeded if it was disinfected (SM p528, #5d&5e2: SSM p38).

12. Do any samples have a toxic effect on the BOD test? No
Specific modifications are probably necessary (SM p528, #5d: SSM p37).

13. How are DO concentrations measured? YSI
If with a meter, how is the meter calibrated? when used
Air calibration is adequate. Use of a barometer to determine saturation is desirable, although not manditory. Checks using the Winkler method of samples found to have a low DO are desirable to assure that the meter is accurate over the range of measurements being made.

How frequently is the meter calibrated? when used
The meter should be calibrated before use.

14. Is a dilution water blank run? poor 3, run 1
A dilution waater blank should always be run for quality assurance (SM p527, #5b: SSM p40, #3).

What is the usual initial DO of the blank? 8.5-8.7
The DO should be near saturation; 7.8 mg/L @ 4000 ft, 9.0 mg/L @ sea level (SM p528, #5b). The distilled or deionized water used to make the dilution water may be aged in the dark at ~20 degrees C for a week with a cotton plug in the opening prior to use if low DO or excess blank depletion is a problem .

What is the usual 5 day blank depletion? <0.2 usual
The depletion should be 0.2 mg/L or less. If the depletion is greater, the cause should be found (SM p527-8, #5b: SSM p41, #6).

15. How many dilutions are made for each sample? one
At least two dilutions are recommended. The dilutions should be far enough apart to provide a good extended range (SM p530, #5f: SSM p41).

16. Are dilutions made by the liter method or in the bottle?
Either method is acceptable (SM p530, #5f).

17. How many bottles are made at each dilution? 3
How many bottles are incubated at each dilution? 2
When determining the DO using a meter only one bottle is necessary. The DO is measured, then the bottle is sealed and incubated (SM p530, #5f).
When determining the DO using the Winkler method two bottles are necessary. The initial DO is found of one bottle and the other bottle is sealed and incubated (Ibid.).

18. Is the initial DO of each dilution measured? ✓
What is the typical initial DO? 8.1-8.5
The initial DO of each dilution should be measured. It should approximate saturation (see #14).

19. What is considered the minimum acceptable DO depletion after 5 days? ✓
What is the minimum DO that should be remaining after 5 days? ✓
The depletion should be at least 2.0 mg/L and at least 1.0 mg/L should be left after 5 days (SM p531, #6: SSM p41).

20. Are any samples seeded? all
Which?
What is te seed source? new grab of Effluent - settle during morning
Primary effluent or settled raw wastewater is the preferred seed. Secondary treated sources can be used for inhibited tests (SM p528, #5d: SSM p41).

How much seed is added to each sample? 30ml to 6L
Adequate seed should be used to cause a BOD uptake of 0.6 to 1.0 mg/L due to seed in the sample (SM p529, #5d).

How is the BOD of the seed determined? seed blank
Dilutions should be set up to allow the BOD of the seed to be determined just as the BOD of a sample is determined. This is called the seed control (SM p529, #5d: SSM p41).

21. What is the incubator temperature? 20°C
The incubator should be kept at 20 +/- 1 degree C (SM p531, #5i: SSM p40, #3).

How is incubator temperature monitored? ✓
A thermometer in a water bath should be kept in the incubator on the same shelf as the BODs are incubated.

How frequently is the temperature checked? log - daily
The temperature should be checked daily during the test. A temperature log on the incubator door is recommended.

How often must the incubator temperature be adjusted? seldom
Adjustment should be infrequent. If frequent adjustments (every 2 weeks or more often) are required the incubator should be repaired.

Is the incubator dark during the test period? yes
Assure the switch that turns off the interior light is functioning.

22. Are water seals maintained on the bottles during incubation? ✓
Water seals should be maintained to prevent leakage of air during the incubation period (SM p531, #5i: SSM p40, #4).

23. Is the method of calculation correct? seed control to be used

Check to assure that no correction is made for any DO depletion in the blank and that the seed correction is made using seed control data.

Standard Method calculations are (SM p531, #6):

for unseeded samples;

$$BOD\ (mg/L) = \frac{D1 - D2}{P}$$

for seeded samples;

$$BOD\ (mg/L) = \frac{(D1 - D2) - (B1 - B2)f}{P}$$

Where:
- D1 = DO of the diluted sample before incubation (mg/L)
- D2 = DO of diluted sample after incubation period (mg/L)
- P = decimal volumetric fraction of sample used
- B1 = DO of seed control before incubation (mg/L)
- B2 = DO of seed control after incubation (mg/L)

$$f = \frac{\text{amount of seed in bottle D1 (mL)}}{\text{amount of seed in bottle B1 (mL)}}$$

Total Suspended Solids Test Review

Preparation

1. What reference is used for the TSS test? Std methods

2. What type of filter paper is used? purchase next time
   Std. Mthds. approved papers are: Whatman 934AH (Reeve Angel), Gelman A/E, and Millipore AP-40 (SM p95,footnote: SSM p23)

3. What is the drying oven temperature? 103-105
   The temperature should be 103-105 degrees C (SM p96, #3a: SSM p23).

4. Are any volatile suspended solids tests run? yes
   If yes--What is the muffle furnance temperature? ✓
   The temperature should be 550+/- 50 degrees C (SM p98, #3: SSM p23).

5. What type of filtering apparatus is used?
   Gooch crucibles or a membrane filter apparatus should be used (SM p95, #2b: SSM p23). modified gooch

6. How are the filters pre-washed prior to use? ✓
   The filters should be rinsed 3 times with distilled water (SM p23, #2: SSM p23, #2).

   Are the rough or smooth sides of the filters up? ✓
   The rough side should be up (SM p96, #3a: SSM p23, #1)

   How long are the filters dried? 24 hrs
   The filters should be dried for at least one hour in the oven. An additional 20 minutes of drying in the furnance is required if volatile solids are to be tested (Ibid).

   How are the filters stored prior to use? ✓
   The filters should be stored in a dessicator (Ibid).

7. How is the effectiveness of the dessicant checked? ✓
   All or a portion of the dessicant should have an indicator to assure effectiveness.

Test Procedure

8. In what is the test volume of sample measured? 100ml
   The sample should be measured with a wide tipped pipette or a graduated cylinder.

9. Is the filter seated with distilled water? - should do
   The filter should be seated with distilled water prior to the test to avoid leakage along the filter sides (SM p97, #3c).

10. Is the entire measured volume always filtered? ✓
The entire volume should always be filtered to allow the measuring vessel to be properly rinsed (SM p97, #3c: SSM p24, #4).

11. What are the average and minimum volumes filtered?

	Volume	
	Minimum	Average
Influent		~100
Effluent		300-400

12. How long does it take to filter the samples?

	Time
Influent	
Effluent	fast

13. How long is filtering attempted before deciding that a filter is clogged? ✓
Prolonged filtering can cause high results due to dissolved solids being caught in the filter (SM p96, #1b). We usually advise a five minute filtering maximum.

14. What do you do when a filter becomes clogged? ✓
The filter should be discarded and a smaller volume of sample should be used with a new filter.

15. How are the filter funnel and measuring device rinsed onto the filter following sample addition? ✓
Rinse 3x's with approximately 10 mLs of distilled water each time (? ?).

16. How long is the sample dried? 1½-2 hrs
The sample should be dried at least one hour for the TSS test and 20 minutes for the volatile test (SM p97, #3c; p98, #3: SSM p24, #4). Excessive drying times (such as overnight) should be avoided.

17. Is the filter thoroughly cooled in a dessicator prior to weighing? yes
The filter must be cooled to avoid drafts due to thermal differences when weighing (SM p97, #3c: SSM p97 #3c).

18. How frequently is the drying cycle repeated to assure constant filter weight has ben reached (weight loss <0.5 mg or 4%, whichever is less: SM p97, #3c)? occassional
We recommend that this be done at least once every 2 months.

19. Do calculations appear reasonable? ✓
Standard Methods calculation (SM p97, #3c).

$$\text{mg/L TSS} = \frac{(A - B) \times 1000}{\text{sample volume (mL)}}$$

where: A= weight of filter + dried residue (mg)
B= weight of filter (mg)

Fecal Coliform Test Review

1. Is the Membrane Filtration (MF) or Most Probable Number (MPN) technique used?
This review is for the MF technique.

2. Are sterile techniques used? ✓

3. How is equipment sterilizated? autoclave
Items should be either purchased sterilized or be sterilized. Steam sterilization, 121 degrees C for 15 to 30 minutes (15 psi); dry heat, 1-2 hours at 170 degrees C; or ultraviolet light for 2-3 minutes can be used. See Standard Methods for instruclions for specific items (SSM p67-68).

4. How is sterilization preserved prior to item use? leave in autoclave
Wrapping the items in kraft paper or foil before they are sterilized protects them from contamination (Ibid.). ✓

5. How are the following items sterilized?

	Purchased Sterile	Sterilized at Plant
Collection bottles		✓
Phosphate buffer		✓
Media	✓	
Media pads	✓	
Petri dishes	✓	
Filter apparatus		✓
Filters	✓	
Pipettes		
Measuring cylinder	100ml	
Used petri dishes		

6. How are samples dechlorinated at the time of collection? ✓
Sodium thiosulfate (1 mL of 1% solution per 120 mLs (4 ounces) of sample to be collected) should be added to the collection bottle prior to sterilization (SM p856, #2: SSM p68, sampling).

7. Is phosphate buffer made specifically for this test? ✓
Use phosphate buffer made specifically for this test. The phosphate buffer for the BOD test should not be used for the coliform test (SM p855, #12: SSM p66).

8. What kind of media is used? ✓
M-FC media should be used (SM p896, SSM p66).

9. Is the media mixed or purchased in ampoules?
Ampoules are less expensive and more convient for under 50 tests per day (SSM p65, bottom).

10. How is the media stored? 2-3 months
The media should be refrigerated (SM p897, #1a: SSM p66, #5).

11. How long is the media stored?
Mixed media ahould be stored no longer than 96 hours (SM p897, #1a: SSM p66, #5). Ampoules will usually keep from 3-6 months -- read ampoule directions for specific instructions.

12. Is the work bench disinfected before and after testing? ✓
This is a necessary sanitazation procedure (SM p831, #1f).

13. Are forceps dipped in alcohol and flamed prior to use? ✓
Dipping in alcohol and flaming are necessary to sterilize the forceps (SM p889, #1: SSM p73, #4).

14. Is sample bottle thoruoghly shaken before the test volume is removed? The sample should be mixed thoroughly (SSM p73, #5). ✓

15. Are special procedures followed when less than 20 mLs of sample is to be filtered? never
10-30 mLs of sterile phosphate buffer should be put on the filter. The sample should be put into the buffer water and swirled, then the vacuum should be turned on. More even organism distribution is attained using this technique (SM p890, #5a: SSM P73, #5).

16. Are special procedures followed when less than 1 mL of sample is to be filtered? never
Sample dilution is necessary prior to filtration when <1 mL is to be tested (SM p864, #2c: SSM p69).

17. Is the filter apparatus rinsed with phosphate buffer after sample filtration? ✓
Three 20-30 mL rinses of the filter apparatus are recommended (SM p891, #5b: SSM p75, #7).

18. How soon after sample filtration is incubation begun? right away
Incubation should begin within 20-30 minutes (SM p897, #2d: SSM p77, #10 note).

19. What is the incubation temperature? ✓
44.5 +/- 0.2 degrees C (SM p897, #2d: SSM p75, #9).

20. How long are the filters incubated? ✓
24 +/- 2 hours (Ibid.).

21. How soon after incubation is complete are the plate counts made? ✓
The counts should be made within 20 minutes after incubation is complete to avoid colony color fading (SSM p77, FC).

22. What color colonies are counted? ✓
The fecal coliform colonies vary from light to dark blue (SM p897, #2e: SSM p78).

23. What magnification is used for counting? ✓
10-15 power magnification is recommended (SM p898, #2e: SSM p78).

24. How many colonies blue colonies are usually counted on a plate?
Valid plate counts are between 20 and 60 colonies (SM p897, #2a: SSM p78).

25. How many total colonies are usually on a plate?
The plate should have <200 total colonies to avoid inhabition due to crowding (SM p893, #6a: SSM p63, top).

26. When calculating results, how are plates with <20 or >60 colonies considered when plates exist with between 20 and 60 colonies?
In this case the plates with <20 or >60 colonies should not be used for calculations (SM p898, #3: SSM p78, C&R).

27. When calculating results how are results expressed if all plates have < 20 or > 60 colonies?
Results should be identified as estimated.
The exception is when water quality is good and <20 colonies grow. In this case the lower limit can be ignored (SM p893, #6a: SSM p78, C&R).

28. How are results calculated?
Standard Methods procedure is (SM p893, #6a: SSM p79):

$$\text{Fecal coliforms/100 mL} = \frac{\text{\# of fecal coliform colonies counted}}{\text{sample size (mL)}} \times 100$$