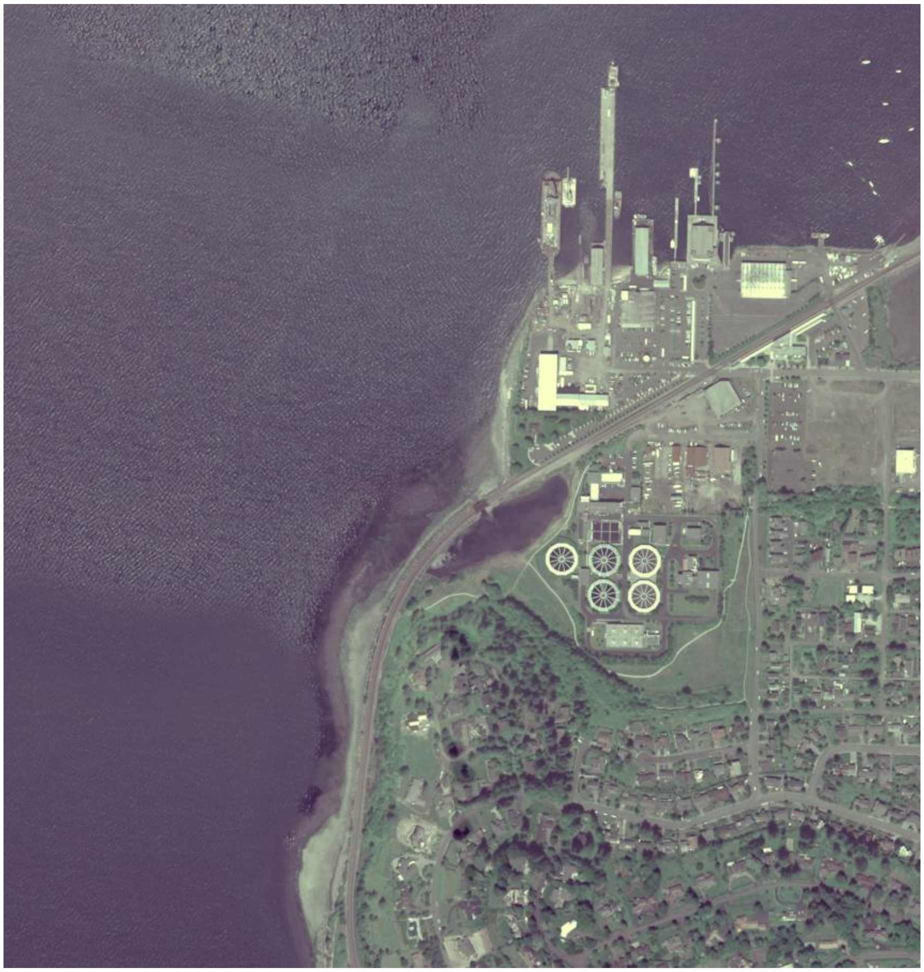
Assessment of Sediment Toxicity near Post Point (Bellingham Bay)



June 2008

Publication No. 08-03-016

DEPARTMENT OF
ECOLOGY
State of Washington

Publication Information

This report is available on the Department of Ecology's website at
www.ecy.wa.gov/biblio/0803016.html

Data for this project are available on Ecology's Environmental Information Management (EIM) website at www.ecy.wa.gov/eim/index.htm. Search User Study ID, PPTox07.

Ecology's Project Tracker Code for this study is 08-033.

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Cover photo: Aerial photograph of the Harris Avenue Shipyard (upper) and Post Point Wastewater Treatment Plant (lower).

Assessment of Sediment Toxicity near Post Point (Bellingham Bay)

by
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Waterbody No. WA 01-0050

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Abstract

Previous studies near 2 Post Point Wastewater Treatment Plant outfalls in Bellingham Bay, Bellingham, Washington, have suggested sediment toxicity related to sulfides. The purpose of this study was to determine if the incidence and severity of toxicity near the outfalls warrants more detailed cleanup investigations.

During 2007, the Washington State Department of Ecology tested surface sediment from 8 locations for toxicity using 4 bioassay protocols. Levels of total sulfides in sediment and porewater were measured in the same samples. Results showed little observable toxicity despite elevated sulfides in both sediment and porewater of some samples. Only the Microtox luminosity test results for 2 samples exceeded the Sediment Quality Standards.

Results indicated a possible dose-response relationship between total sulfides and Microtox toxicity, but different from relationships calculated using previous results. Levels of total sulfides also explained some of the variability in amphipod and larval test toxicity results. Losses of sulfides from porewater appeared to occur during setup procedures and tests themselves more than during sample storage.

As a result of this study, it is recommended that future monitoring of sediment toxicity in the Post Point area be limited. Results also suggest that improved sample handling and toxicity testing protocols should be developed for evaluating areas of high sediment sulfide.

Acknowledgements

The author of this report would like to thank the following people for their contributions to this study:

- Peter Adolphson, Nigel Blakley, Randy Coots, Mark Henderson, Dale Norton, Mary O'Herron, George Onwumere, Daniel Sherratt, Janice Sloan, Joan LeTourneau, and Cindy Cook (Ecology).
- Stuart Magoon and staff (Ecology, Manchester Environmental Laboratory).
- Freeman (Fritz) Anthony (City of Bellingham).
- Sue Dunihoo and staff (Analytical Resources, Inc.).
- Andy Peterson (Squalicum Marina).
- Michele Redmond (Northwest Aquatic Sciences).
- Mike Stoner (Port of Bellingham).

The author also offers thanks to Mr. Richard Caldwell, Dr. Peter Chapman, Mr. Bill Gardiner, Mr. Bryn Phillips, and Dr. Jack Word for valuable discussion and advice about how to assess sediment toxicity due to sulfides.

Introduction

Previous studies

Many studies of sediment quality have been conducted in Bellingham Bay. Most of these have been associated with the former Georgia Pacific pulp mill, commercial waterways, and downtown marinas. There have also been studies of sediment contamination near the Harris Avenue Shipyard and Post Point Wastewater Treatment Plant (WWTP), both in the southern part of the bay (see cover photo).

The Port of Bellingham began investigating contamination near the Harris Avenue Shipyard in the 1990s (GeoEngineers, 1996; RETEC, 1998). Detailed cleanup investigations and a dredging project study followed between 2000 and 2004 (RETEC, 2003; 2004). Results showed sediment toxicity at some sampling locations near the shipyard, but apparently not related to shipyard-derived contaminants. Instead, the toxicity was ascribed to the elevated ammonia and sulfides that were present. These parameters were presumed due to decomposition of organic matter originating from the nearby Post Point WWTP outfall.

The City of Bellingham evaluated sediment toxicity at 9 locations near the Post Point outfalls in 2003 (Anchor, 2004). Two locations close to the secondary outfall contained nearly 5,000 mg/kg total sulfides and showed significant sediment toxicity. The 7 other locations lacked toxicity but contained 240 mg/kg - 4,200 mg/kg total sulfides.

Because of uncertainty about sediment quality in this area, the Washington State Department of Ecology conducted a sediment study in 2004. The study had 2 objectives:

- Determine the spatial distribution of elevated total sulfide concentrations, especially between the Post Point WWTP outfalls and Harris Avenue Shipyard.
- Evaluate the sediments in the study area for compliance with biological criteria in the Sediment Management Standards (SMS; Ecology, 1995).

Results showed sediment toxicity exceeded the cleanup screening level (CSL) at one location and the sediment quality standard (SQS) at 3 other locations. The 4 samples having significant toxicity were from locations nearest the shoreline (Blakley, 2006). There appeared to be a dose-response relationship between Microtox toxicity and sediment sulfide levels. However, there was still insufficient evidence for listing the area as a sediment cleanup site.

The 2007 Study

The current study was intended to determine if sediments near the Post Point WWTP outfalls had significant impacts on biological resources (exhibited toxicity) that warranted a full remedial investigation. Primary objectives included:

- Collect sediment samples from appropriate locations at a time of year when biologically-available sulfides are expected to be maximal.
- Assess toxicity of each sediment sample based on results from 2 acute tests and 2 chronic tests listed in the SMS rule.

The 2007 study also explored improved ways to collect and handle sediment samples to be tested for toxic sulfides. To this end, procedures were designed to collect samples more reflective of *in-situ* conditions. This meant revising field procedures to minimize loss of volatile sulfides and oxidation of hydrogen sulfide. Secondary objectives included:

- Measure the fraction of total sulfides present in porewater.
- Estimate changes in levels of porewater sulfides due to storage and laboratory handling of samples prior to starting toxicity tests.

The study was not designed to positively identify sources of observed toxicity. It was designed to allow analysis of relationships between toxicity and total sulfides.

Methods

Sampling

Navigation and positioning of Ecology's vessel, the *RV Skookum*, was done using procedures described in the Quality Assurance Project Plan. Surface sediment was successfully collected from 8 locations near Post Point outfalls, but often not at the planned locations (Figure 1). Field notes and target and final sampling locations are listed in Appendix B. Sampling highlights include:

- Coordinates for target locations PPTox01 and PPTox02 were difficult to attain due to strong currents and tides.
- Strong winds and 2'-3' seas on the second day made vessel positioning and sampling difficult.
- No sediment was collected at or near target location PPTox04 because cobble or shell material prevented the van Veen sampler from closing properly.
- Construction activities hindered safe sampling at 4 target locations near the secondary outfall (Figures 1 and 2).
- Coordinates for PPTox11 and BBY10 (Blakley, 2006) became alternate target locations for samples PPTox06 and PPTox07, respectively.

As a result of currents, tides, construction, wind, and seas, the 3-meter positioning accuracy required in the QA Project Plan was nearly impossible to achieve. Overall, the average distance between target and final sampling locations was about 17 meters (>50 feet). This was mostly due to the inaccuracy positioning the vessel for locations PPTox01 and PPTox02. Subsequent positioning accuracy was about 9 meters (30 feet). Only in the Carr Inlet reference area, with excellent field conditions, was the final sampling location within the 3 meters of the target.

The collection, handling, and storage of all sediment samples followed the QA Project Plan (Gries, 2007). Ecology field staff:

- Used a double van Veen grab (0.2m^2) to sample surface sediments (Figure 1b and 1c).
- Collected sediment from the top 10 cm of grab samples containing 11-17 cm of sediment.
- Recorded field conditions and observations of sediment grabs.
- Handled all sediment samples using pre-cleaned stainless steel equipment, and followed decontamination procedures.
- Placed unmixed subsamples in pre-labeled containers for analysis of total sulfides.
- Mixed remaining sediment so as to minimize changes to levels of total sulfides.
- Placed subsamples for analysis of other conventional parameters and sediment toxicity into pre-cleaned and pre-labeled containers.

- Placed samples on ice (4^oC) while stored in the field, at Ecology offices, during transfer/shipping, at Manchester Environmental Laboratory, and at contractor facilities.
- Transferred subsamples for analysis of total solids, grain size, total organic carbon, and total sulfides (in whole sediment and porewater) within 48 hours of sample collection.
- Transferred subsamples for analysis of sediment toxicity within 1 week of sample collection.
- Maintained full chain of custody.

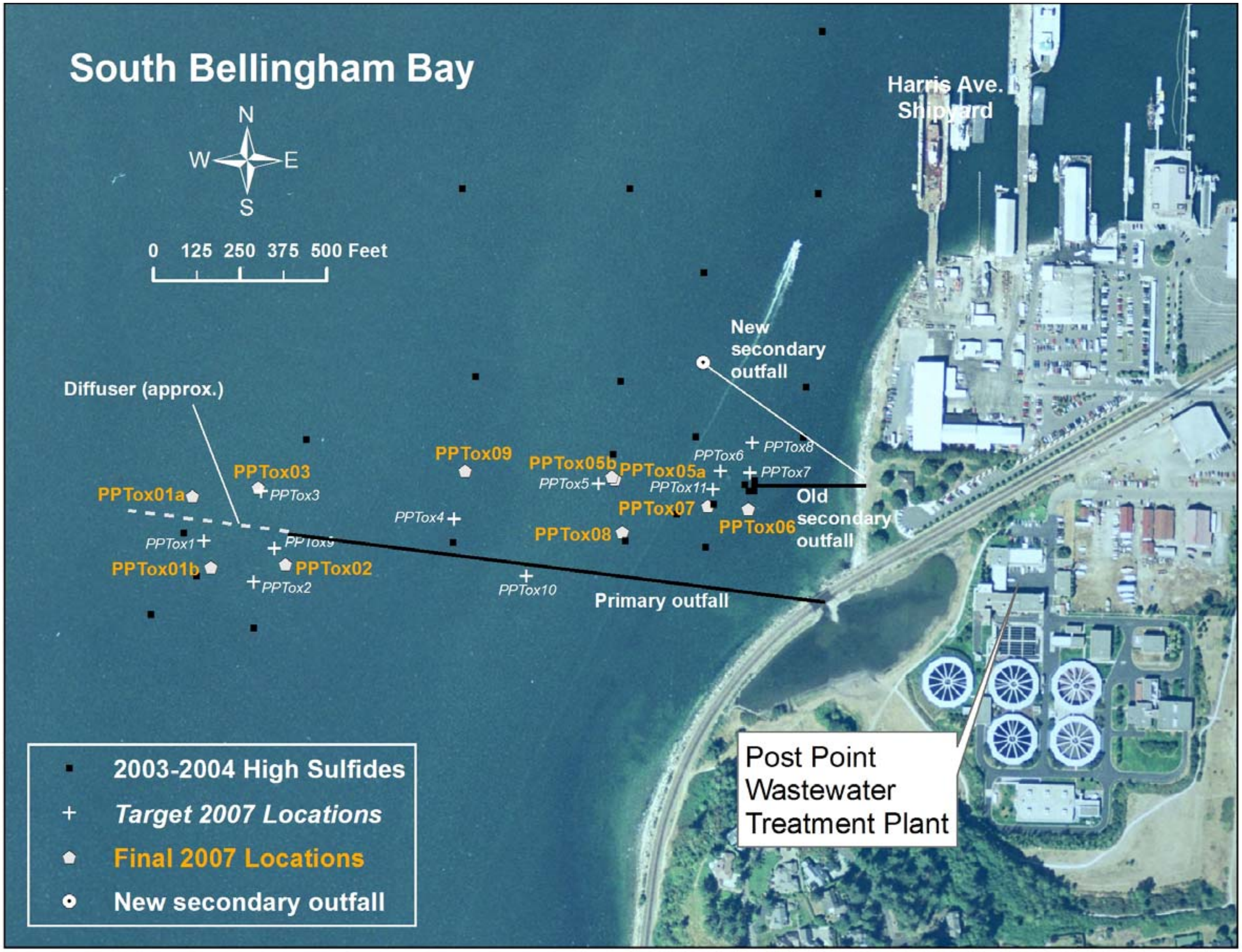

Figure 1. Target and final sediment sampling locations near Post Point WWTP outfalls.
Construction to replace the alternate outfall was occurring near the 4 eastern target locations.

Figure 2. Sampling sediment near Post Point.
(Photos taken 9/19/08 by Mary O'Herron.)

Top: Replacing the secondary outfall for the Post Point Wastewater Treatment Plant.
Lower left: Retrieving/deploying the van Veen grab sampler.
Lower right: Surface sediment in 2 sides of the double van Veen (0.1 m^2 each).

Analysis

The parameters measured for this study, along with methods used and laboratories involved, are summarized in Table 1.

Table 1. Summary of parameters measured and test methods.

Parameter	Method	Reference	Laboratory
Conventionals			
Total solids	Mass difference after drying (70°C and 104°C)	PSEP 1986	MEL and ARI
Total Organic Carbon	Conversion to CO_2, measured by non-dispersive infrared detector	PSEP 1986	MEL
Total sulfides[1] (sediment, porewater)	Colorimetric, absorbance ∝ level of methylene blue (from reaction with sulfide)	EPA Method 376.2	ARI
Toxicity[2]			
Amphipod	10-day survival and reburial	PSEP 1995	NAS
Sediment larval	96-hour survival and development	PSEP 1995	NAS
Juvenile polychaete	20-day survival and growth	PSEP 1995	NAS
Microtox	15-minute luminosity	Ecology 2003	CH2M Hill

[1] Samples were not preserved with zinc acetate until after porewater was separated from whole sediment.
[2] Toxicity labs measured total sulfides in porewater upon receipt of subsamples, in porewater at start and end of tests, and in overlying water of test beakers.

ARI = Analytical Resources, Inc.; MEL = Ecology's Manchester Environmental Laboratory; NAS = Northwest Aquatic Sciences; PSEP = EPA's Puget Sound Estuary Program Protocols and Guidelines.

The methods and standard operating procedures used to measure conventional parameters and toxicity for this study were consistent with the QA Project Plan. The lone exception was that Analytical Resources, Inc. used EPA Method 376.2 to measure total sulfides. This was an acceptable performance-based, alternative method.

A review of sample results showed sediment conventional results met data quality objectives and were usable with few qualifiers. One sample of sandy sediment (PPTox05) lacked sufficient mass of fine particles (silts and clays) to be measured by the settling method. For this sample, % fines was calculated as 100% minus the combined % of sands and gravel.

The QA Project Plan required toxicity testing using 2 acute and 2 chronic protocols commonly used throughout the region. Northwest Aquatic Sciences (NAS) conducted 3 of these toxicity tests according to PSEP (1995) and DMMP (1990-2007). Test organisms and type of endpoint were:

- *Eohaustorius estuarius* amphipods (acute test).
- Larvae of the mussel *Mytilus galloprovincialis* (acute test)
- Juvenile *Neanthes arenicola* polychaetes (marine worms, chronic test).

Tests were set up and conducted with sample mixing and aeration kept to a minimum.

Review of quality control results for these toxicity tests (available on request) showed:

- Water quality parameters in test exposures were well controlled.
- Negative control responses were within performance limits.
- Test organisms showed normal sensitivity to reference toxicants[1].
- The reference sample response met the performance standards.

All results for these 3 tests met data quality objectives and could be interpreted according to standards (Ecology, 1995).

CH2M Hill conducted the fourth toxicity test protocol, Microtox, using *Vibrio fischeri* (Ecology, 2003). Pre-test mixing and aeration were minimized, but 3 samples (PPTox06, PPTox08, and PPTox09) needed aeration to raise dissolved oxygen to >50% saturation before starting the tests.

Review of Microtox test results revealed data quality objectives were satisfied with one exception. The mean negative control light output after 15 minutes was less than the required 80% of the mean initial light output. Despite this, test sample results were deemed acceptable because:

- Mean negative control light output after 5 minutes was > 80% of mean initial light output.
- Statistical comparisons were made to the reference sample.
- Mean reference sample light output at 15 minutes was > 80% of the mean initial light output.

Results of all toxicity tests were analyzed statistically and interpreted by contract laboratory staff. This was done according to requirements of the SMS rule (Ecology, 1995) and associated guidance (Ecology, 2003; Michelsen and Shaw, 1996). The contractor's methods, calculations, and findings were confirmed by the principal investigator.

Statistical analyses of results were mainly performed with SYSTAT software for Windows 11.0 (SYSTAT, 2004).

[1] The EC_{50} value is the level of a toxic agent in water that causes 50% of the test organisms to show a specified effect, e.g., ½ of the organisms die. Normal sensitivity means that the EC_{50} value reported by NAS was within 3 standard deviations of the historic mean EC_{50} value reported by the laboratory.

Results

Sediment conventionals

Results for conventional sediment parameters are summarized in Table 2. Three of the 8 sediment samples collected near Post Point had more than >75% sand (PPTox05-06, PPTox09). Sediment from the other 5 locations (PPTox01-03 and PPTox07-08) had 73 - 85% fines (silt and clay particles). The reference sample for toxicity tests (PPTox10) had 82% fines.

TOC at the study site ranged from 0.24 - 2.54%. The 3 sandy locations had the lowest TOC, ranging from 0.24 to 1.23%. Silty sediments collected near Post Point had 2.1 - 2.6% TOC, but the high silt reference sample only had 0.67% TOC.

ARI measured total sulfides in subsamples of sediment 4-6 days after sampling. Results showed that total sulfides in the 8 sediment samples varied by more than 2 orders of magnitude (Table 2, Figure 3). Sandy sediments with relatively low TOC had total sulfides ranging from 6 to 538 mg/kg. Silty sediments, with greater TOC, had 793 – 2,630 mg/kg total sulfides. The reference sediment had total sulfides of 109 mg/kg.

Porewater was extracted from each whole sediment sample by centrifugation under a nitrogen atmosphere. Results showed total porewater sulfides were less than 0.2 mg/L in all but 3 samples. Sediments from locations PPTox06 - PPTox08 had porewater with 2.0 to 30.9 mg/L total sulfides.

The toxicity testing laboratory, NAS, received sediment subsamples within 6 days of collection dates. Laboratory staff measured porewater sulfides using a similar method approximately 3 days later. The same 3 samples continued to have detectable levels of porewater sulfides. Porewater sulfide levels in the 3 samples often decreased during the setup of *Eohaustorius* and *Neanthes* toxicity tests (Table 3). By the end of the 2 tests, porewater sulfides were almost always undetectable.

Table 2. Parameters measured in sediments near Post Point.

Shaded results are for the 3 sampling locations with appreciable levels of total sulfides in porewater.

Sampling Location	Total Solids[1]	TOC	Gravel	Sand	Silt	Clay	Fines	Total Preserved Solids[2]	Total Sulfides Post-Sampling (ARI)[3]	
									Sediment	Porewater
PPTox01	31.4	2.12	6.7	8.7	40.4	44.2	84.6	33.3	1,630	0.11
PPTox02	38.9	2.11	1	25.4	32.3	41.6	73.9	42.2	1,590	0.08
PPTox03	35.3	2.54	2.2	19.0	35.8	42.8	78.6	39.5	793	<0.06 u
PPTox05	78.7	0.24	0.5	96.7	--	--	2.8	74.7	5.95	<0.12 u
PPTox06	54.0	1.23	1.5	75.8	11.6	11.1	22.7	58.7	538	7.25
PPTox07	34.0	2.48	1.8	15.2	50.4	32.6	83.0	35.4	2,630	30.9
PPTox08	41.1	2.13	3	24.1	41.5	31.4	72.9	39.9	1,500	2.00
PPTox09	68.6	0.66	1.2	82.4	7.1	9.3	16.4	70.8	201	<0.50 u
PPTox10 (reference)	59.8	0.67	0.2	18.2	73.1	8.6	81.7	60.7	109	<0.25 u

1 Applies to subsamples used to measure TOC (MEL).

2 Applies to subsamples preserved with zinc acetate after arrival at laboratory (Analytical Resources, Inc. or ARI) and used to measure total sulfides.

3 Measured in samples received by Analytical Resources Inc. (ARI) within 4-6 days of sample collection.

Units of measure:
Total Solids and Total Preserved Solids = % of wet weight.
TOC through Fines = % of dry weight.
Total sulfides, whole sediment = mg/kg dry weight.
Total sulfides, porewater = mg/L.

TOC = Total organic carbon.
u = undetected at value shown (reporting limit).

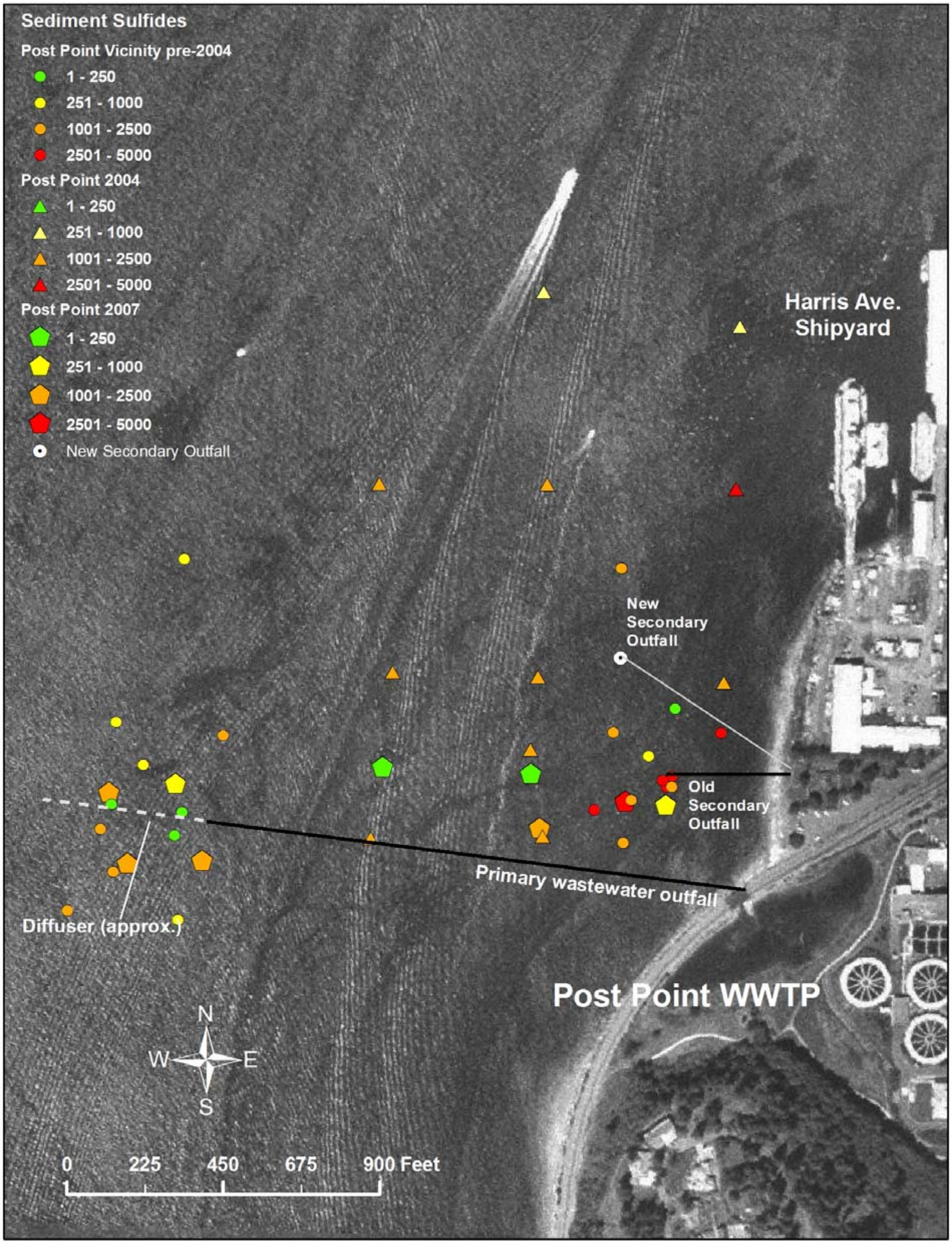

Figure 3. Total sulfides in sediments near Post Point and Harris Avenue Shipyard.

Table 3. Levels of total sulfides in Post Point sediment samples over time.

Units of measure are: mg/Kg dry wt. for sediment samples, and mg/L for porewater samples. PPTox06-PPTox-09 were the only locations that had appreciable levels of total porewater sulfides.

Sampling Location	Total Sulfides[1] (ARI)		Total Sulfides (mg/L) (NAS)				
	Sediment	Initial Porewater	Initial Porewater[2]	Porewater during Toxicity tests[3]			
				Eohaustorius		Neanthes	
	9/24/07	9/24/07	9/27/07	9/28/07	10/8/07	9/28/07	10/18/07
PPTox01	1,630	0.11	<0.1	<0.1	<0.1	0.2	<0.1
PPTox02	1,590	0.08	<0.1	<0.1	<0.1	0.2	<0.1
PPTox03	793	<0.06 u	<0.1	<0.1	<0.1	0.2	<0.1
PPTox05	5.95	<0.12 u	<0.1	<0.1	<0.1	<0.1	<0.1
PPTox06	538	7.25	2.8	2.6	<0.1	4.9	0.2
PPTox07	2,630	30.9	32.1	5.5	<0.1	13.0	0.2
PPTox08	1,500	2.00	7.4	0.2	<0.1	4.4	<0.1
PPTox09	201	<0.50 u	<0.1	0.1	0.1	0.2	<0.1
PPTox10 (Carr Inlet 20)	109	<0.25 u	<0.1	<0.1	<0.1	0.2	<0.1

[1] Received by Analytical Resources Inc. (ARI) within 24-72 hours of sample collection (September 21, 2007) and measured within about 72 hours of sample receipt (September 24, 2007).

[2] Received by Northwest Aquatic Sciences (NAS) within 4-6 days of sample collection (September 24, 2007) and measured within 72 hours of sample receipt.

[3] Measured by Northwest Aquatic Sciences at start of toxicity tests, begun within 7-9 days of sample collection and <4 days of sample receipt.

u = undetected at value shown (reporting limit).

Sediment toxicity

NAS was responsible for conducting 4 sediment toxicity tests using subsamples from the 8 Post Point locations and the Carr Inlet reference sediment (PPTox10). Procedures used for all tests involved minimal mixing and aeration of sediment. Exposure conditions were well-controlled, as shown by daily measurements of temperature, pH, dissolved oxygen, ammonia, and total sulfides in the overlying water. Control and reference samples met applicable performance standards, except as noted for the Microtox test (see Methods). All results were usable and interpretable (see Appendix C).

A summary of toxicity test results is presented in Table 3. Additional details are provided in Appendix C, and complete results available on request (NAS, 2007). There was no significant toxicity measured using the amphipod, larval, or juvenile polychaete test protocols. Microtox toxicity, measured as significantly decreased light output after a 15-minute exposure to porewater, exceeded the SQS at locations PPTox07 and PPTox08 only.

Table 4. Toxicity of sediments collected near Post Point WWTP outfalls.

Sampling Location	*Eohaustorius* % Mortality	*Mytilus* % NCMA	*Neanthes* Growth (mg/worm/day)	Microtox Luminosity (15 min)		Final Toxicity (SMS)
PPTox01	23.0*	14.8	0.67	113.0	--	< SQS
PPTox02	24.0*	15.7	0.82	99.8	--	< SQS
PPTox03	8.0	17.4	0.88	69.8	--	< SQS
PPTox05	5.0	5.6	1.02	--	114.4	< SQS
PPTox06	15.0	11.6	0.87	75.4	--	< SQS
PPTox07	14.0	31.9	0.95	--	73.4*	**> *SQS***
PPTox08	10.0	20.7	0.82	--	62.2*	**> *SQS***
PPTox09	10.0	19.6	0.77	--	95.4	< SQS
PPTox10 (reference)	13.0	22.5	0.79	Batch 1 91.0	Batch 2 97.0	--

***** = test sample was statistically different from reference sample result ($p<0.05$).

NCMA = normalized combined abnormality and mortality, SMS = Sediment Management Standards (Ecology, 1995), < SQS = passes interpretive guidelines for all toxicity tests, >***SQS*** = fails SQS criteria for one toxicity test.

The larval development test protocol was conducted starting on September 27, 2007. All test samples passed at the SQS level. No test sediment had mean normal survivorship significantly different from or <85% of that observed in the reference sediment. In addition, no test sample had a mean % normalized combined mortality and abnormality (NCMA) significantly greater than that observed in the reference sediment.

The 10-day amphipod toxicity tests began on September 28, 2007. Test results showed all samples passed the SQS (Ecology, 1995). Samples PPTox01 and PPTox02 had significantly higher mortality than the reference sediment, but did not have mean percent mortality greater than 25%.

NAS also began the 20-day juvenile polychaete growth tests on September 28, 2007. The mean weight of individual worms at the start of the test was somewhat low (0.45 mg), but more than the minimum allowed (0.25 mg). None of the juvenile polychaete test samples had a mean individual growth rate significantly different from, or 50% lower than, that observed in the reference sediment. Therefore, all test sediments passed the SQS.

The Microtox test results showed the following. After 15-minute exposures of *Vibrio* to porewater from samples, only PPTox07 and PPTox08 had < 80% of the light output of, and were significantly different from, the reference sample. Therefore, these 2 samples failed to meet the SQS.

Data analysis

In addition to confirming the regulatory interpretations of toxicity test results, relationships between various parameters of interest were explored. Supporting information can be found in Appendix D.

Potential relationships, mainly between conventional sediment parameters and toxicity test results, were explored mainly using Spearman rank correlation and regression analysis. Results are described below, and supporting information can be found in Appendix D.

Using only results of this study, significant Spearman rank correlations were found between different pairs of parameters. The correlations of most interest included:

- % fines and total sulfides ($p<0.05$).
- % TOC and total sulfides ($p<0.05$).
- % TOC and Microtox luminosity ($p< 0.05$) (Figure 4).
- Total sulfides and *Eohaustorius* mortality ($p<0.10$).

Correlations between total sulfides and both *Eohaustorius* survival and *Mytilus* development became highly significant ($p<0.02$) when combined with results of Blakley (2006).

Spearman rank correlations do not reveal the ‘best fit’ model for a set of observations. Therefore, linear and logistic regression models were used to further examine results. Table 5 shows simple (least squares) linear regression results for some relationships of interest.

Table 5. Linear regression results for Post Point sediment toxicity study.

Independent variable or predictor (X)	Dependent variable or predicted (Y)	r^2	Slope	Intercept
Fines (%)	TOC (%)	0.94	0.0247	0.348
TOC (%)	Total sulfides (mg/kg)	0.66	820	-274
Total sulfides (mg/L)	*Eohaustorius* mortality	0.29	0.0042	8.96
	Mytilus NCMA	0.56	0.0065	9.91

NCMA = normalized combined mortality and abnormality, TOC = total organic carbon.

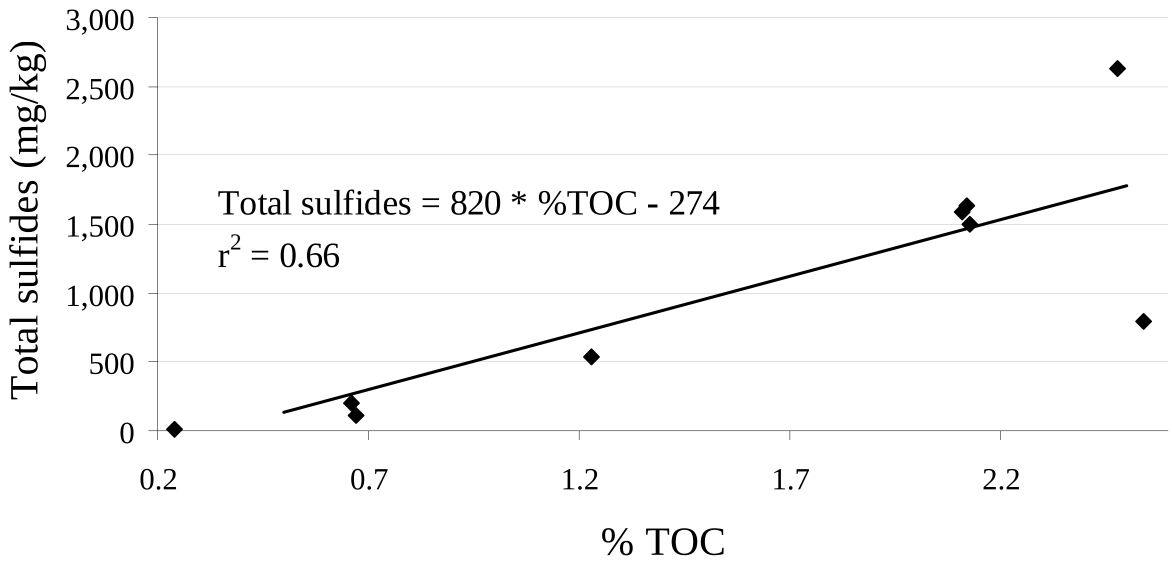

Figure 4. Relationship between total sulfides and % TOC in sediments near Post Point.

Results showed the following:

- Variability in % fines explained about 94% of the variability in levels of TOC in sediments.
- Variability in % TOC explained about 66% of the variability in levels of total sulfide. This is shown in Figure 5.
- Levels of total sulfides in whole sediment explained 29% and 56% of the variability in *Eohaustorius* mortality and *Mytilus* toxic responses, respectively.
- Relationships between various conventional sediment parameters and toxic response in Microtox and juvenile polychaete tests were relatively poor.

Linear relationships between total sulfides and toxic responses observed in this study were often poor, so a logistic regression model was used to explore possible dose-response relationships. This was mainly done using levels of total sulfides (dose) and Microtox toxicity results (response) [2]. The 2 samples that showed significantly reduced luminosity (>SQS) were assigned a score of 1, and the remaining samples (<SQS) were given a score of 0. The resulting logistic relationship was described by:

$$pT_m = e^{\theta}/(1+ e^{\theta}), \text{ where}$$

pT_m = probability of significant toxicity (0.0-1.0) and

$\theta = 0.004[S] - 6.574$

The equation yields 83% accurate predictions. Only the level of total sulfide found at location PPTox08 (1,500 mg/kg) misclassified the sample as nontoxic. Based on toxicity results from this study alone, the EC_{50} calculated for total sulfides was 1,763 mg/L.

[2] The analysis was limited to Microtox luminosity because it was the only test showing significant responses.

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Discussion

Sample representativeness

Difficult conditions for field sampling reduced the accuracy of vessel positioning and altered sampling plans. Despite this, the 8 sediment samples likely represent what was observed to be a patchy benthic environment near the Post Point outfalls. One indication of this is that levels of total sulfides in the 8 samples, that span nearly 3 orders of magnitude, are within the range of approximately 90% of all other area results (Figure 5).

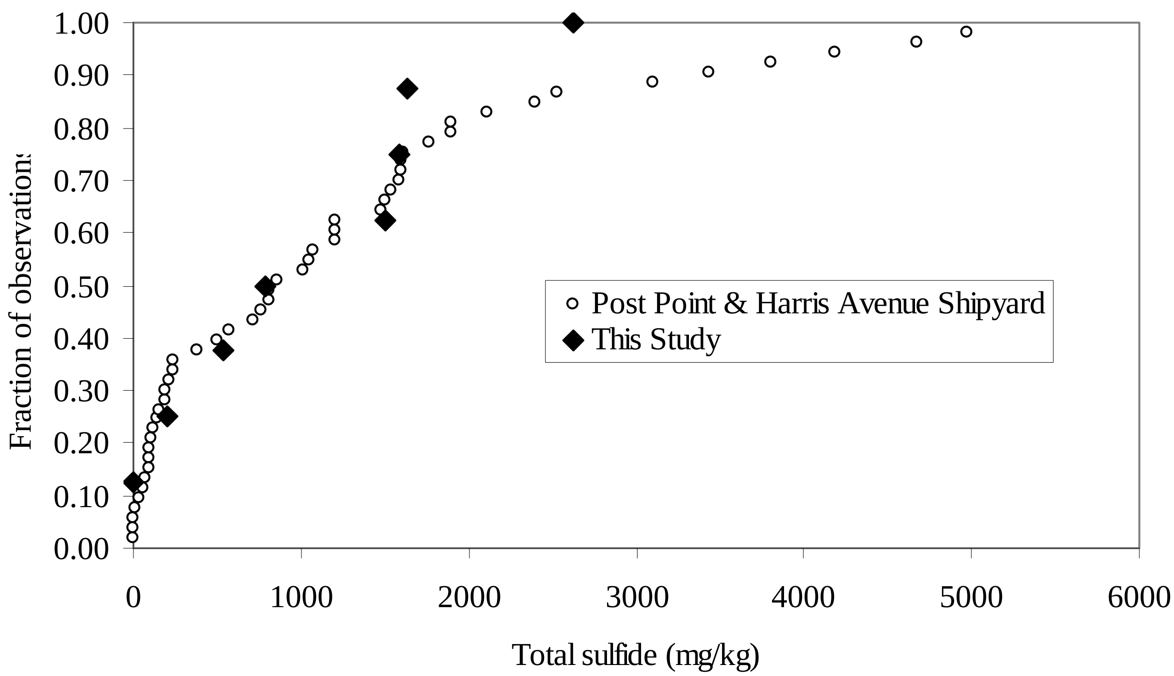

Figure 5. Cumulative distribution of total sulfide levels in sediments near Post Point and Harris Avenue Shipyard.

Sediment conventionals

Results of this study show a dichotomy of sediment types near the Post Point outfalls:

- Sandy sediments with relatively low % TOC and levels of total sulfides.
- Silty sediments with relatively low % TOC and levels of total sulfides.

This dichotomy yielded correlations with greater significance, and regressions explaining more variability, than analogous ones based on larger data sets. Regardless, results showed a clear relationship between fine-grained sediment and organic loading (% TOC). In addition, organic loading, whether from the Post Point outfalls or natural sources, explained much of the variability in levels of total sulfides. Finally, results of correlation and regression analysis from this study supported the presumption that total sulfides contributed to the toxicity expressed by amphipod survival, mussel development, and Microtox luminosity tests.

While previous studies showed % TOC and levels of total sulfides to be elevated, this 2007 study found:

- The maximum % TOC measured in sediments near Post Point (2.54%) is not unusual for urban sediments in Puget Sound (Aasen, 2007).
- The mean level of total sulfides measured for this study is not different from previous studies near Post Point or locations near the inner Bellingham Bay shoreline.

The latter finding results from statistically comparing the mean level of total sulfides from this 2007 study to mean levels from previous studies. The first comparison was to the mean level of total sulfides near Post Point and Harris Avenue Shipyard (only). The second comparison was to the mean level for surface sediments within about 500 meters of the inner Bellingham Bay shoreline.

Ecology's EIM database was queried, and MyEIM [3] was used to extract and compile results for total sulfides in whole sediment samples. The 8 samples collected for the current study averaged 1,000 mg/kg total sulfides. The mean level of total sulfides in 82 previous sediment samples collected near Post Point was 1,306 mg/L. The comparable mean was 1,234 mg/L for 136 other sampling locations within approximately 500 meters of the Bellingham Bay shoreline. Results for individual sampling locations are shown in Figure 6.

None of the data sets was normally distributed, even after being transformed. Therefore, the nonparametric Mann-Whitney test was used to compare means. The null hypothesis was that any 2 group means were the same. The probability values obtained ($p<0.71$ - $p<0.93$) gave little reason to reject this hypothesis. The sample means are not different.

Porewater sulfides measured in samples collected for this study were never more than 1.4% of the total sulfides in the whole sediment. This small proportion may be common for Puget Sound sediments. Alternatively, it may reflect losses of porewater sulfides during sample collection and handling that occurred, despite implementing procedures to minimize changes in total sulfides. Regardless, losses during sampling and early handling could not be quantified.

Results in the Sediment Conventionals section (Table 3) did show:

- Short-term storage may cause little change in levels of total sulfides. Levels measured in porewater samples extracted from 2 subsamples by 2 contract laboratories after 2 holding times (3 and 6 days) were qualitatively similar.
- Standard protocols for setting up and conducting toxicity tests, even with reduced mixing and aeration, decreased levels of porewater total sulfides in 2 of the 3 samples that had appreciable levels (PPTox07 and PPTox08).

[3] MyEIM is a new application designed by Ecology to facilitate retrieval, compilation, and analysis of EIM data.

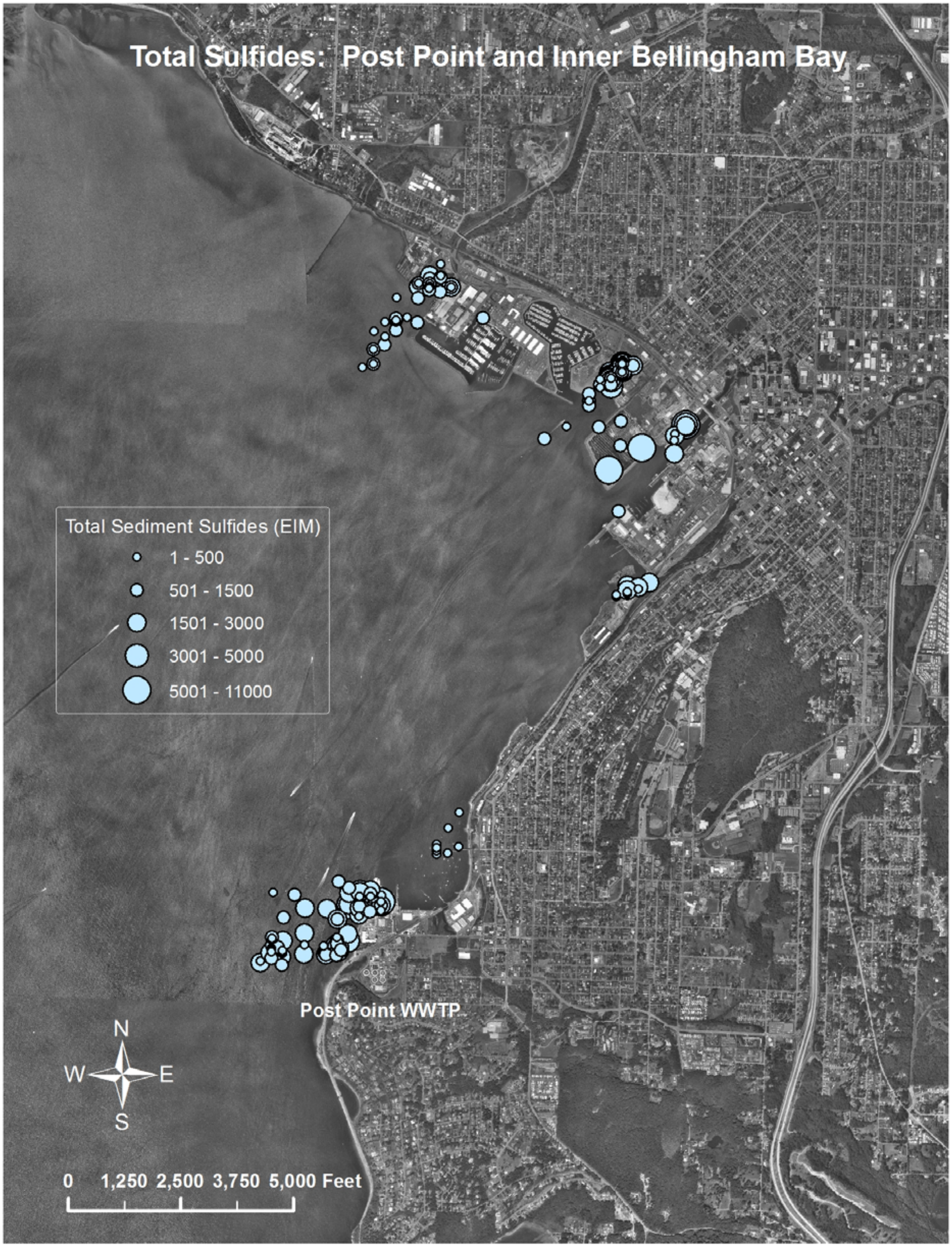

Figure 6. Total sulfides in sediments near Post Point and elsewhere in inner Bellingham Bay.

Toxicity

Since 1990, at least 123 toxicity tests have been conducted using sediment collected from at least 36 sample locations near Post Point (RETEC, 1998; 2004; Anchor, 2004; Blakley, 2006; this study). Results from all studies show that 17 of the 36 sampling locations had significant toxicity (Table 6). Nearly 50% of all sampling locations potentially classified as toxic (>SQS) may be a reason for concern. However, other findings should also be considered:

- Only about 1 in 5 of all possible test outcomes (25/123) showed significant toxicity.[4]
- Only 12 of 94 (12.8%) amphipod, sediment larval, and polychaete test outcomes were toxic.[4]
- Several locations and samples classified as toxic were later found not to show significant toxicity through re-sampling or repeated testing (sometimes prompted by QA/QC failures).
- There was no spatial pattern of Microtox toxicity.

Table 6. Summary of sediment toxicity studies near Post Point and Harris Avenue Shipyard.

Study (sampling years)	Sampling locations	Test Organism			
		Amphipod	Larval	Microtox	Polychaete
RETEC, 2004 (2000-2004)	9	1/8	7/20	--	0/6
Anchor, 2004 (2003)	9	1/9	0/9	2/9	--
Blakley, 2006 (2004)	10	1/10	0/10	3/10	--
Gries, 2008 (this 2007 study)	8	0/8	0/8	2/8	0/8
Totals	36	3/35	7/47	7/27	0/14

Fractions are *unique* locations with significant effects / total number of samples (including retests). RETEC (2004) featured repeated samplings of the same locations and repeated testing.

Possible explanations for the lack of extensive or severe toxicity included:

- Toxic compounds (hydrogen sulfide; H_2S) were not present or unavailable at levels causing significant effects.
- The test organisms used were not sensitive to total or porewater sulfides, or were able to reduce their exposures by means of various behaviors.

The first of these explanations was examined by calculating the fraction of porewater sulfides predicted to be present as toxic H_2S at the start and end of the *Eohaustorius* and *Neanthes* toxicity tests. This was done using the equation and constants given in Wang and Chapman (1999) or Savenko (1977). The basis for calculations is presented in Appendix D.

Calculated levels of H_2S were compared to EC_{50} values derived from responses of various test organisms to water-only exposures of H_2S (Gardiner, 2007). The authors cited EC_{50} values for *Eohaustorius* and *Neanthes* of 0.33 and 0.10-0.78 mg/L H_2S, respectively. Results showed that one or both test organisms should have responded to levels of H_2S in samples PPTox06-08 (Tables 7 and 8).

[4] The value cited includes samples found toxic by >1 test and samples found toxic >1 time by repeated testing.

There is no basis for suggesting that *Eohaustorius* and *Neanthes* were insensitive to the levels of H_2S likely present in some test sediments. The lack of response by these test organisms may indicate:

- Levels of H_2S in porewater declined rapidly during the tests.
- Test organisms either had greater tolerance of H_2S than suggested by Gardiner (2007) or reduced their exposures by means of various behaviors.

Table 7. Calculated levels of H_2S for *Eohaustorius* toxicity test exposures.

Values in bold font exceed relevant EC_{50} values reported by Gardiner (2007).

Sampling Location	Porewater Total Sulfides (measured)	Porewater H_2S (calculated)		Overlying Water Total sulfides (measured)	Overlying Water H_2S (calculated)	
	Day 0 (pH 7.6)	Day 0 pH 7.6	Day 10 pH 7.1	Day 0 pH 7.5	Day 1 pH 8.2	Day 10 pH 8.7
PPTox01	<0.1	<0.02	<0.04	<0.1	<0.004	<0.001
PPTox02	<0.1	<0.02	<0.04	<0.1	<0.004	<0.001
PPTox03	<0.1	<0.02	<0.04	<0.1	<0.004	<0.001
PPTox05	<0.1	<0.02	<0.04	<0.1	<0.004	<0.001
PPTox06	2.6	***0.5***	<0.04	<0.1	<0.004	<0.001
PPTox07	5.5	***1.0***	<0.04	<0.1	<0.004	<0.001
PPTox08	0.2	0.04	<0.04	<0.1	<0.004	<0.001
PPTox09	0.1	0.02	0.04	<0.1	<0.004	<0.001
PPTox10	<0.1	<0.02	<0.04	<0.1	<0.004	<0.001

Table 8. Calculated levels of H_2S for *Neanthes* toxicity test exposures.

Values in bold font exceed at least one relevant EC_{50} value reported by Gardiner (2007).

Sampling Location	Porewater Total Sulfides (measured)	Porewater H_2S (calculated)		Overlying Water H_2S (calculated)	
	Day 0 pH 7.6	Day 0 pH 7.6	Day 20 pH 6.9	Day 0 pH 8.1	Day 20 pH 8.0
PPTox01	0.2	0.04	<0.04	<0.001	<0.001
PPTox02	0.2	0.04	<0.04	<0.001	<0.001
PPTox03	0.2	0.04	<0.04	<0.001	<0.001
PPTox05	<0.1	<0.02	<0.04	<0.001	<0.001
PPTox06	4.9	***0.8***	0.08	<0.001	<0.001
PPTox07	13.0	***2.1***	0.08	<0.001	<0.001
PPTox08	4.4	***0.7***	<0.04	<0.001	<0.001
PPTox09	0.2	0.04	<0.04	<0.001	<0.001
PPTox10	0.2	0.04	<0.04	<0.001	<0.001

In addition to the logistic regression results reported in this report and in Blakley (2006), a similar analysis was conducted using other sets of results. Table 9 and Figure 7 showed each set of Microtox results yielded a significant logistic regression equation. The strength of the relationship (correlation coefficient), predictive accuracy, and apparent EC_{50} values depended on the study data used. The Anchor (2004) results best fit the logistic regression model and yielded the most accurate predictions of toxicity, but showed the highest $EC_{50.}$ The lower apparent sensitivity of Microtox to total sulfides in that study may have been due to:

- Differences in levels of total sediment sulfides present in samples.
- Greater loss of biologically available sulfides during sampling and handling than occurred in other the studies.

Table 9. Logistic regression model for total sulfides and Microtox toxicity.

Survey(s)	Sample number	r	Predictive accuracy	Equation	Apparent LC_{50} (mg/L)
Anchor 2004	9	1.00	100	$pT_m = e^{\theta}/(1+ e^{\theta})$ where $\theta = 0.047[S] – 207.8$	4,430
Blakley 2006	9	0.56	68.4%	$pT_m = e^{\theta}/(1+ e^{\theta})$ where $\theta = 0.003[S] – 6.264$	1,850
Gries 2007	9	0.72	83.0%	$pT_m = e^{\theta}/(1+ e^{\theta})$ where $\theta = 0.004[S] – 6.574$	1,760
Blakley 2006 + Gries 2007	19	0.64	75.4%	$pT_m = e^{\theta}/(1+ e^{\theta})$ where $\theta = 0.003[S] – 6.255$	1,830
All surveys	28	0.46	69.1%	$pT_m = e^{\theta}/(1+ e^{\theta})$ where $\theta = 0.001[S] – 2.748$	2,860

r = correlation coefficient.
LC_{50} = concentration of total sulfide that is 'lethal' to 50% of the test organisms.
mg/L = milligrams per liter.
pT_m = the probability of exceeding the SQS for Microtox luminosity.
$e \cong 2.71828$.
[S] = measured concentration of total sulfides.

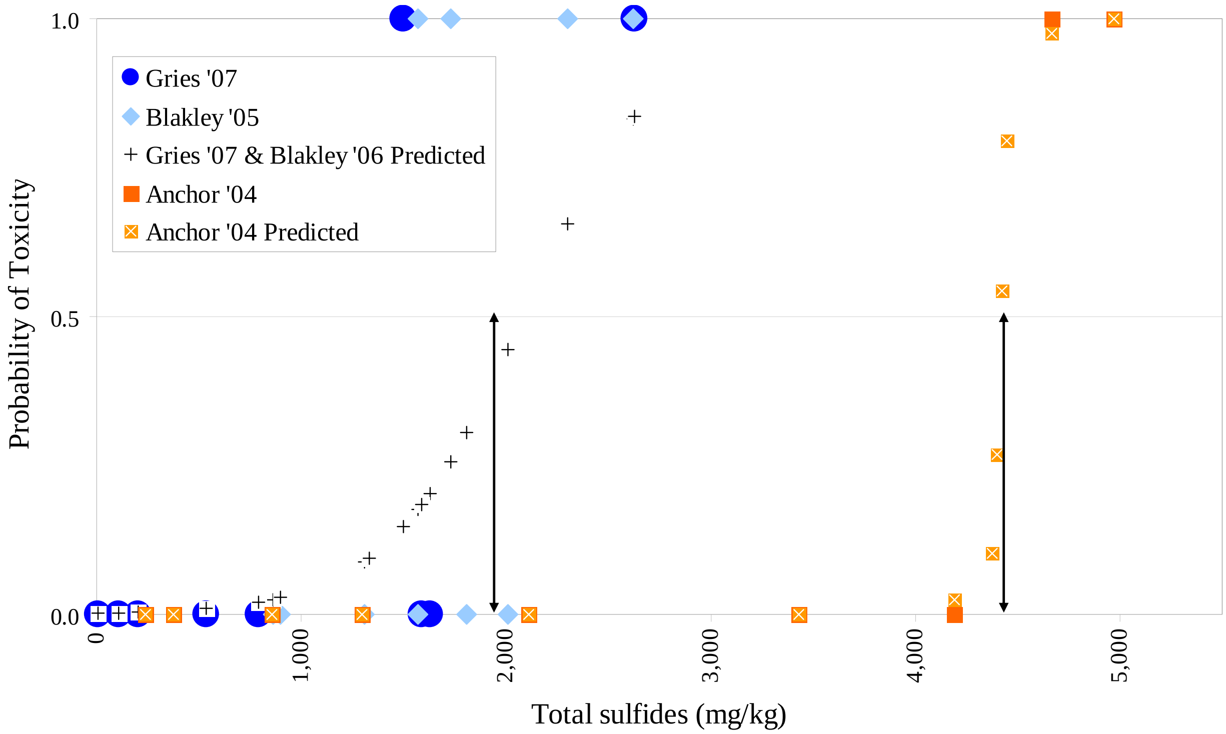

Figure 7. Microtox responses (toxic = 1, not toxic = 0) fit to logistic regression model.

Conclusions

As a result of this 2007 study, the following 4 conclusions are made:

1. Results of this study do not seem to support detailed remedial investigations or monitoring of sediment quality near the Post Point WWTP outfalls.

Reasons for this conclusion include the following:

- Evidence of widespread or severe sediment toxicity is lacking.
- Sediment contamination near the outfalls, when reported by other studies, is generally below SQS levels.
- Sediment sulfides in the vicinity of outfalls, presumed a cause of toxicity, do not appear different from levels measured in other areas of inner Bellingham Bay.
- Sediment containing high total sulfides does not always result in observable toxicity.
- Levels of organic carbon associated with fine-grained sediments near the Post Points outfalls are not unusually high for urban areas of Puget Sound.
- The fraction of organic carbon in sediments near the Post Point outfalls that comes from natural sources (such as nearby eel grass beds) is not known.

2. Total sulfide contributed to, but did not explain all of, the toxicity observed in several tests.

This was shown by a correlation analysis using results from the present study alone or combined with previous results. It was also indicated by logistic regressions between total sulfides and toxic responses (absolute or toxic/not toxic).

3. Sulfide toxicity assessed with laboratory toxicity tests should be viewed with caution until specialized protocols are available to minimize loss of sulfides.

- Standard protocols for collecting surface sediment, handling samples, and testing toxicity were not designed to maintain *in-situ* conditions.
- Some test organisms may reduce their exposure to H_2S by means of normal or avoidance behaviors.

4. Even limited mixing and aeration of sediment samples during test setup and exposures led to substantial losses of total sulfides from porewater. Losses of total sulfides from porewater due to sample collection or early handling and storage protocols could not be quantified. Short-term sample storage did not seem to greatly alter levels of total sulfide in porewater.

Recommendations

As a result of this 2007 study, 3 recommendations are made:

1. Results of this study do not indicate that extensive follow-up studies are necessary.

Based on various lines of evidence (% TOC, levels of total sulfides, toxicity test results), detailed remedial investigations seem unwarranted. Future monitoring that may occur should confirm that outfall discharges do not substantially increase sediment TOC, sulfides, or toxicity.

2. Investigations in areas where sediment sulfides may contribute to sediment toxicity should use a set of new field and laboratory methods.

New sample handling and toxicity testing protocols should be developed that better capture *in-situ* conditions. Several approaches may be envisioned:

- Expose toxicity test organisms to sediment in 3-4 inch diameter sediment cores that are:
  - Collected manually from grab samplers.
  - Not mixed or homogenized.
  - Overlain with seawater from the site.
  - Aerated only when the reference sample dissolved oxygen drops below a threshold.
- Collect undisturbed sediment from a grab sampler placed under a nitrogen atmosphere in the field, and test organisms in a greater number of replicate containers.
- Measure direct indicators of *in-situ* benthic community health (abundance, diversity, richness, sulfide-tolerant species) instead of its frequent substitute, laboratory toxicity (Wang and Chapman, 1999; Blakley, 2006).

3. Ecology should evaluate different methods of estimating the fraction of man-made organic carbon in sediment.

This study and others provide evidence that total sulfides may contribute to sediment toxicity at some locations near Post Point outfalls. However, sulfide-induced toxicity is ultimately caused by organic loading. Organic loading, in turn, may come from man-made sources (outfall discharges, wood waste), natural sources (rivers, nearshore runoff, aquatic vegetation), or both. Currently, regulations do not define field or laboratory methods that help distinguish between natural and man-made sources of organic loading. A list of such methods should be compiled.

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Appendices

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Appendix A. Acronyms and abbreviations

Following are acronyms and abbreviations used frequently in this report:

ARI	Analytical Resources Incorporated
Ecology	Washington State Department of Ecology
EIM	Environmental Information Management database (Department of Ecology)
EPA	U.S. Environmental Protection Agency
H_2S	Hydrogen sulfide
MEL	Manchester Environmental Laboratory (Department of Ecology)
NAS	Northwest Aquatic Sciences
PSEP	Puget Sound Estuary Program
QA	Quality assurance
QC	Quality control
SMS	Sediment Management Standards
SQS	Sediment quality standard
TOC	Total organic carbon
WWTP	Wastewater treatment plant

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Appendix B. Field notes and vessel positioning

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Table B-1. Field sampling notes.

Station Location	Date	Time	MEL ID	TarLat	TarLon	Latitude NAD 83	Long NAD 83	Wheel Depth	Sounder Depth	Conditions	Temp	Salinity	Sediment Depth	RPD?	Sediment Type	Color?	Odor?
PPTox01	18-Sep	1415 1455	384020	48.71876	122.524092	48.71911 48.718555	122.524245 122.524002	25.9 25.9		Overcast Breeze	<13.4	30.0	16-17 17	2 mm 1 mm	Silt-Clay	Br/Ol+Gr Same (darker w/ depth)	No
PPTox02	18-Sep	1530	384021	48.718449	122.523483	48.71859	122.523113	23		Overcast Calm	13.0	30.0	16-17	Na	Silt-Clay	Br/Ol+Gr	No
PPTox03	18-Sep	1605	384022	48.719167	122.523422	48.719195	122.523457			Partly sunny Calm	13.0	30.0	15-17	1 mm	Silt-Clay	Br/Ol+GrBl	No
~~PPTox04~~	19-Sep		~~384023~~	48.718987	122.521114	Sample could not be collected											
PPTox05	19-Sep	1020 1100	384024	48.719294	122.519401	48.719333 48.719350	122.519203 122.519238	10.5 12.5		Clear Calm	12.6	30.0	9-11	None	Sandy	GrBr/GrBr	No
PPTox06	19-Sep	1140	384025	48.719421	122.517946	48.719125	122.517602	8.5	9	Clear Calm	12.8	30.0	15-17	2 mm	Silt-Clay Some sand	Br/OlBl	Slight H_2S?
PPTox07	19-Sep	1425	384026	48.719412	122.517591	48.71914	122.518088	12- 12.5	11.9	Clear Choppy	13.5	30.0	16-17	0 mm	Silt-Clay	Bl/Bl	Moderate H_2S
PPTox08	19-Sep	1500	384027	48.719647	122.517578	48.718915	122.519098	12	11.8	Clear Choppy	Na	Na	16-17	0 mm	Silt-Clay	Bl/Bl	Moderate H_2S
PPTox09	19-Sep	1600	384028	48.718716	122.52325	48.719368	122.520992	Na	Na	Clear Choppy	12.8	Na	11-12	2-10 mm	Silt-Clay Some sand	Br/Gr	No
PPTox10 Carr Inlet	20-Sep	1130	384029	48.718548	122.520235	47.333335	122.670655	16.5	16.7	Overcast Calm	13.5	30.0	12.5-13	None	Silt-Clay	Ol/OlGr	No

There were 2 grab samples taken at PPTox01 and PPTox05.

Table B-1 continued. Field sampling notes.

Station Location	Comments
PPTox01	Sampler full to top but sediment not pressed into door screen. Overlying water not turbid. Top 2" more watery than bottom 2" (more congealed). Some small shell fragments. Sediment in 2nd cast very similar to that in first.
PPTox02	Appeared very similar to PPTox01. Could see very narrow oxidized zone at surface and overlying water only somewhat turbid in one quadrant of van Veen.
PPTox03	All 3 samples very similar. PPTox more gelatinous in texture than PPTox01. Each has 1-2 mm oxidized zone/RPD.
~~PPTox04~~	No samples acquired. Field target moved to BB09: 3 casts yielded cobble that prevented closure. Same 100' south of target. Same 100' north of target.
PPTox05	Consistent sandy material. 2 grabs left gallon containers with approx. 1" of headspace.
PPTox06	Sample collected near eelgrass beds.
PPTox07	2'-4' swells. Black mayonnaise.
PPTox08	2'-4' swells. Odor somewhat less than PPTox07. Similar to PPTox07.
PPTox09	2'-4' swells - difficult conditions. Top few mm washed - overlying water turbid. Surface biota (small worm tubes and 5 mm gastropods) unaffected.
PPTox10 Carr Inlet	Nice reference sediment. Maldanids, crinoids observed.

There were 2 grabs taken at PPTox01 and PPTox05.

Table B-2. Distance between target and final sampling locations near Post Point outfalls.

Distance from target was derived using the calculator found at http://jan.ucc.nau.edu/cvm-cgi-bin/latlongdist.pl. Final sampling locations are shown as '--' where sediment could not be collected (target location PPTox-04) or where alternate target were chosen PPTox06-10). The latter location codes were assigned to samples collected at locations chosen in the field.

Target Sampling Location	Latitude	Longitude	Final Sampling Locations	Latitude (°N)	Longitude (°W)	Distance From Target (m)	Depth Uncorr. (m)	Tidal Elev. MLLW m(ft)	Depth Corr. MLLW (m)
PPTox01	48.718760	122.524092	PPTox01 Repl a	48.719110	122.524245	40.5	25.9	0.9 (3.3)	25.0
			PPTox01 Repl b	48.718555	122.524002	23.8	27.0	1.2 (4.2)	25.8
PPTox02	48.718449	122.523483	PPtox02	48.718590	122.523113	31.4	23.0	1.4 (5.0)	21.6
PPTox03	48.719167	122.523422	PPTox03	48.719195	122.523457	4.0	25.1	1.65 (5.8)	23.4
PPTox04	48.718987	122.521114	--[a]	--	--	na	na	Na	Na
PPTox05	48.719294	122.519401	PPTox05 Repl a	48.719333	122.519203	15.2	10.5	0.3 (1.3)	10.2
			PPTox05 Repl b	48.719350	122.519238	13.5	12.5	0.15 (0.9)	12.3
PPTox06	48.719421	122.517946	--	--	--	na	na	Na	Na
PPTox07	48.719412	122.517591	--	--	--	na	na	Na	Na
PPTox08	48.719647	122.517578	--	--	--	na	na	Na	Na
PPTox09	48.718716	122.523250	--	--	--	na	na	Na	Na
PPTox10	48.718548	122.520235	--	--	--	na	na	Na	Na
--	--	--	PPTox06	48.719125	122.517602	na	9.0	0.1 (0.6)	8.9
PPTox11	48.719273	122.518028	PPTox07	48.719140	122.518088	15.4	12.25	0.6 (2.2)	11.6
BBY10	48.718847	122.519065	PPTox08	48.718915	122.519098	7.9	12.0	0.8 (2.9)	11.2
--	--	--	PPTox09	48.719368	122.520992	na	na	1.2 (4.3)	Na
Carr Inlet 20	47.333312	122.670658	PPTox10	47.333335	122.670655	2.6	16.5	na	Na

MLLW = mean lower low water
[a] = sample could not be collected because cobble and shell material prevented sampler from closing
Repl. = field replicate coordinates
na = not applicable or not available

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Appendix C. Sediment toxicity data

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Table C-1. Results of 10-day amphipod toxicity tests (NAS, September 28-October 8, 2007).

DESCRIP	REPL	INIT	SURV	MORT	PSURV	PMORT			SURV	MORT	PSURV	PMORT
PPTox01	1	20	16	4	80.0	20.0						
PPTox01	2	20	12	8	60.0	40.0		Mean	15.4	4.6	77.0	23.0
PPTox01	3	20	17	3	85.0	15.0		SD	2.1	2.1	10.4	10.4
PPTox01	4	20	15	5	75.0	25.0		n	5	5	5	5
PPTox01	5	20	17	3	85.0	15.0						
PPTox02	1	20	14	6	70.0	30.0						
PPTox02	2	20	14	6	70.0	30.0		Mean	15.2	4.8	76.0	24.0
PPTox02	3	20	17	3	85.0	15.0		SD	1.6	1.6	8.2	8.2
PPTox02	4	20	14	6	70.0	30.0		n	5	5	5	5
PPTox02	5	20	17	3	85.0	15.0						
PPTox03	1	20	18	2	90.0	10.0						
PPTox03	2	20	19	1	95.0	5.0		Mean	18.4	1.6	92.0	8.0
PPTox03	3	20	18	2	90.0	10.0		SD	0.5	0.5	2.7	2.7
PPTox03	4	20	18	2	90.0	10.0		n	5	5	5	5
PPTox03	5	20	19	1	95.0	5.0						
PPTox05	1	20	19	1	95.0	5.0						
PPTox05	2	20	19	1	95.0	5.0		Mean	19.0	1.0	95.0	5.0
PPTox05	3	20	19	1	95.0	5.0		SD	0.0	0.0	0.0	0.0
PPTox05	4	20	19	1	95.0	5.0		n	5	5	5	5
PPTox05	5	20	19	1	95.0	5.0						
PPTox06	1	20	16	4	80.0	20.0						
PPTox06	2	20	16	4	80.0	20.0		Mean	17.0	3.0	85.0	15.0
PPTox06	3	20	17	3	85.0	15.0		SD	1.7	1.7	8.7	8.7
PPTox06	4	20	16	4	80.0	20.0		n	5	5	5	5
PPTox06	5	20	20	0	100.0	0.0						
PPTox07	1	20	14	6	70.0	30.0						
PPTox07	2	20	17	3	85.0	15.0		Mean	17.2	2.8	86.0	14.0
PPTox07	3	20	20	0	100.0	0.0		SD	2.4	2.4	11.9	11.9
PPTox07	4	20	16	4	80.0	20.0		n	5	5	5	5
PPTox07	5	20	19	1	95.0	5.0						
PPTox08	1	20	14	6	70.0	30.0						
PPTox08	2	20	18	2	90.0	10.0		Mean	18.0	2.0	90.0	10.0
PPTox08	3	20	19	1	95.0	5.0		SD	2.3	2.3	11.7	11.7
PPTox08	4	20	20	0	100.0	0.0		n	5	5	5	5
PPTox08	5	20	19	1	95.0	5.0						
PPTox09	1	20	16	4	80.0	20.0						
PPTox09	2	20	19	1	95.0	5.0		Mean	18.0	2.0	90.0	10.0
PPTox09	3	20	18	2	90.0	10.0		SD	1.2	1.2	6.1	6.1
PPTox09	4	20	18	2	90.0	10.0		n	5	5	5	5
PPTox09	5	20	19	1	95.0	5.0						
PPTox10	1	20	18	2	90.0	10.0						
PPTox10	2	20	19	1	95.0	5.0		Mean	17.4	2.6	87.0	13.0
PPTox10	3	20	17	3	85.0	15.0		SD	1.1	1.1	5.7	5.7
PPTox10	4	20	16	4	80.0	20.0		n	5	5	5	5
PPTox10	5	20	17	3	85.0	15.0						
control	1	20	20	0	100.0	0.0						
control	2	20	18	2	90.0	10.0		Mean	19.4	0.6	97.0	3.0
control	3	20	20	0	100.0	0.0		SD	0.9	0.9	4.5	4.5
control	4	20	20	0	100.0	0.0		n	5	5	5	5
control	5	20	19	1	95.0	5.0						

Abbreviations in Table C-1:

INIT = initial number
SURV = number survivors
MORT = number dead = INIT - SURV
PSURV = % survival = 100(SURV/INIT
PMORT = % mortality = 100(MORT/INIT)

Table C-2. Results of 96-hour sediment larval (*Mytilus galloprovincialis*) toxicity tests (NAS, September 27-29, 2007).

Location Code	Replicate	Initial	Normal	Abnorm	Total	% Mort	% Abnorm	% Comb	Norm % Mort	Norm % Comb		Normal	% Mort	% Abnorm	% Comb	Norm % Mort	Norm % Comb
PPTox01	1	270.8	206	8	214	20.97	3.74	23.93	13.71	13.30	Mean	202.40	20.75	5.77	25.26	13.47	14.81
PPTox01	2	270.8	203	6	209	22.82	2.87	25.04	15.73	14.56	S.D.	16.98	4.94	3.26	6.27	5.40	7.15
PPTox01	3	270.8	219	12	231	14.70	5.19	19.13	6.85	7.83	n	5.00	5.00	5.00	5.00	5.00	5.00
PPTox01	4	270.8	210	13	223	17.65	5.83	22.45	10.08	11.62							
PPTox01	5	270.8	174	22	196	27.62	11.22	35.75	20.97	26.77							
PPTox02	1	270.8	210	14	224	17.28	6.25	22.45	9.68	11.62	Mean	200.20	21.42	6.21	26.07	14.19	15.74
PPTox02	2	270.8	204	10	214	20.97	4.67	24.67	13.71	14.14	S.D.	31.15	9.09	6.27	11.50	9.92	13.11
PPTox02	3	270.8	193	1	194	28.36	0.52	28.73	21.77	18.77	n	5.00	5.00	5.00	5.00	5.00	5.00
PPTox02	4	270.8	240	7	247	8.79	2.83	11.37	0.40	-1.01							
PPTox02	5	270.8	154	31	185	31.68	16.76	43.13	25.40	35.19							
PPTox03	1	270.8	196	18	214	20.97	8.41	27.62	13.71	17.51	Mean	196.20	24.08	4.67	27.55	17.10	17.42
PPTox03	2	270.8	223	6	229	15.44	2.62	17.65	7.66	6.14	S.D.	21.46	7.01	3.20	7.93	7.65	9.03
PPTox03	3	270.8	200	4	204	24.67	1.96	26.14	17.74	15.82	n	5.00	5.00	5.00	5.00	5.00	5.00
PPTox03	4	270.8	199	5	204	24.67	2.45	26.51	17.74	16.25							
PPTox03	5	270.8	163	14	177	34.64	7.91	39.81	28.63	31.40							
PPTox05	1	270.8	206	7	213	21.34	3.29	23.93	14.11	13.30	Mean	224.20	14.92	2.72	17.21	7.10	5.64
PPTox05	2	270.8	240	8	248	8.42	3.23	11.37	0.00	-1.01	S.D.	18.58	6.54	0.99	6.86	7.14	7.82
PPTox05	3	270.8	215	5	220	18.76	2.27	20.61	11.29	9.51	n	5.00	5.00	5.00	5.00	5.00	5.00
PPTox05	4	270.8	212	8	220	18.76	3.64	21.71	11.29	10.77							
PPTox05	5	270.8	248	3	251	7.31	1.20	8.42	-1.21	-4.38							
PPTox06	1	270.8	224	8	232	14.33	3.45	17.28	6.45	5.72	Mean	210.00	18.39	5.01	22.45	10.89	11.62
PPTox06	2	270.8	212	8	220	18.76	3.64	21.71	11.29	10.77	S.D.	9.08	2.36	1.61	3.35	2.58	3.82
PPTox06	3	270.8	210	10	220	18.76	4.55	22.45	11.29	11.62	n	5.00	5.00	5.00	5.00	5.00	5.00
PPTox06	4	270.8	201	15	216	20.24	6.94	25.78	12.90	15.40							
PPTox06	5	270.8	203	14	217	19.87	6.45	25.04	12.50	14.56							
PPTox07	1	270.8	171	9	180	33.53	5.00	36.85	27.42	28.03	Mean	161.80	31.17	13.65	40.25	24.84	31.90
PPTox07	2	270.8	161	41	202	25.41	20.30	40.55	18.55	32.24	S.D.	33.03	10.58	7.72	12.20	11.55	13.90
PPTox07	3	270.8	203	18	221	18.39	8.14	25.04	10.89	14.56	n	5.00	5.00	5.00	5.00	5.00	5.00
PPTox07	4	270.8	163	22	185	31.68	11.89	39.81	25.40	31.40							
PPTox07	5	270.8	111	33	144	46.82	22.92	59.01	41.94	53.28							
PPTox08	1	270.8	178	21	199	26.51	10.55	34.27	19.76	25.08	Mean	188.40	26.14	5.81	30.43	19.35	20.71
PPTox08	2	270.8	178	12	190	29.84	6.32	34.27	23.39	25.08	S.D.	21.62	7.82	3.35	7.98	8.54	9.10

Location Code	Replicate	Initial	Normal	Abnorm	Total	% Mort	% Abnorm	% Comb	Norm % Mort	Norm % Comb		Normal	% Mort	% Abnorm	% Comb	Norm % Mort	Norm % Comb
PPTox08	3	270.8	227	9	236	12.85	3.81	16.17	4.84	4.46	n	5.00	5.00	5.00	5.00	5.00	5.00
PPTox08	4	270.8	178	3	181	33.16	1.66	34.27	27.02	25.08							
PPTox08	5	270.8	181	13	194	28.36	6.70	33.16	21.77	23.82							
PPTox09	1	270.8	163	16	179	33.90	8.94	39.81	27.82	31.40	Mean	191.00	26.44	4.13	29.47	19.68	19.61
PPTox09	2	270.8	180	1	181	33.16	0.55	33.53	27.02	24.24	S.D.	19.43	6.95	3.58	7.17	7.59	8.18
PPTox09	3	270.8	204	15	219	19.13	6.85	24.67	11.69	14.14	n	5.00	5.00	5.00	5.00	5.00	5.00
PPTox09	4	270.8	211	5	216	20.24	2.31	22.08	12.90	11.20							
PPTox09	5	270.8	197	4	201	25.78	1.99	27.25	18.95	17.09							
PPTox10	1	270.8	209	26	235	13.22	11.06	22.82	5.24	12.04	Mean	184.20	27.55	6.02	31.98	20.89	22.47
PPTox10	2	270.8	210	6	216	20.24	2.78	22.45	12.90	11.62	S.D.	26.57	10.87	3.82	9.81	11.87	11.18
PPTox10	3	270.8	169	6	175	35.38	3.43	37.59	29.44	28.87	n	5.00	5.00	5.00	5.00	5.00	5.00
PPTox10	4	270.8	148	15	163	39.81	9.20	45.35	34.27	37.71							
PPTox10	5	270.8	185	7	192	29.10	3.65	31.68	22.58	22.14							
swcontrol	1	270.8	247	10	257	5.10	3.89	8.79	-3.63	-3.96	Mean	237.60	8.42	4.20	12.26	0.00	0.00
swcontrol	2	270.8	237	8	245	9.53	3.27	12.48	1.21	0.25	S.D.	6.31	2.07	0.77	2.33	2.26	2.66
swcontrol	3	270.8	233	11	244	9.90	4.51	13.96	1.61	1.94	n	5.00	5.00	5.00	5.00	5.00	5.00
swcontrol	4	270.8	231	13	244	9.90	5.33	14.70	1.61	2.78							
swcontrol	5	270.8	240	10	250	7.68	4.00	11.37	-0.81	-1.01							

Initial = number of inoculated embryos (from average of zero-time counts) = 270.8
Normal = number normal
Abnorm = number abnormal
Total = Normal + Abnormal
% Mort = % mortality = 100((Initial - Total)/Initial)
% Abnorm = % abnormality = 100(Abnormal/Total)
% Comb = combined percent mortality and abnormality = 100((Initial - Normal)/Initial)
Norm % Mort = normalized percent mortality = 100(1 - (Total/SWTotal)),
where SW = mean total larvae in seawater controls = 248
Norm % Comb = normalized % combined mortality and abnormality = 100(1 - (Normal/SWNorm)),
where SWNorm = average of normal larvae counted in seawater controls = 237.6

Table C-3. Results of 20-day juvenile polychaete (*Neanthes arenicola*) toxicity tests.

(NAS, September 28 – October 18, 2007).

Location Code	Replicate	Initial Number	Dead Number	Weight Count	Initial Weight	Total Weight	Mean Indiv. Weight	Growth (mg/worm/day)		Total Weight	Mean Indiv. Weight	Growth (mg/worm/day)
PPTox01	1	5	0	5	0.448	44.09	8.818	0.419	Mean	66.45	13.86	0.67
PPTox01	2	5	1	4	0.448	56.66	14.165	0.686	S.D.	15.43	2.89	0.14
PPTox01	3	5	0	5	0.448	78.56	15.712	0.763	n	5	5	5
PPTox01	4	5	0	5	0.448	78.55	15.71	0.763				
PPTox01	5	5	0	5	0.448	74.41	14.882	0.722				
PPTox02	1	5	0	5	0.448	77.46	15.492	0.752	Mean	84.31	16.86	0.82
PPTox02	2	5	0	5	0.448	92.23	18.446	0.900	S.D.	12.73	2.55	0.13
PPTox02	3	5	0	5	0.448	81.48	16.296	0.792	n	5	5	5
PPTox02	4	5	0	5	0.448	101.45	20.29	0.992				
PPTox02	5	5	0	5	0.448	68.93	13.786	0.667				
PPTox03	1	5	0	5	0.448	114.63	22.926	1.124	Mean	92.09	17.95	0.88
PPTox03	2	5	0	5	0.448	82.15	16.43	0.799	S.D.	17.12	4.24	0.21
PPTox03	3	5	0	5	0.448	101.5	20.3	0.993	n	5	5	5
PPTox03	4	6	0	6	0.448	70.24	11.707	0.563				
PPTox03	5	5	0	5	0.448	91.92	18.384	0.897				
PPTox05	1	5	0	5	0.448	104.05	20.81	1.018	Mean	104.21	20.84	1.02
PPTox05	2	5	0	5	0.448	89.97	17.994	0.877	S.D.	10.18	2.04	0.10
PPTox05	3	5	0	5	0.448	102.17	20.434	0.999	n	5	5	5
PPTox05	4	5	0	5	0.448	106.4	21.28	1.042				
PPTox05	5	5	0	5	0.448	118.45	23.69	1.162				
PPTox06	1	5	0	5	0.448	74.7	14.94	0.725	Mean	88.93	17.79	0.87
PPTox06	2	5	0	5	0.448	93.39	18.678	0.912	S.D.	12.41	2.48	0.12
PPTox06	3	5	0	5	0.448	107.65	21.53	1.054	n	5	5	5
PPTox06	4	5	0	5	0.448	83.23	16.646	0.810				
PPTox06	5	5	0	5	0.448	85.66	17.132	0.834				
PPTox07	1	5	0	5	0.448	86.01	17.202	0.838	Mean	96.79	19.36	0.95
PPTox07	2	5	0	5	0.448	98.79	19.758	0.966	S.D.	10.55	2.11	0.11
PPTox07	3	5	0	5	0.448	113.33	22.666	1.111	n	5	5	5
PPTox07	4	5	0	5	0.448	96.17	19.234	0.939				
PPTox07	5	5	0	5	0.448	89.64	17.928	0.874				
PPTox08	1	5	0	5	0.448	82.98	16.596	0.807	Mean	77.49	16.93	0.82
PPTox08	2	5	0	5	0.448	74.1	14.82	0.719	S.D.	7.72	1.44	0.07

Location Code	Replicate	Initial Number	Dead Number	Weight Count	Initial Weight	Total Weight	Mean Indiv. Weight	Growth (mg/worm/day)		Total Weight	Mean Indiv. Weight	Growth (mg/worm/ day)
PPTox08	3	5	1	4	0.448	75.13	18.7825	0.917	n	5	5	5
PPTox08	4	5	0	5	0.448	87.41	17.482	0.852				
PPTox08	5	5	1	4	0.448	67.85	16.9625	0.826				
PPTox09	1	5	0	5	0.448	94.83	18.966	0.926	Mean	79.24	15.85	0.77
PPTox09	2	5	0	5	0.448	72.81	14.562	0.706	S.D.	11.76	2.35	0.12
PPTox09	3	5	0	5	0.448	64.71	12.942	0.625	n	5	5	5
PPTox09	4	5	0	5	0.448	86.59	17.318	0.844				
PPTox09	5	5	0	5	0.448	77.25	15.45	0.750				
PPTox10	1	5	0	5	0.448	77.92	15.584	0.757	Mean	74.28	16.17	0.79
PPTox10	2	5	0	5	0.448	87.11	17.422	0.849	S.D.	13.51	2.71	0.14
PPTox10	3	5	0	5	0.448	74.61	14.922	0.724	n	5	5	5
PPTox10	4	5	1	4	0.448	80.22	20.055	0.980				
PPTox10	5	5	1	4	0.448	51.55	12.8875	0.622				
control	1	5	0	5	0.448	138.51	27.702	1.363	Mean	93.12	19.37	0.95
control	2	5	0	5	0.448	111.23	22.246	1.090	S.D.	31.42	5.94	0.30
control	3	5	0	5	0.448	80.89	16.178	0.787	n	5	5	5
control	4	5	0	5	0.448	60.49	12.098	0.583				
control	5	5	1	4	0.448	74.5	18.625	0.909				

Weight Count = no. of worms weighed at test end
Initial Weight = mean day zero weight of worms (mg)
Total Weight = tared weight of worms in Weight Count
Mean Individual Weight = Total Weight / Weight Count
Growth = mean individual growth rate = (MIW/20)

Table C-4. Results of 5-minute and 15-minute Microtox luminosity tests using *Vibrio fischeri*. (CH2M Hill, October 1, 2007).

Batch	Client Sample Location	Replicate	Light Output		
			Initial Value	Final 5 Minutes	Final 15 Minutes
1	PPTOX01	A	117	112	108
1	PPTOX01	B	124	123	115
1	PPTOX01	C	116	124	114
1	PPTOX01	D	119	124	114
1	PPTOX01	E	120	120	114
1	PPTOX02	A	110	105	100
1	PPTOX02	B	118	116	107
1	PPTOX02	C	106	108	99
1	PPTOX02	D	101	101	97
1	PPTOX02	E	106	105	96
1	PPTOX03	A	66	68	66
1	PPTOX03	B	72	72	74
1	PPTOX03	C	70	67	70
1	PPTOX03	D	70	70	70
1	PPTOX03	E	66	65	69
1	PPTOX06	A	87	81	72
1	PPTOX06	B	88	84	73
1	PPTOX06	C	92	86	74
1	PPTOX06	D	93	87	79
1	PPTOX06	E	94	89	79
1	PPTOX10	A	102	102	99
1	PPTOX10	B	99	99	93
1	PPTOX10	C	89	89	84
1	PPTOX10	D	95	95	88
1	PPTOX10	E	95	95	91
1	control	A	81	68	55
1	control	B	78	65	57
1	control	C	88	71	59
1	control	D	82	69	57
1	control	E	82	72	59
2	PPTOX05	A	134	130	116
2	PPTOX05	B	129	125	114
2	PPTOX05	C	120	121	115
2	PPTOX05	D	126	125	110
2	PPTOX05	E	121	122	117
2	PPTOX07	A	95	87	75
2	PPTOX07	B	93	86	73
2	PPTOX07	C	96	89	75
2	PPTOX07	D	91	85	70
2	PPTOX07	E	92	87	74
2	PPTOX08	A	73	67	61
2	PPTOX08	B	78	72	67
2	PPTOX08	C	74	68	62
2	PPTOX08	D	73	68	61
2	PPTOX08	E	72	65	60
2	PPTOX09	A	106	107	96
2	PPTOX09	B	106	103	96
2	PPTOX09	C	102	105	97

Batch	Client Sample Location	Replicate	Light Output		
			Initial Value	Final 5 Minutes	Final 15 Minutes
2	PPTOX09	D	104	103	95
2	PPTOX09	E	100	102	93
2	PPTOX10	A	117	119	107
2	PPTOX10	B	106	104	100
2	PPTOX10	C	98	96	91
2	PPTOX10	D	103	104	93
2	PPTOX10	E	98	97	94
2	control	A	91	76	61
2	control	B	84	72	57
2	control	C	91	78	62
2	control	D	84	72	58
2	control	E	86	73	59

Appendix D. Additional analyses

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Table D-1. Descriptive statistics for 2007 study of sediment toxicity near Post Point.

	Total Solids (%)	Sand (%)	Silt (%)	Clay (%)	Fines (%)	TOC (%)	Total Sulfides (mg/kg)
N of cases	9	9	8	8	9	9	9
Minimum	31.4	8.7	7.1	8.6	2.8	0.24	5.95
Maximum	78.7	96.7	73.1	44.2	84.6	2.54	2630
Range	47.3	88	66	35.6	81.8	2.3	2624
Sum	441.8	365.5	292.2	221.6	516.6	14.18	8997
Median	41.1	24.1	38.1	32	73.9	2.11	793
Mean	49.09	40.61	36.53	27.70	57.40	1.58	1000
95% CI Upper	62.11	66.77	54.05	40.77	82.91	2.25	1683
95% CI Lower	36.07	14.45	19.00	14.63	31.89	0.90	316
Std. Error	5.65	11.35	7.41	5.53	11.06	0.29	296
Standard Dev	16.94	34.04	20.96	15.63	33.19	0.88	889
Variance	286.8	1158.5	439.4	244.3	1101.7	0.78	791076
C.V.	0.35	0.84	0.57	0.56	0.58	0.56	0.89
Skewness	0.71	0.88	0.22	-0.35	-0.90	-0.43	0.60
SE Skewness	0.72	0.72	0.75	0.75	0.72	0.72	0.72
Kurtosis	-0.90	-1.27	0.36	-2.10	-1.30	-1.73	-0.52
SE Kurtosis	1.40	1.40	1.48	1.48	1.40	1.40	1.40
SW Statistic	0.90	0.79	0.95	0.82	0.76	0.87	0.91
SW P-Value	0.23	0.02	0.72	0.05	0.01	0.11	0.34
Percentile values:							
5%	31.4	8.7	7.1	8.6	2.8	0.24	5.95
10%	32.4	11.3	8.5	8.8	8.2	0.41	47.2
25%	35.0	17.5	22.0	10.2	21.1	0.67	178
50%	41.1	24.1	38.1	32.0	73.9	2.11	793
75%	62.0	77.5	46.0	42.2	82.0	2.22	1600
90%	74.7	91.0	66.3	43.8	84.0	2.52	2230
95%	78.7	96.7	73.1	44.2	84.6	2.54	2630

TOC = Total organic carbon
CI = Confidence Interval
Dev = Deviation
C.V. = Coefficient of Variation

Table D-2a-d. Spearman rank correlations between parameters measured for 2007 study of sediment toxicity near Post Point.

n = sample count, df = degrees of freedom
Values in *italics* = $p < 0.10$, in **bold** = $p < 0.05$, and in ***bold italics*** = $p < 0.02$.

a. This 2007 study, excluding reference sample: n = 8, df = 6

	Neanthes growth	*Mytilus* NCMA	Microtox luminosity	*Eohaustorius* mortality	Total Sulfides (mg/L)	TOC (%)	Fines (%)	Clay (%)	Silt (%)	Sand (%)	Total Solids (%)
Neanthes growth	1										
Mytilus NCMA	0.228	1									
Microtox luminosity	*-0.695*	-0.429	1								
Eohaustorius mortality	-0.187	-0.443	*0.683*	1							
Total Sulfides (mg/L)	0.275	0	0.048	0.443	1						
TOC (%)	*0.623*	0.143	-0.548	-0.204	*0.667*	1					
Fines (%)	0.012	0.214	0.19	0.287	0.548	0.476	1				
Clay (%)	0.12	-0.381	0.143	0.263	**0.762**	**0.714**	0.524	1			
Silt (%)	0.132	*0.667*	-0.286	-0.06	0.214	0.357	**0.714**	-0.024	1		
Sand (%)	-0.012	-0.286	-0.048	-0.156	-0.524	-0.524	***-0.976***	-0.476	**-0.786**	1	
Total Solids (%)	-0.252	0.119	-0.048	-0.323	***-0.881***	**-0.786**	**-0.762**	***-0.905***	-0.31	**0.738**	1

b. This 2007 study, including reference sample: n = 9, df = 7

	Fines (%)	TOC (%)
Neanthes growth	-0.293	0.134
Microtox luminosity	-0.167	**-0.683**
Mytilus NCMA	0.45	0.4
Eohaustorius mortality	0.502	0.159
Total Sulfides (mg/L)	**0.683**	**0.767**

c. This 2007 study, including reference sample: n = 9, df = 7

	Total Sulfides (mg/L)
Neanthes growth	-0.109
Microtox luminosity	-0.267
Mytilus NCMA	0.3
Eohaustorius mortality	*0.611*

d. Blakley (2004) and this study: n = 19, df = 17

	Total Sulfides (mg/L)
Microtox luminosity	-0.327
Mytilus NCMA	***0.566***
Eohaustorius mortality	***0.562***

Table D-3. Linear regression results for 2007 Post Point sediment toxicity study.

Independent variable or predictor (X)	Dependent variable or predicted (Y)	r	Slope	Intercept
Total solids (%)	Fines (%)	-0.97	-1.88	144
Fines (%)	TOC (%)	0.97	0.0247	0.348
Total solids (%)	Total sulfides (mg/L)	-0.82	-41.1	3073
Fines (%)	Total sulfides (mg/L)	0.84	21.8	-74.5
TOC (%)	Total sulfides (mg/L)	0.81	820	-274
Total sulfides (mg/L)	*Eohaustorius* mortality	0.54	0.0042	8.96
Total sulfides (mg/L)	*Mytilus* NCMA	0.75	0.0065	9.91
Total sulfides (mg/L)	*Neanthes* growth	-0.16	$-1.9x10^{-05}$	0.87
Total sulfides (mg/L)	Microtox luminosity	-0.29	-0.0067	95.4

Calculation of levels of H_2S as a fraction of total sulfides.

Toxic hydrogen sulfide dissolved in water dissociates into ionic forms:

$$H_2S = H^+ + HS^-$$

At normal acidity (pH), the equilibrium favors the non-ionic species:

$$([H^+] \times [HS^-]) / [H_2S] = K = 10^{-7.02} = 0.0000000955$$

The level of hydrogen sulfide can be calculated by:

$$[H_2S] = ([H^+] \times [HS^-]) / K$$

But $[HS^-]$ is not measured or known, so the fraction of total sulfides that is toxic $[H_2S]$ can be expressed as a ratio:

$$[H_2S] / [HS^-] = [H^+] / K$$

which depends on the pH of the sample ($-\log[H^+]$)

Percent H_2S is given by:

$$\% H_2S = 100 \times (([H_2S] / ([H_2S] + [HS^-]))$$

where $[HS^-]$ can be expressed in terms of $[H_2S]$ by rearranging the previous equation

Table D-4. Calculation of % total sulfides in porewater that is highly toxic H_2S.

pH	$[H^+]$	K	$[H^+]/K$	$[H_2S]/[HS^-]$	$\%[H_2S]$
6	0.0000010000	0.0000000955	10.471285	10.5	91.3
7	0.0000001000	0.0000000955	1.047129	1.05	51.2
7.25	0.0000000562	0.0000000955	0.588844	0.59	37.1
7.5	0.0000000316	0.0000000955	0.331131	0.33	24.9
7.75	0.0000000178	0.0000000955	0.186209	0.19	15.7
8	0.0000000100	0.0000000955	0.104713	0.10	9.5
8.25	0.0000000056	0.0000000955	0.058884	0.059	5.6
8.5	0.0000000032	0.0000000955	0.033113	0.033	3.2
8.75	0.0000000018	0.0000000955	0.018621	0.019	1.8
9	0.0000000010	0.0000000955	0.010471	0.010	1.0

Final calculations were made using test pH and K values that reflected test temperatures and salinities (Savenko, 1977).