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BIOMONITORING REPORT FOR FY 88

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INTRODUCTION

Ecology received an EPA 104(b)(3) grant in 1986 to support development and implementation of a biomonitoring program through the NPDES permit system. Biomonitoring is part of Ecology's toxics control strategy that combines chemical and biological monitoring. The main objective of this two year study was to develop expertise and experience in performing bioassays and interpreting their results. The grant funded first-year hiring of additional field and laboratory staff in the Environmental Investigations and Laboratory Services (EILS) Program. Contract laboratory services for bioassays were also provided during the first year.

This report documents the second year of biomonitoring testing and evaluation for the two year grant period. Testing of marine species for both industrial and municipal discharges into Puget Sound was emphasized. A previous report described results and conclusions from the first year (Bernhardt, 1988).

METHODS

Thirteen bioassay organisms were tested during eleven Class II inspections conducted by the EILS Compliance Monitoring Section (Table 1). All inspections, except one, included both effluent monitoring and outfall near-field sediment collection with bioassays and companion chemical analyses. The exception was sediment samples in the Puyallup River, where sediment deposition did not occur in the vicinity of the Puyallup wastewater treatment plant (WTP) outfall.

General bioassay information for each inspection, including test descriptions, endpoints, references, and the laboratories used are listed in Table 2. Further details on effluent volumes and sampling locations, etc. can be found in the individual Class II inspection reports listed in the Appendix.

Effluent

Sampling for chemical parameters consisted of forty-eight grabs at thirty minute intervals for a 24-hour composite. All equipment used, including ISCO portable samplers, lines, and containers, were cleaned following appropriate sampling protocols (Huntamer, 1986). Bioassay sample collection consisted of three grabs composited over twenty-four hours. This method was necessary due to the large sample volume needed, especially the 10-15 gallons for the trout test. Two exceptions are noted. For Weyerhaeuser at Everett, effluent samples were collected during their normal four hour discharge period, and the bioassays run on a combination of ISCO composite and grab composited samples. Secondly, at Pennwalt the bioassay samples were taken directly from the ISCO sample because of the relatively small volume needed for those tests. All samples were placed on ice immediately following collection and were delivered to Ecology's Manchester Laboratory within 24 hours.

Table 1. List of Ecology Class II biomonitoring inspections for FY '88: 1988 Biomonitoring Report.

		Acute					Chronic					Mutagenicity	Sediment	
Inspection	Dates of Field Work	Rainbow Trout	Micro-tox	Daphnia pulex	Mysid Shrimp	Fathead Minnow	Daphnia magna 7-day	Cerio-daphnia	Pacific Oyster	Bay Mussel	Echino-derm	Ames Test	Hyal-lela	Rhepox-inius
Bellingham WTP	8/25-26/87	X	X						X					X
Pt. Townsend Paper Co.	12/1-2/87	X	X						X			X		X
Puyallup WTP	1/19-20/88	X	X					X						
Bremerton WTP	1/25-27/88	X	X					X						X
Ferndale WTP	2/22-24/88	X	X					X					X	
Pennwalt	4/5-6/88		X								X			X
Weyco, Everett	4/18-20/88	X	X		X					X	X	X		X
Kalama Chemical	5/2-4/88	X	X				X	X					X	
Weyco, Cosmopolis	5/23-25/88	X		X	X				X			X		X
ITT-Rayonier, Hoq	5/23-25/88	X		X	X				X			X		X
Texaco, Anacortes	6/20-22/88	X		X		X			X		X			X
		10	8	3	3	1	1	4	5	1	3	4	2	8

Table 2. Bioassay test information: 1988 Biomonitoring Report.

Bioassay	Description	Endpoint(s)	Ref.	Laboratory	Inspection(s)
Rainbow Trout (Oncorhyncus mykiss, formerly Salmo gairdneri)	96 hour survival, static, acute, juvenile fish.	% mortality	(1)	Ecology E.V.S.(a)	Bellingham, PTPC, Bremerton, Ferndale, Weyco(both), Kalama, ITT, Texaco. Puyallup
Microtox (Photobacterium phosphoreum)	decreased light production of luminescent marine bacterium	EC50	(2)	Biochem(b) Ecology	Bellingham PTPC, Puyallup, Bremerton, Ferndale, Pennwalt, Weyco-Ev., Kalama.
Daphnia pulex	48 hr., acute, survival	% mortality	(3)	Ecology	Weyco-Cos., ITT, Texaco.
Ceriodaphnia dubia	10 day, chronic, survival and reproduction.	NOEC, LOEC	(4)	Ecology E.V.S.	Kalama. Bremerton, Ferndale, Puyallup
Daphnia magna	7 day, chronic, survival and reproduction.	NOEC, LOEC	(5)	Ecology	Kalama
Pacific oyster (Crassostrea gigas)	48 hour oyster larvae mortality and/or abnormality.	LC50, EC50	(6)	E.V.S.(b)	Bellingham, PTPC, Weyco, ITT, Texaco.
Bay Mussel (Mytilus edulis)	same as oyster	LC50, EC50	(6)	E.V.S.	Weyco- Everett.
Echinoderm: Purple sea urchin- Strongylocentrotus purpuratus	Fertilization inhibition test, ~ 2 hours. Choice of sand dollar or green or purple sea urchin: depends on seasonal spawning cycle.	EC50	(7)	Ecology	Pennwalt, Weyco-Ev.
Sand dollar- Dendraster excentricus			(7)	Ecology	Texaco.
Mysid Shrimp (Mysidopsis bahia)	96-hour survival.	LC50	(3)	E.V.S	Weyco(both), ITT.
Fathead Minnow (Pimephales promelas)	48 hr. survival of larvae.	% mortality	(3)	EA(c)	Texaco.
Ames Test	mutagenic activity test with Salmonella typhimurium	mutagenic response	(8) (9)	BCCRC(d) SRI Int.(e)	PTPC. Weyco(both), ITT.
Hyallela azteca	freshwater amphipod survival, 10 days.	% survival	(10)	Ecology	Ferndale, Kalama
Rhepoxinius abronius	marine benthic amphipod, survival, 10 days	% survival	(11)	E.V.S. Ecology	Bellingham, PTPC, Bremerton, Weyco-both, ITT, Texaco. Pennwalt.

Table 2 continued

1- Dept. of Ecology "Static Acute Fish Toxicity Test", Biological Testing Methods, July 1981 revision. DOE 80-12.

2- Microtox System Operating Manual by Beckman. Microbics Corporation, Carlsbad, Ca.

3- EPA (1985) "Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms." EPA/600/4-85/013.

4- EPA (1985) "Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms." EPA/600/4-85/014.

5- EPA, 1987. "A Short-Term Chronic Toxicity Test Using Daphnia magna." EPA Technical Report. EPA/600/D-87/080, March 1987.

6- ASTM, 1986. Standard practice for conducting static acute toxicity tests with larvae of four species of bivalve mollusks. pp. 368-384. In: Annual Book of ASTM Standards, Water and Environmental Technology, Volume 11.04. American Society for Testing and Materials, Philadel. Pa.

7- Dinnel, P.A., et.al, 1987. Improved Methodology for a Sea Urchin Sperm Cell Bioassay for Marine Waters. Arch. Environ. Contam. Toxicol., 16, 23-32.

8- Ames, B.N., J.McCann, and F. Yamasaki, 1975. Methods for Detecting Carcinogens and Mutagens with the Salmonella/mammalian Microsome Mutagenicity Test. Mutat. Res. 31:347.

9- Maron, D.M., and B.N. Ames, 1983. Revised Methods for the Salmonella Mutagenicity Test. Mutat. Res. 113, 173-215.

10-Nebeker,A.V., et al., 1984. Biological Methods for Determining Toxicity of Contaminated Freshwater Sediments to Invertebrates. Env. Tox. and Chemistry, vol.3.

11-Tetra Tech, Inc. 1986. Recommended Protocols for Conducting Laboratory Bioassays on Puget Sound Sediments. EPA, Seattle, Wa.

a- E.V.S. Consultants, Seattle, Wa.

b- BioChem Environmental Services, Inc., Seattle, Wa.

c- EA Engineering, Science, and Technology, Inc., Lafayette, Ca.

d- British Columbia Cancer Research Centre, Vancouver, B.C.

e- SRI International, Menlo Park, Ca.

EC50 - the `effective concentration' at which half of the test organisms are affected by the response of interest.

LC50 - the concentration of effluent that causes mortality to half of the test organisms.

NOEC - the concentration of an effluent which produces no statistically significant response by the test organism- a safe concentration below which no impact is expected to occur.

LOEC - the lowest observable effect concentration that is shown to cause a statistically significant response by the test organism.

Species selection was based on several criteria. First, freshwater species were used for freshwater discharges to freshwater receiving bodies. For marine receiving waters, combinations of freshwater and saltwater organisms were usually assayed. An effort was made to try various combinations for comparison purposes. One discharger, Pennwalt, discharges a saltwater effluent. In this case, only saltwater organisms were used.

Sediment

Sediment collection consisted of two or more grab composites using a 0.1m2 van Veen sampler, except for Ferndale, where a petite Ponar sampler was used. The top 2 cm surface layer from each grab was pooled, homogenized, and split into subsamples, following Puget Sound Protocols (Tetra Tech, 1986a). The exception was VOAs, which were taken from a single grab. Samples were immediately placed on ice and delivered within 24 hours to Manchester Laboratory. All equipment was cleaned prior to use following appropriate protocols (Tetra Tech, 1986a).

The marine amphipod *Rhepoxinius abronius* was used for all marine sediments. *Hyallela azteca*, a freshwater amphipod, was used on the two freshwater sediments.

EFFLUENT RESULTS

A summary of effluent bioassay results are shown in Table 3. A review of the eleven inspections follows.

1. Bellingham WTP: A wide range of toxicities were noted. No acute toxicity was noted by the trout test at 65 percent effluent; moderate amount by Microtox; and quite significant chronic toxicity in the oyster larvae test. Possible causative agents were ammonia, chlorine, and/or several heavy metals.

2. Port Townsend Paper Company: No toxicity was indicated by trout at 65 percent effluent or Microtox, but quite significant chronic toxicity by the oyster larvae test. No mutagenic activity was indicated by the Ames Test. Chemical analyses indicated somewhat elevated levels of cyanide, mercury, and copper.

3. Puyallup WTP: No toxicity was shown in the trout test at 100 percent effluent, slight toxicity from *Ceriodaphnia*, but high toxicity in Microtox. Chlorine and copper were noted as being high.

4. Bremerton WTP: No trout toxicity was exhibited at 100 percent effluent, and a small amount from Microtox and *Ceriodaphnia*. Copper, mercury, ammonia, and chlorine may have contributed.

5. Ferndale WTP: Toxicity was high in all tests: trout, Microtox, and *Ceriodaphnia*. Chlorine was shown to contribute part of the toxicity, as toxicity decreased in Microtox and the trout test after dechlorination. Lead, mercury, and cyanide were elevated.

Table 3. Summary of effluent bioassay results: 1988 Biomonitoring Report.

	Acute					Chronic							
	Rainbow Trout	Micro-tox	Daphnia pulex	Mysid Shrimp	Fathead Minnow	Daphnia magna 7-day	Cerio-daphnia	Pacific Oyster		Bay Mussel		Echino-derm	Ames Test
	% survival	EC50	% survival	LC50	% survival	NOEC/LOEC	NOEC/LOEC	mortal. LC50	abnorm. EC50	mortal. LC50	abnorm. EC50	EC50	response
Bellingham WTP	100	34.7%						8.8%	3.5%				
Pt. Townsend Paper Co.	100	>100%						**	3.0%				none
Puyallup WTP	100*	11.4%					50%/100%						
Bremerton WTP	100*	70.1%					50%/100%(-)						
Ferndale WTP	0*	11.7%					13%/25%						
Pennwalt		>100%										20%(+)	
Weyco, Everett	67	58.9%		>100%						0.5%	0.5%	2.4%	none
Kalama Chemical	0*	25.9%				10%/30%	10%/30%(-)						
Weyco, Cosmopolis	100		75	58%				**	0.3%				none
ITT-Rayonier, Hoq.	100		100	>100%				**	0.2%				none
Texaco, Anacortes	100		80		100							>100%	

* - trout tests conducted at 100% effluent: all others at 65% effluent.
** - LC50 could not be calculated because the highest concentrations had <50% lethality.
(+) - results inconclusive due to wide confidence limits.
(-) - controls averaged less than 15 offspring/adult: thus did not meet validation requirements.

6. Pennwalt Corp.: Two saltwater species were used because Pennwalt's discharge is saltwater. Microtox showed no toxicity. Chronic toxicity was fairly high in the echinoderm test, but excessively high statistical confidence limits rendered this result inconclusive. Effluent silver concentration was several orders of magnitude above EPA's water quality criterion.

7. Weyerhaeuser, Everett: A wide range of toxicity was indicated by the species with this effluent. The mysid shrimp test indicated no acute toxicity, but a relatively high amount was found in the trout test. Microtox indicated moderate toxicity, but very high chronic toxicity was found by the bay mussel and echinoderm tests. No mutagenic activity was indicated in the Ames test. Mill personnel identified a surfactant as the causative agent. Only very small amounts of other chemicals were identified.

8. Kalama Chemical: All species tested (*Ceriodaphnia*, *D. magna*, Microtox) showed a significant toxic response to Kalama's effluent. *Ceriodaphnia* and the chronic *Daphnia magna* test responded nearly identically. Chemical tests did not indicate a clear causative agent, although cyanide and ammonia were possibilities.

9. Weyerhaeuser, Cosmopolis: In acute tests, trout showed no response to 65 percent effluent, *Daphnia pulex* had 25 percent mortality, and the EC50 for mysid shrimp was 58 percent effluent. Pacific oyster larvae indicated very high chronic toxicity. No mutagenic activity was revealed by the Ames test. Fluoranthene was found at about the chronic toxicity criterion.

10. ITT Rayonier, Hoquiam: Rainbow trout, *Daphnia pulex*, and mysids showed no acute toxicity to ITT's effluent. However, a very high chronic effect occurred in the oyster larvae test. Chromium slightly exceeded the water quality criterion.

11. Texaco, Anacortes: Very little effect was seen in the three acute tests (trout, *D. pulex*, and fathead minnow) or the Echinoderm chronic test. No chemical parameters were detected at levels believed to cause toxicity.

EFFLUENT DISCUSSION

Several points are noted by this set of bioassay results. First, biological toxicity was indicated in many cases where no toxicity was expected, either due to the type of discharge or prior chemical analyses. However, chemical testing often did not reveal the cause(s) of the toxicity. Of the eleven sets of bioassays, some level of chronic and/or acute toxicity was found in all but three (Bremerton and Texaco, and inconclusive at Pennwalt). Of these eight, possible/probable causative agent(s) were identified by chemical testing in four, or one-half, of the cases. Another observation is that the test organisms frequently differed in their sensitivities. For a particular effluent, the level of toxic response varied greatly in many cases. Also, most organisms tested showed significant responses to some effluents but not others.

An objective of this study was to examine the issue of relative sensitivities between specific tests. Bioassay relationships for FY '88 results were compared in Table 4. For this analysis, the tests were ranked by their relative degree of toxicity shown for each effluent. Not surprisingly, tests with a chronic component showed the greatest overall sensitivity. Several observations are noted. The bivalve larvae tests (oyster and mussel) were the most sensitive for every effluent tested. Also, *Daphnia magna*, the one time used, got essentially identical results with *Ceriodaphnia*. This test was piloted as a possible substitute for *Ceriodaphnia*, which has been criticized for having several chronic problems, including inadequate control reproduction and being labor-intensive. This comparison table, while interesting, used a fairly limited data base and the ranking scheme was somewhat arbitrary. Therefore, no hard-and-fast rules should be implied by this comparison.

Bernhardt (1988) raised several points in the first biomonitoring summary report. Among these was the impact of chlorine and ammonia on bioassay results. In this set of bioassays, chlorine was a potential toxicant in many cases. On several occasions, two bioassays were conducted: one on the whole effluent, and the second after chlorine neutralization with sodium thiosulfate. This proved to be an effective way to assess the role of chlorine in the overall toxicity of the sample. It also is a good idea to have the chlorine checked at the lab as the residual may have changed significantly from field conditions. Interpretation of the bioassay results could then be based on actual chlorine concentration at the start of the test. Unfortunately, a similarly convenient method for testing the role of ammonia on bioassay results is not available. Therefore, existing data on ammonia toxicity, either water quality criteria or species-specific data, were used to predict ammonia's possible role in any observed toxicity.

The many advantages of bioassays for detecting toxicity have been well documented in the literature. Cumulative effects of complex discharges are measured. Also, the bioavailability of pollutants are assessed, and interactions of the effluent constituents are considered.

Also documented is that bioassays have certain limitations. For the sampling performed during these inspections, questions not addressed include: Does toxicity change over time? Does the causative agent(s) differ over time?

Establishing a cause-and-effect relationship between bioassay response and chemical analyses can be difficult. The typical 126-item priority pollutant scan covers only a fraction of potential toxicity-causing compounds. EPA has stated that "limiting the search to these 126 compounds will result in failure to identify the cause of toxicity in most cases" (Mount *et al.*, 1988). In addition to looking for the 'wrong' compounds, the cause(s) of toxicity may be found at concentrations below the detection limits of existing lab equipment. Also, we do not always know what type of compound to look for and, therefore, cannot specify the appropriate type of analysis. Effluents can be a very complicated 'soup': a GC/MS chromatogram of these mixtures may have overlapping peaks that can hide smaller peaks. "Trying to pinpoint the cause of toxicity in such a complex mixture is likely to fail because this approach does not include matrix effects and toxicant bioavailability" (Mount *et al.*, 1988).

Table 4. Ranking(a) of effluent bioassays by relative sensitivity during Ecology Class II inspections: 1988 Biomonitoring Report.

Inspection	Acute: Rainbow Trout	Acute: Microtox	Acute: Daphnia pulex	Acute: Mysid Shrimp	Acute: Fathead Minnow	Chronic: Daphnia magna 7-day	Chronic: Ceriodaphnia	Chronic: Pacific Oyster	Chronic: Bay Mussel	Chronic: Echinoderm
Bellingham WTP	5	3						1		
Pt. Townsend Paper Co.	4	4						1		
Puyallup WTP	5	1					3			
Bremerton WTP	5	3					1			
Ferndale WTP	1	3					5			
Pennwalt		4								2
Weyco, Everett	4	3		5					1	2
Kalama Chemical	1	5				3	3			
Weyco, Cosmopolis	5		3.67	2.33				1		
ITT-Rayonier, Hoq.	3.67		3.67	3.67				1		
Texaco, Anacortes	4		2		4			1		4
Total # tests:	10	8	3	3	1	1	4	5	1	3
Total score:	37.7	26	9.3	11	4	3	12	5	1	8
Average ranking:	3.8	3.3	3.1	3.7	4	3	3	1	1	2.7
Relative Sensitivity(b):	7	5	4	6	8	3	3	1	1	2

(a): Scores based on 1 through 5: 1=most sensitive, 5=least sensitive.
Scores for four tests: #1=1, #2=2.33, #3=3.67, #4=5.
Scores for three tests: #1=1, #2=3, #3=5.
Scores for two tests: #1=2, #2=4.

In case of a tie, tied tests given average of test rankings: other tests given regular ranking.

(b): on scale of 1 to 8: 1=most sensitive, 8=least sensitive.

An ambitious biomonitoring schedule is planned for compliance inspections in FY '89, as listed in Table 5. Several comparisons between the chronic *Daphnia magna* test and *Ceriodaphnia* are scheduled. The chronic, 7-day fathead minnow test is also being used several times in late FY '89 and 90. Also, the sediment Microtox test has been piloted on several inspections. These results should add to our data base in the future. Included in this set are about four sets of bioassays made available through an EPA contract lab in San Diego in late FY '89 and early FY '90.

In summary, toxicity was found in effluents where it was not expected, and vice versa. Possible cause(s) were suggested by chemical testing for about one-half of the effluents, but tentatively identified for only one effluent. Also, predicting which organisms will be sensitive to a particular effluent did not appear to be straight-forward.

SEDIMENT RESULTS

Sediment bioassay results are listed in Table 6. In no case was significant sediment toxicity found in relation to the field control sample. Outfall near-field sediment toxicity, as compared to the laboratory control sample, occurred in four of the ten inspections. Each set of results are now briefly discussed. For further information, consult the individual inspection reports (Appendix).

1. Bellingham WTP: Both outfall samples and the field control sample exhibited toxicity as compared to the lab control sample. Therefore, the toxicity was not necessarily effluent-related. Chromium and nickel were reported as above the 90th percentile for Puget Sound non-reference sediments (Tetra Tech, 1986b).

2. Port Townsend Paper Company: Both outfall samples (but not the field control) indicated toxicity when compared to the laboratory control sample. Chemical analyses did not indicate a causative agent. Low levels of some PNAs were found.

3. Bremerton WTP: Significant mortality was not noted, although high levels of some compounds were found. In particular, phthalates were high, along with nickel, chromium, and some PCBs.

4. Ferndale WTP: Results were confusing. The upstream control site had the highest mortality, with the two outfall samples having the least. Nickel was high in all samples. However, nickel was highest in the outfall samples that had the lowest mortality.

5. Pennwalt Corp.: The control sample was designed to be outside the outfall deposition zone, but not to be a 'clean' reference site. Many compounds were detected at high concentrations, including PNAs and pesticides. All three field samples exhibited toxicity compared to the laboratory control.

Table 5. List of Ecology Class II biomonitoring inspections scheduled for FY '89: 1988 Biomonitoring Report.

		Acute					Chronic								
Inspection	Dates of Field Work	Rainbow Trout	Micro-tox	Daphnia pulex	Fathead minnow	Hyallela azteca	Daphnia magna 7-day	Cerio-daphnia	Pacific Oyster	Bay Mussel	Fathead minnow 7-day	Echino-derm	Muta-genicity Ames Test	Sediment Micro tox	Rhepox-inius
Intalco, Ferndale	7/26/88	X		X				X	X			X			X
Ellensburg WTP	8/8/88	X	X	X				X							
Georgia-Pacific,Bell.	8/23/88	X		X	X							X	X		X
Spokane WTP	9/21/88	X	X	X			X								
Okanogan WTP	10/18/88	X		X				X							
Selah WTP	10/26/88		X												
Daiashowa, Port Angeles	11/15/88	X	X	X			X		X					X	
Central Kitsap WTP	11/29/88	X	X				X								
Lilyblad, Tacoma	12/88	X	X												
Scott Paper, Everett	1/31/89	X	X	X					X						
Shell Oil, Anacortes	2/22/89	X	X									X			X
Burlington	3/28/89	X	X				X								
Edmonds WTP	4/18/89	X	X							X		X			X
Boise Cascade, Steilacoom	4/25/89	X	X				X		X			X		X	
British Petroleum	5/9/89	X	X									X		X	X
Tacoma Central WTP	6/27/89	X	X		X				X			X			X
US Oil, Tacoma	6/14/89	X	X			X	X	X			X	X			

6. Weyerhaeuser, Everett: No significant mortality was found in any sample, even though survival was less in the first outfall sample. Low concentrations of several contaminants were found, including phthalates, copper, chromium, some guaiacols and resin acids.

7. Kalama Chemical: Survival decreased near the outfall as compared to the field control, but was not statistically significant, and was no lower than the laboratory control. Cadmium and nickel were slightly elevated.

8. Weyerhaeuser, Cosmopolis: No significant response was noted. Chromium and nickel were somewhat elevated.

9. ITT Rayonier, Hoquiam: The ITT sediment samples did not show an adverse effect. Chromium and nickel exceeded the most stringent proposed Apparent Effects Threshold (AET) values, and were very similar to Weyco, Cosmopolis results.

10. Texaco, Anacortes: A significant difference in survival was seen in Texaco's #1 outfall sample when compared to the laboratory control. No chemical parameters were detected at high concentrations, although chromium and nickel were slightly elevated.

SEDIMENT DISCUSSION

Results of the marine and freshwater sediment bioassays are listed in Table 6. *Rhepoxinius* passed the quality assurance criterion of greater than or equal to 90 percent survival in the lab control sediment for all test lots. In the ten tests, two laboratory problems occurred, a technical error and an equipment failure. Table 7 lists chemical concentration excursions exceeding proposed AET values. Therefore, depending on which set of AETs are used, these sediments would be classified as failing sediment AET standards. However, the test sediments all passed the amphipod test. To be reinstated as passing the standards, the sediments would have to also pass the bivalve larvae test and one of the following tests: benthic infaunal abundance, Microtox, or a chronic effects test to be named later.

AETs successfully predicted *Rhepoxinius* results in six of eight test sets. At Pennwalt, in Commencement Bay, the amphipods survived even though arsenic, benzofluoranthenes, and total PCBs exceeded the amphipod AETs. These are listed in Table 8. In the other anomoly, the Bellingham samples had greater than 25 percent absolute mortality at concentrations less than AET values. DeWitt, *et al.* (1988) have shown that elevated amphipod mortalities can occur due to effects of a high percentage of fine-grained material in a sample. At Bellingham, grain size was not run. However, existing data suggests that the percent of fine material may have been very high. Elevated mortality due to percent fines has been suggested in previous samples from Bellingham Bay (Barrick, *et al.*, 1988).

Puget Sound Protocols (Tetra Tech, 1986a) do not require reburial as part of the test procedure. For the data sets listed, percent reburial was insignificant in all cases. Also, the confidence limits for avoidance (emergence from the sediment) exceeded the observed avoidance value. Thus, the usefulness of these two parameters are unknown.

Table 6. Summary of sediment bioassay results: 1988 Biomonitoring Report

Rhepoxinius:	Outfall Sample #1				Outfall Sample #2				Field Control				Laboratory Control		
	Survival(1)	Avoidance(2)	% Reburial(3)	% Fines	Survival	Avoidance	% Reburial	% Fines	Survival	Avoidance	% Reburial	% Fines	Survival	Avoidance	% Reburial
Bellingham WTP	14.4+/-2.7*	0.2+/-0.5	-	-	13.0+/-2.0*	0.2+/-0.5	-	-	12.8+/-3.0*	0.1+/-0.5	-	-	19.0+/-.7	0.4+/-0.5	-
Pt. Townsend Paper Co.	16.4+/-2.6*	1.1+/-1.6	98.8	49	16.8+/-1.8*	0.4+/-0.6	98.8	74	18.6+/-0.5	0.4+/-0.7	100	15	19.8+/-0.4	1.1+/-1.0	98
Bremerton WTP	18.0+/-0.8	0.02+/-0.1	100	74	19.2+/-0.8	0.1+/-0.3	97.9	87	18.8+/-1.8	0.02+/-0.1	97.9	87	19.2+/-0.8	0.8+/-0.6	93.8
Pennwalt	16.2+/-3.2*	**	**	49	17.8+/-1.4*	**	**	97	17.2+/-0.4*	**	**	56	19.8+/-0.4	**	**
Weyco, Everett	17.0+/-3.4	0.6+/-0.8	100	45	19.8+/-0.4	0.3+/-0.4	98.0	1.5	19.2+/-0.8	0.4+/-0.5	98.9	2.2	19.8+/-0.4	0	100
Weyco, Cosmopolis	19.4+/-0.9	0.1+/-0.4	100	21	18.6+/-0.5	0.3+/-0.7	100	58	18.6+/-1.1	0.1+/-0.3	98.9	50	18.8+/-1.6	0.7+/-1.1	100
ITT-Rayonier, Hoq.	18.8+/-1.3	0.3+/-0.5	98.9	82	17.2+/-1.1	0.2+/-0.4	98.9	87	18.6+/-1.1	0.1+/-0.3	98.9	50	18.8+/-1.6	0.7+/-1.1	100
Texaco, Anacortes	17.0+/-1.9*	0.04+/-0.2	***	94.4	17.8+/-1.6	0.3+/-0.5	***	97.4	19.2+/-0.4	0.2+/-0.4	***	98.6	19.6+/-0.5	0.2+/-0.4	***

Hyallela:	Outfall		Downstream		Field Control(upstream)		Laboratory Control
	Survival, %	% Fines	Survival	% Fines	Survival	% Fines	Survival
Ferndale WTP	100	3	90	0.6	77	28	87
Kalama Chemical	83	14.2	90	14.9	93	9.0	83

* - statistically significant difference as compared to the laboratory control sample.
** - data not available due to laboratory equipment problems.
*** - data not available due to laboratory error.

1 - Average of twenty ampmhipods per replicate: five replicates per sample.

2 - Number of amphipods on liquid surface per day, out of twenty.

3 - Number of amphipods able to rebury in clean sediment at end of test period.

Table 7. Sediment priority pollutant results exceeding candidate Puget Sound sediment chemical standards (AET's)*: 1988 Biomonitoring Report.

Parameter	LAET-UTOX(1)	New LAET(2)	ACR NOEC(3)	PSDDA SL(4)	Bellingham WTP #1	Bellingham WTP #2	Bellingham WTP Ctrl.	PTPC #1	PTPC #2	PTPC Ctrl.	Bremerton WTP #1	Bremerton WTP #2	Bremerton WTP Ctrl.	Pennwalt #1	Pennwalt #2	Pennwalt Ctrl.	Weco, Ev. #1	Weco, Ev. #2	Weco, Ev. Ctrl.	Weco, Cos. #1	Weco, Cos. #2	Weco, Cos. Ctrl.	ITT Rayonier #1	ITT Rayonier #2	ITT Rayonier Ctrl.	Texaco #1	Texaco #2	Texaco Ctrl
METALS (mg/kg dw)																												
Arsenic	57	57	57	70										145	127	129												
Cadmium	5.1	5.1	0.96	0.96							1.1	1.3		1.3	1.2	0.8												1.1
Chromium	260	260	27	NA	80	80	77	30.3	31.8	31.4	136	78.8	61	35.8	41.4	28.1	32	13	159	28	29	30	30.7	35	30	30		
Copper	390	390	130	81							218	208	140	381	223	224												
Lead	450	450	66	66							137	121	89	231	138	142												
Mercury	0.59	0.41	0.21	0.21	0.66	0.38	0.46				0.43	0.39	0.37	0.38	0.29	0.29												
Nickel	>140	>140	14	28	98	106	97	32.8	31.1	40.8	61.2	46.9	43	51.3	46.4	44.1	37	17	21	62	58	56	55.4	57.7	56	46	44	31
Silver	>0.56	>0.56	0.61	1.2							1.6	2.7	2.3	0.62	0.22	0.85												
Zinc	410	410	160	160							273	225	160	261	282	233												
ORGANICS (ug/kg dw)																												
LPAH(a)	5200	5200	2400	610										2140	3390	1520												
Fluorene	540	540	360	64										230	260	170												
Phenanthrene	1500	1500	690	320										1400	2700	920												
Anhracene	960	960	1300	130										510	430	430												
HPAH(b)	17000	12000	6900	1800							1950			36,650	34,400	15,090												
Fluoranthene	2500	1700	3000	630							320 J			4300	5800	1200												
Pyrene	3300	2600	1600	430							370 J			4500	6000	2000												
Benz(a)anthra	1600	1300	510	450										3400	2600	970												
Chrysene	2800	1400	920	670										4800	3800	2200												
Benzofluoranthenes	3600	3200	990	800							860 M			14,200	11,600	6800												
Benzo(a)pyrene	1600	1600	360	680										2400	1700	970												
Indeno(1,2,3,-c,d,) pyrene	690	600	260	69										1000	710	450												
Benzo(g,h,i)perylen	720	670	260	540										750	690	500												
1,2-Dichlorobenzene	50	35	11	19																								
Bis(2-ethylhexyl) phthalate 1300	1300	310	3100								4000	5000																
Di-n-octyl phthalat	>420	>420	620	69000										1300	1500	2200												
Phenol	420	420	120	120						320																		
Dibenzofuran	540	540	170	54										160														
Hexachlorobutadiene	11	11	11	29										160														
Total PCB's	1000	130	310	130										6600	3530	590												

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(1) 1988 Lowest Apparent Effects Threshold Value excluding the Microtox value
(2) 1988 Lowest Apparent Effects Threshold Value
(3) Acute to Chronic Ratio No Observable Effects Concentration as reported in Contaminated Sediments Criteria Report, August 1988, PTI Environmental Services, i.e. Highest Apparent Effects Threshold Value, whichever is lower
(4) Puget Sound Dredged Disposal Analysis Screening Level (SL), ie, the 1986 Highest Apparent Effects Threshold value divided by 10. The SL is defined as no lower than mean reference area values and no higher than the 1986 lowest apparent effects threshold value

a - sum of low molecular weight polynuclear aromatic hydrocarbons, i.e. naphthalene, acenaphthalene, acenaphthene, fluorene, phenanthrene, and anthracene.
b- sum of high molecular weight polynuclear aromatic hydrocarbons, i.e. fluoranthene, pyrene, benz(a)anthracene, chrysene, benzofluoranthenes, benzo(a)pyrene, indeno(1,2,3-c,d)pyrene, dibenzo(a,h)anthracene, and benzo(g,h,i)perylene.

* - candidate AET's were compiled by Brett Betts of Ecology's Sediment Management Unit.

Table 8. Cases where Class II inspection results exceeded amphipod AET values: 1988 Biomonitoring Report.

Parameter	Amphipod	Pennwalt		
METALS (mg/kg dw)	AET*	#1	#2	Ctrl.
Arsenic	93	145	127	129
ORGANICS (ug/kg dw)				
Benzofluoranthenes	7,800	14,200	11,600	6,800
Total PCB's	3,100	6,600	3,530	590

* - from PTI, 1988

The purpose of Ecology's draft sediment standards (Ecology 1988) is to define unacceptable contaminant concentrations in Puget Sound sediments. The main tool to do this is AET, or apparent effects threshold: "the sediment concentration of a contaminant above which statistically significant adverse effects for a particular biological indicator are always expected relative to appropriate reference conditions" Barrick, *et al*., 1988). Exceedance of any of these standards (10 metals and 43 organics) causes the sediment to be classified. The standards set forth several biological tests to then confirm this classification. These include three acute tests -- *Rhepoxinius*, bivalve larvae, and Microtox -- plus chronic tests including benthic infaunal abundance and future tests as developed.

The *Rhepoxinius* test must meet several test performance criteria to be valid. First, the bioassay must pass the quality assurance criterion of greater than or equal to 90 percent survival in the laboratory control sediment. The standards then call for comparing reference sample and test sample results. Failure of *Rhepoxinius* is signified by both 1) statistically significant mortality in the test sample as compared to the reference sample, and 2) greater than 25 percent absolute mortality for the test sample.

The reference samples are to be collected from designated areas not yet specified by the Department. Therefore, interpretation of criterion #1 for this data set is not possible since the reference samples were not 'official'. Criterion #2, greater than 25 percent absolute mortality, was only exceeded in one instance. At Bellingham, greater than 25 percent mortality occurred in both test samples and the field control.

Training and familiarity with sediment bioassays has occurred and will continue. For FY '89 and '90 inspections, other sediment tests such as Microtox (saline extraction method) are planned. Official sites will be used for reference sediments as these sites are developed by the Sediment Management Unit and the Sediment Ambient Monitoring personnel.

Potential problems may include identification of official station locations without sophisticated equipment, such as LORAN C, and the expense of collecting reference samples at relatively distant stations.

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APPENDIX

List of Class II inspection reports for FY'88: 1988 Biomonitoring Report.

Reif, D., 1988. Bellingham Post Point Pollution Control Plant Class II Inspection. WA State Dept.of Ecology, Water Quality Investigations Section, Olympia, WA. February 1988.

Reif, D., 1988. Port Townsend Paper Company Class II Inspection. WA State Dept. of Ecology, Environmental Investigations and Laboratory Services Program, Compliance Monitoring Section, Olympia, WA. November 1988.

Hallinan, P., 1988. Puyallup Wastewater Treatment Plant Class II Inspection, January 19-20, 1988. WA State Dept. of Ecology, Compliance Inspections Section, Olympia, WA. May 1988.

Reif, D., 1988. Bremerton Wastewater Treatment Plant Class II Inspection. WA State Dept.of Ecology, Water Quality Investigations Section, Olympia, WA. August 1988.

Ruiz, C. E., 1989. Ferndale Wastewater Treatment Plant Class II Inspection. WA State Dept. of Ecology, Environmental Investigations and Laboratory Services Program, Compliance Monitoring Section, Olympia, WA. February 1989.

Heffner, M., 1989. Pennwalt Class II Inspection Report. WA State Dept. of Ecology, Environmental Investigations and Laboratory Services Program, Compliance Monitoring Section, Olympia, WA. April 1989.

Ruiz, C. E., 1989. Weyerhaeuser, Everett Class II Inspection. WA State Dept. of Ecology, Environmental Investigations and Laboratory Services Program, Compliance Monitoring Section, Olympia, WA. In publication.

Heffner, M., 1989. Kalama Chemical Inc. May 1988 Class II Inspection Report. WA State Dept. of Ecology, Environmental Investigations and Laboratory Services Program, Compliance Monitoring Section, Olympia, WA. In publication.

Hallinan, P., 1989. Weyerhaeuser, Cosmopolis Class II Inspection. WA State Dept. of Ecology, Environmental Investigations and Laboratory Services Program, Compliance Monitoring Section, Olympia, WA. May 1989.

Reif, D., 1989. ITT Rayonier, Hoquiam Class II Inspection. WA State Dept. of Ecology, Environmental Investigations and Laboratory Services Program, Compliance Monitoring Section, Olypmpia, WA. In publication.

Ruiz, C. E., 1989. Texaco, Anacortes Class II Inspection. WA State Dept. of Ecology, Environmental Investigations and Laboratory Services Program, Compliance Monitoring Section, Olympia, WA. In publication.