Squalicum Harbor (Bellingham Bay) Tributyltin (TBT) Investigation

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Cover photo: Inner Squalicum Harbor Marina, Bellingham Bay, Bellingham, Washington (photo by Nigel Blakley)

Squalicum Harbor (Bellingham Bay) Tributyltin (TBT) Investigation

by
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April 2007

Waterbody Number: WA-01-0050

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Acronyms and Abbreviations

CSL	Cleanup Screening Level
MEL	Manchester Environmental Laboratory
PSEP	Puget Sound Estuary Protocols
QA/QC	Quality Assurance and Quality Control
RI/FS	Remedial Investigation and Feasibility Study
RPD	Relative Percent Difference
RSD	Relative Standard Deviation
SMS	Sediment Management Standards
SOP	Standard Operating Procedure
SQS	Sediment Quality Standard
TBT	Tributyltin
TOC	Total Organic Carbon
WAC	Washington Administrative Code

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Abstract

During 2005, sediments from the inner portion of the Squalicum Harbor Marina in Bellingham were analyzed for tributyltin (TBT) in bulk sediment and sediment pore water. The highest TBT concentrations occurred close to an area of the marina where bottom scraping and painting of boats has been conducted.

Regulatory limits for TBT concentrations in bulk sediment have not been established. However, TBT concentrations detected in this study were not higher than proposed regulatory limits for TBT concentrations in pore water.

Biological effects of the TBT contamination were examined. The potential for TBT bioaccumulation was studied using *Macoma* clams exposed to the sediment under controlled laboratory conditions. TBT concentrations in the clam tissues did not exceed levels of concern found in the scientific literature.

The abundance and diversity of benthic marine invertebrates (e.g., clams, worms) at sediment sampling locations were evaluated. Invertebrate abundance and diversity were reduced at sampling stations with higher TBT concentrations in sediment (0.12-0.26 mg/kg dw, and 1.16 mg/kg in one subsample) compared to stations with lower concentrations (0.04 mg/kg or less). However, there may be other factors correlated with the TBT concentrations that affect these invertebrate communities. These results indicate a need to further investigate the effects of TBT contamination in sediments on benthic invertebrate communities.

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- Mike Stoner of the Port of Bellingham and Squalicum Harbormaster Mike Endsley were extremely helpful with arrangements for sampling under difficult conditions in the marina.
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- David Hope of Pacific Rim Laboratories, Inc. conducted the TBT analyses.
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    - Gene Ruff performed taxonomic work (Annelida).
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    - Pete Adolphson, Mary O'Herron and Dale Norton provided helpful review comments. The final report was formatted by Joan LeTourneau.

Introduction

Background

Squalicum Harbor, in Bellingham Bay, Washington (Figure 1), contains a marina with tributyltin (TBT)-contaminated sediments. A Remedial Investigation and Feasibility Study (RI/FS) of the harbor's inner boat basin reported up to 10.68 mg TBT ion/Kg in dry sediment, and up to 1.1 µg TBT ion/L in sediment pore water (ThermoRetec, 2001). The area of contamination described in the report surrounds two boat lifts in the western portion of the marina formerly operated by Marine Services Northwest[1] for small boat maintenance, including bottom scraping and repainting.

Regulatory limits for TBT have not been established under Washington State's Sediment Management Standards regulation (SMS; Chapter 173-204 WAC). However, TBT is classified as a "deleterious substance" under the SMS and may be regulated case-by-case using biological or other appropriate testing methods.

A report on issues relating to the development of SMS standards for TBT (Michelsen et al., 1996) has proposed regulatory criteria that are based on pore water concentrations, rather than on bulk sediment. The report proposed a pore water concentration of 0.05 µg/L as "conceptually equivalent" to the Sediment Quality Standard (SQS). Under the SMS regulation, SQS are "no adverse biological effects" levels used as a sediment quality goal for Washington State sediments.

The SMS regulation also establishes Cleanup Screening Levels (CSLs), which are "minor adverse effects" levels used as an upper regulatory level for source control and as minimum cleanup levels. The recommended TBT equivalent for the CSL is 0.15 µg/L in pore water.

Three stations sampled in the Marine Services Northwest RI/FS investigation had pore water TBT concentrations higher than these recommended TBT SQS and CSL values. These stations near the Marine Services Northwest boat lifts also had the highest bulk sediment TBT concentrations.

Under the tiered SMS sediment evaluation process, exceedance of a numerical CSL can be overridden by a demonstration that biological effects criteria are not exceeded. Similarly, a finding of no exceedances, based on chemical criteria, can be overridden by a demonstration of adverse biological effects exceedances. However, biological effects of the marina TBT-contaminated sediments were not evaluated in the 2000/2001 Marine Services Northwest RI/FS investigation.

[1] The boat lifts are currently operated by Boondocks Boats and Motors, a division of Marine Services Northwest, Inc. The location property owner is the Port of Bellingham.

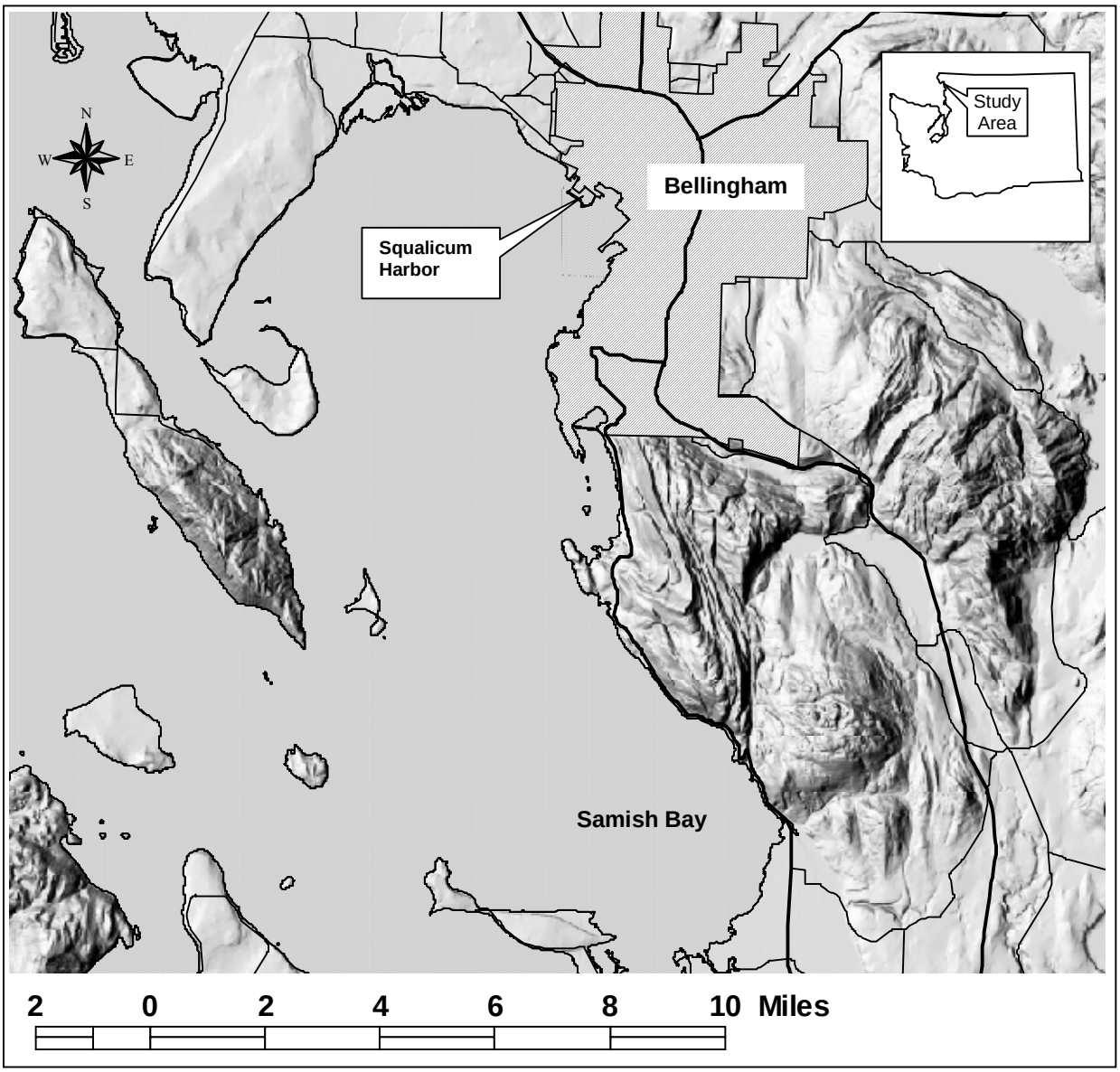

Figure 1: Location Map for Squalicum Harbor

Study Objectives

This investigation was requested by the Washington State Department of Ecology Bellingham Field Office to assist with sediment cleanup decisions. The results complement data from the Marine Services Northwest RI/FS sampling conducted in 2000 (ThermoRetec, 2001).

The primary objectives of the investigation were to (1) supplement the limited data available on TBT bulk and pore water concentrations in the sediments, and (2) evaluate the effects of the TBT contaminated sediment on benthic infauna.

Additional objectives were to (1) obtain toxicity and tissue bioaccumulation information that may be useful to the Department of Ecology in developing appropriate methods for regulating TBT contamination under the SMS regulation, and (2) understand the relationship between benthic infaunal effects, bioaccumulation, and TBT concentrations in bulk sediment and pore water. The importance of data from TBT biological testing conducted in connection with cleanup projects has been noted in a Puget Sound Dredge Disposal Analysis (PSDDA) Issue Paper (Michelsen et al., 1996).

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Methods

Sampling Design

The sampling locations chosen for this investigation are shown in Figure 2, including a reference station (SH8) in Samish Bay about 11 miles south of the study area. The objective in selecting the locations was to sample from a gradient of TBT sediment concentrations previously documented in the study area (ThermoRetec, 2001). The Samish Bay reference station location was selected because it has been previously found to be uncontaminated and supporting a healthy benthic invertebrate community (Long et al., 1999).

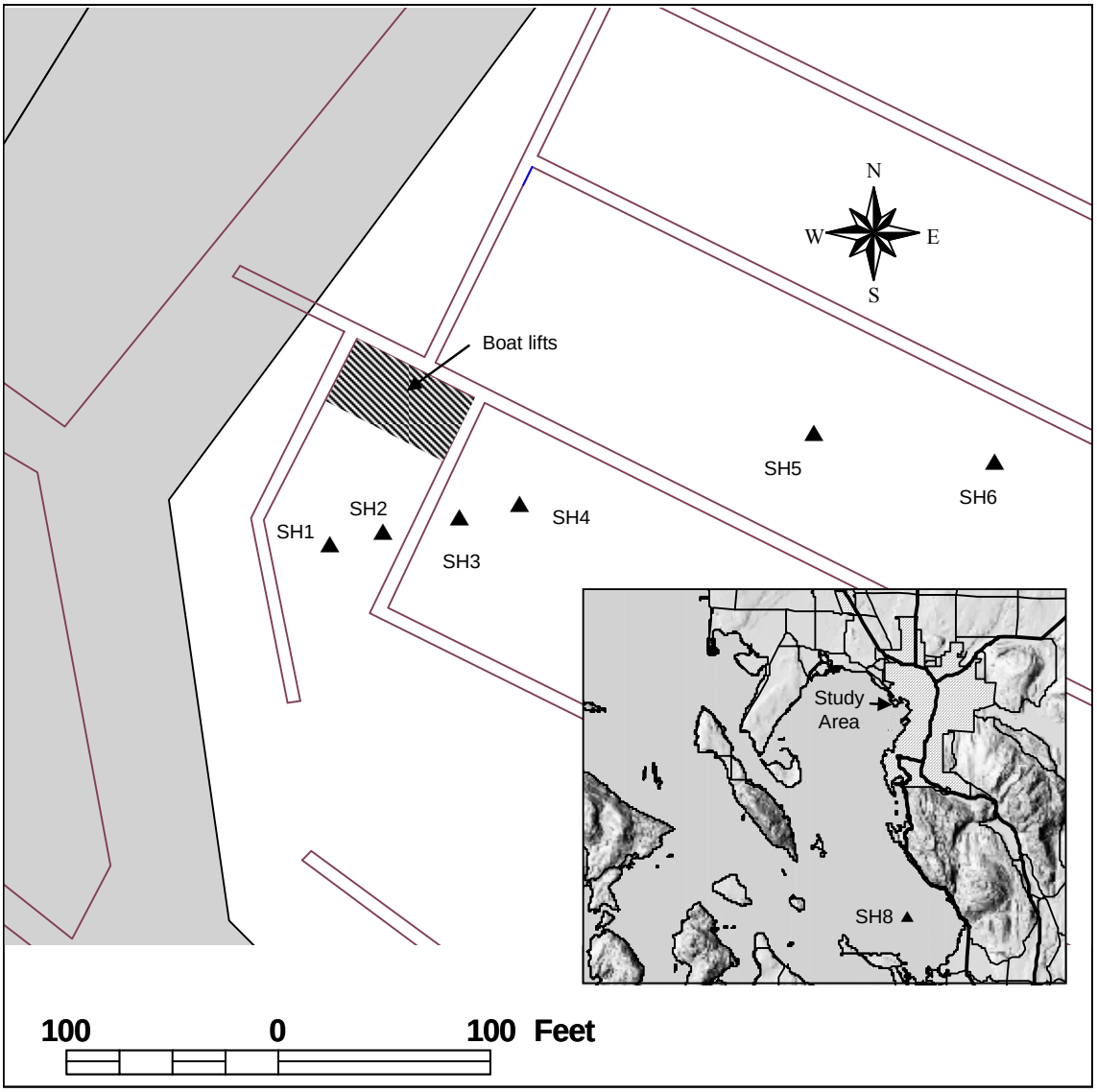

Figure 2. Inner Squalicum Harbor Marina Sampling Stations and SH8 Reference Station Location (Inset).

Sampling Methods

Sampling was conducted September 19-21, 2005. Where applicable, sampling methods followed Puget Sound Estuary Protocols (PSEP, 1996) and requirements of Ecology's Sediment Management Standards (Chapter 173-204 WAC; Ecology 2003). Samples were collected from Ecology's 26-foot research vessel *R.V. Skookum* using a grab sampler deployed and retrieved with a hydraulic winch. Station positions were located using a Northstar GPS (Global Positioning System) Receiver with differential correction. A field log, with location information and physical descriptions of the samples collected, was maintained during sampling (Appendix A).

All utensils used to manipulate the samples (stainless steel spoons and mixing bowls) were precleaned by washing with Liquinox® detergent, followed by sequential rinses with tap water, deionized water, and pesticide-grade acetone. The equipment was then air-dried and wrapped in aluminum foil until used in the field. The grab sampler was precleaned with Liquinox® detergent and deionized water. Between stations, the grab sampler was cleaned by thoroughly brushing with on-site seawater.

Sediment samples were collected with a double 0.1 m^2 stainless-steel modified vanVeen grab sampler. To ensure synoptic data, sediment for chemical analyses was collected simultaneously with sediment collected for the benthic community analyses. Upon retrieval of the sampler, the contents were visually inspected to determine if the sample was acceptable (jaws closed, no washout, clear overlying water, sufficient depth of penetration). If the sample was unacceptable, it was dumped overboard at a location away from the station.

Sediment was removed from the grab using a precleaned stainless-steel scoop. One 0.1 m^2 grab sample from one side of the sampler was collected for chemical analyses. From the other side of the sampler, sediment was removed for the benthic infaunal analyses. All infaunal samples were rinsed gently through a 1.0 mm screen, and the organisms retained on the screen were preserved in the field with a 10% aqueous solution of borax-buffered formalin.

The sampler was deployed and retrieved three times at each station. Each grab was taken within a 30-ft. radius of the station coordinates recorded in the field log (Appendix A). Sediment collected from each of the grabs for chemical analyses was combined in a stainless steel bucket and thoroughly mixed using a precleaned, stainless-steel stirring blade attached to an electric drill. The composited sample was then transferred to glass containers and stored in a cooler with ice. Sediment collected from each of the grabs for benthic infaunal analyses was not combined, so that three infaunal samples were obtained at each station.

A field duplicate sample was taken at one station (SH3). Two grabs were collected, and sediment from both sides of the sampler was composited for chemical analyses. None of the field duplicate sediment was retained for benthic infaunal analysis.

At three of the stations (SH2, SH4, and SH6), two additional grabs were taken to obtain sediment for the clam (*Macoma*) bioaccumulation test. For both grabs, sediment from both sides of the sampler was collected and transferred to a 5-gal. HDPE (high density polyethylene) bucket. After delivery to the laboratory, the contents of each bucket were thoroughly mixed and an aliquot taken for TBT analysis prior to beginning the bioaccumulation test. The procedure followed for this test is described in Appendix B.

Analytical Methods

Table 1 summarizes the analytical methods and laboratories used in this study. All samples were analyzed by Manchester Environmental Laboratory (MEL) or by accredited contract laboratories selected by MEL.

Table 1. Analytical Test Methods.

Parameter	Method	Laboratory
Grain Size	PSEP	ARI
Total Organic Carbon	PSEP-TOC (reported on a dry weight basis at 70°C)	MEL
Percent Solids (sediment, clam tissue)	Standard Methods 2540G	MEL
Butyltins (bulk sediment)	Extraction: Jiang et al., 1996. Analysis: SW-846 method 8270-SIM	MEL
Butyltins (pore water)	Pore water extraction: see Appendix C GC-MS: Unger et al., 1986; Unger, M.A., 1996	Pacific Rim
Butyltins (clam tissue)	Extraction: SW-846 method 8323 Analysis: SW-846 method 8270-SIM	MEL

ARI Analytical Resources, Inc. Tukwila, WA.
MEL Manchester Environmental Laboratory (Washington State Department of Ecology, Manchester, WA)

Bioaccumulation testing of sediment samples was conducted with the bent-nose clam (*Macoma nasuta*) under controlled laboratory conditions using a 45-day exposure protocol (Battelle, 2000). Battelle Marine Sciences Laboratory conducted the exposure phase of the testing, with some modifications to the protocol, as described in the laboratory's report (Appendix B). MEL analyzed the clam tissue for TBT following the exposure phase of the test. Data were reported by MEL on a wet-weight basis. Percent solids data also reported by MEL were then used to convert the TBT concentrations to a dry-weight basis.

Data Quality

Physical/Chemical

Data quality was assessed through analysis of field duplicates, laboratory replicates, laboratory control samples, and matrix spikes. Procedural blanks were analyzed to assess laboratory contamination.

Laboratory data quality assessments, based on the reporting laboratories' quality assurance procedures and criteria, are provided in the Case Narratives (Appendix E). With the exception of non-detects, no data quality qualifier flags are attached to any of the sample data for TBT in sediment or clam tissue. However, all sample results for pore water are flagged as estimates (J) because of a potential positive bias due to background contamination.

Data quality objectives established for this project in the sampling plan (Blakley, 2005) were met with the following exceptions (Appendix D, Table D-1):

- Grain size. The precision objective (RSD $\leq$ 20%) was not met for the gravel size class.
- Bulk sediment TBT. The precision objective (RPD $\leq$ 20%) was not met; the RPD from two laboratory duplicates was 72%. TBT recovery in the laboratory control sample was 78%, slightly below the objective of 80-120%.
- Clam tissue TBT. TBT recovery was low in the laboratory control samples (42-53%). This is below the objective of 80-120% but within laboratory in-house limits of 40-130% (Appendix E). Recovery was higher in analyses of a certified reference material (67-69%) and matrix spikes (77-87%).
- Pore water TBT. The precision objective was not met (RPD = 33%, compared with a goal of $\leq$ 20%). TBT recovery in the laboratory control sample was 72%, below the objective of 80-120%. Recovery was higher (97%) for analyses of matrix spike samples. These recovery estimates do not account for potential losses of TBT during pore water extraction from sediment, suggesting that measurements may underestimate actual concentrations. However, there may also be a positive bias in the measurements due to background TBT contamination (detected in the blank), as noted earlier. Holding time for the samples was 16 days, which exceeded the SOP limit of two weeks. However, the laboratory report suggests that the extended holding time is unlikely to affect data quality (Appendix E).

Biological

Sorting QA/QC procedures for benthic invertebrates consisted of resorting 25% of each sample by a second sorter to determine whether a sample sorting efficiency of 95% removal was met. For one sample (SH8), the sampling efficiency was 90.35% and the entire sample was resorted. All other samples met the QA/QC requirement, with efficiencies ranging from 97.25% to 100%.

Results

Data from this study are available electronically from Ecology's Environmental Information Management System database at www.ecy.wa.gov/eim/ and will be included in Ecology's Sediment Quality Information System (SEDQUAL).

Physical Characteristics of Sediments

Sediment samples from the marina consisted of thick, black, silty clay with little variation in visual appearance. The samples also showed little variation in TOC (1.9 – 2.1%; Table 2). Silt and clay were the predominant grain size fractions at all of the marina stations. Solids content ranged from 33-43%. In contrast, the reference station (SH8) sediment had a high sand content (95%), low TOC (0.3%), and high solids content (76%).

Table 2. Sediment Sample Grain Size, Percent Solids and Total Organic Carbon Content.

Station ID	Lab ID	% Solids	TOC (%)	Grain Size (%)			
				Gravel	Sand	Silt	Clay
SH1	384020	34**	1.99	0.1*	10.5*	48.6*	40.8*
SH2	384021	33	2.13	0.0	8.8	47.3	43.9
SH3	384022	38	2.10	0.3	8.3	44.0	47.5
SH3 (field duplicate)	384026	39	1.85	0.4	6.8	44.1	48.7
SH4	384023	40	1.98	0.0	7.1	41.6	51.4
SH5	384024	44	2.04*	0.0	16.2	35.7	48.0
SH6	384025	43	1.97	0.4	13.6	34.8	51.2
SH8 (Samish Bay)	384027	76	0.31	0.0	94.7	2.8	2.5

* Mean of three laboratory replicates.
** Mean of two laboratory replicates.

Tributyltin Concentrations

Sediment and Pore Water

TBT was found in the marina sediments at concentrations ranging from 0.04 to 0.26 mg/kg but was not detected at the SH8 reference station in Samish Bay (Table 3). Within the marina, the two stations closest to the Marine Services Northwest boat lifts, SH1 and SH2, had concentrations an order of magnitude higher than the furthest stations, SH5 and SH6. Variability within locations can be high; SH3 had a concentration of 0.13 mg/kg, while the field duplicate sample taken there had 0.26 mg/kg.

TBT concentrations in sediment pore water ranged from 0.032 µg/L at SH2 to below the detection limit of 0.018 µg/L at other stations. The two stations closest to the boat lifts, SH1 and SH2, had the highest concentrations. The recommended pore water standard of 0.05 µg/L (Michelsen et al., 1996) was not exceeded at any of the stations.

Table 3. Tributyltin Concentrations (as Ion) in Sediment and Pore Water.

Station ID	Bulk sediment (mg/kg dw)	Pore Water** (µg/L)
SH1	0.24	0.027
SH2	0.26	0.032*
SH3	0.13	<0.018
SH3 (field duplicate)	0.26	0.025
SH4	0.12*	0.018
SH5	0.04	<0.018
SH6	0.05	0.020
SH8 (Samish Bay)	<0.002	<0.018

* Mean of two laboratory replicates.
** All pore water data are flagged J: the analyte was positively identified; the associated numerical value is the approximate concentration of the analyte in the sample.

Clam Tissue

TBT concentrations in clams that were experimentally exposed to sediment from the stations under controlled laboratory conditions are show in Table 4.

Prior to the setup of the clam testing chambers, an aliquot of sediment was taken from each sample container and analyzed for TBT. TBT concentrations measured from the sediment samples followed the pattern noted in Table 3, with the highest concentration coming from the station closest to the boat lifts (SH2). However, the value for SH2 (1.16 mg/kg) was considerably higher than the previously measured concentration of 0.26 mg/kg (Table 3), although both analyses were from aliquots of the same original grab sample.

TBT concentrations were highest in clams exposed to the most contaminated sediment (SH2), with a mean tissue concentration of 0.56 µg/kg dw. For the two other test sediments, clams exposed to SH6 sediment had a mean concentration of 0.11 µg/kg, higher than for SH4 (0.06 µg/kg) although the sediment TBT concentrations were similar. TBT was only detected in clams that were exposed to sediments from the marina stations.

Within sediment exposure regimes, clam sample replicates exhibited relatively low variability in TBT concentrations in comparison with differences between regimes. Differences between the three test sediments in their effect on clam TBT levels are therefore almost certainly biologically significant.

Table 4. TBT Tissue Concentrations in Clams (*Macoma nasuta*) after 45-day Laboratory Exposure to Sediment Samples.

Sediment Exposure Regime	Sediment Test Sample[a] TBT (mg TBT ion/ kg dw)	Clam Replicate Sample	Tissue TBT (µg TBT ion/ kg ww)	% solids	Tissue TBT (mg TBT ion/ kg dw)	Mean	RSD[b]
No sediment exposure[c]	—	1	<2.58	16		—	—
		2	<2.40	16			
		3	<2.49	17			
Control sediment[d]	Not analyzed	1	<2.58	15		—	—
		2	<2.58	15			
		3	<2.49	15			
		4	<2.49	15			
		5	<2.67	15			
SH2 sediment	1.16	1	98.02	16	0.61	0.56	15.4%
		2	75.74	16	0.47		
		3	106.93	16	0.67		
		4	89.11	16	0.56		
		5	75.74	16	0.47		
SH4 sediment	0.08	1	9.80	15	0.07	0.06	21.2%
		2	8.29	16	0.05		
		3	8.91	16	0.06		
		4	8.55	16	0.05		
		5	13.37	16	0.08		
SH6 sediment	0.07	1	17.82	17	0.10	0.11	20.4%
		2	12.03	16	0.08		
		3	17.82	16	0.11		
		4	23.17	17	0.14		
		5	18.71	16	0.12		

a Analysis of aliquot taken from sediment sample used in clam exposure testing. Aliquots were not analyzed for TOC.
b RSD - Relative standard deviation
c Tissue from depurated clams not exposed to test sediment
d Sequim Bay sediment

Benthic Invertebrate Community

Benthic invertebrate diversity and abundance were lower at stations with higher TBT concentrations (SH1-SH4) than those with low (SH5-SH6) or undetectable (SH8) concentrations (Table 5 and Figure 3). This pattern is consistent for all of the phyla examined.

Table 5. Benthic invertebrate diversity and abundance.

	SH1	SH2	SH3	SH4	SH5	SH6	SH8
Bulk sediment TBT (mg/kg dw)	0.24	0.26 (1.16 in sample used for clam testing)	0.13 (0.26 in field duplicate)	0.12	0.04	0.05	<0.002
Diversity (# of species)							
Crustacea	3	4	3	2	7	5	28
Mollusca	6	3	6	5	25	23	24
Annelida	8	9	8	11	27	35	43
Other phyla - # of phyla	1	0	0	1	3	4	5
# of species	1	0	0	1	7	5	15
Abundance (# of individuals)							
Crustacea	4	6	10	6	320	14	1,168
Mollusca	121	61	146	223	922	764	1,759
Annelida	1,521	1,960	1,427	4,152	6,184	6,157	2,332
Other phyla	2	0	0	2	45	26	179
Total abundance – all phyla	1,648	2,027	1,583	4,383	7,471	6,961	5,438

Pooled data for 3 replicates per station, each replicate volume = 0.017 m^3
Raw data from the taxonomic analysis of the benthic invertebrate samples is provided in Appendix F.

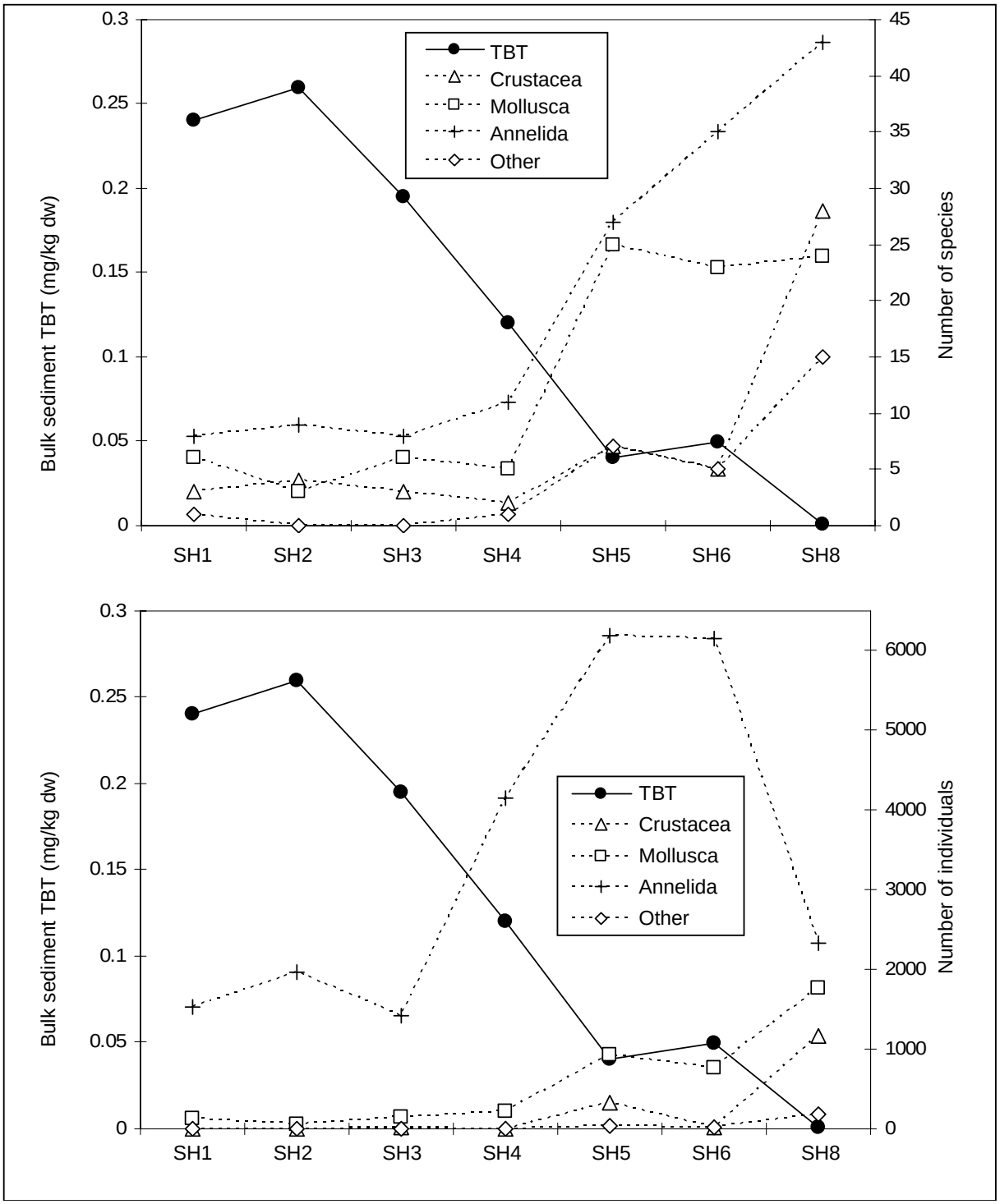

Figure 3. Relationship Between Bulk Sediment TBT Concentration and Benthic Invertebrate Diversity (upper graph) and Abundance (lower graph). Data from Table 5. The TBT concentration for station SH3 is the mean of two field duplicate samples (0.13 and 0.26 mg/kg).

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Discussion

Results from this study show some association between TBT concentrations in sediment, pore water, and clam tissue (Figure 4). However, the relationships between these variables are difficult to quantify because of the variability in sediment and pore water measurements, coupled with small sample sizes.

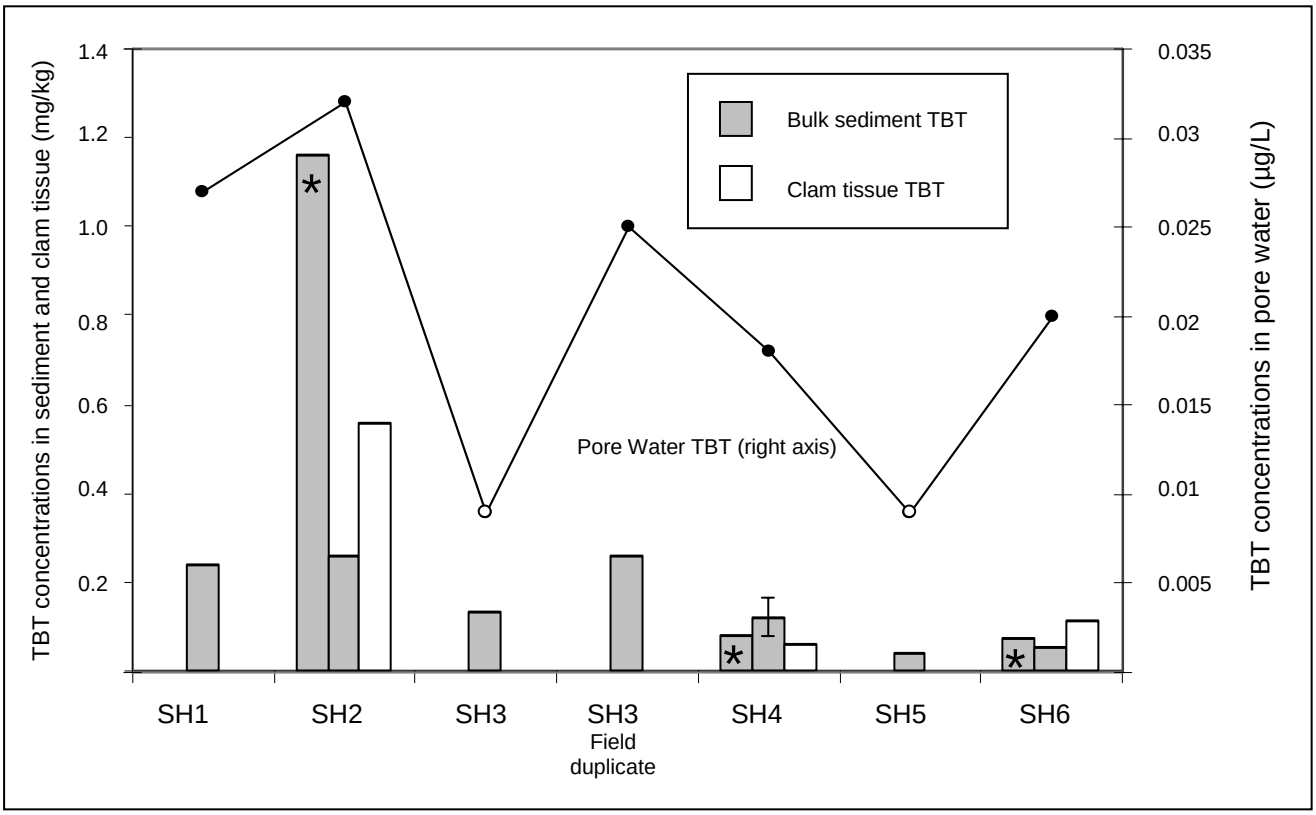

Figure 4. Comparison of TBT Concentrations from Sediment, Pore Water and Clam Tissue. Clam tissue data are available only for Stations SH2, SH4 and SH6.
* TBT concentration in sediment used for clam bioaccumulation study (Table 4).
○ TBT not detected in pore water; value shown is half the detection limit.
I High and low values for laboratory duplicates (SH4).

The high variability in sediment TBT measurements is evident from data for SH3 and SH2. At SH3 the TBT concentration was 0.13 mg/kg, while the field duplicate concentration was twice as high (Table 3). At SH2 the concentration was 0.26 mg/kg (Table 3) but much higher in an aliquot from the SH2 subsample used for the clam exposure test (1.16 mg/kg, Table 4). TBT associated with particulates, such as antifouling paint chips, might account for this variability if the particulates are not uniformly distributed throughout the sediment.

For pore water, TBT concentrations were generally near the detection limit (0.018 µg/L), and this may have contributed to measurement variability. For example, the concentration at SH3 was reported to be below the detection limit, while the field duplicate was just above the limit (0.025 µg/L). Moreover, the laboratory duplicates for SH2 were also near the detection limit and exceeded the project objective for variability in pore water TBT measurements (RPD ≤ 20%).

Despite the data limitations, results from this study are consistent with previous evidence for a gradient in sediment TBT levels centered near the marina boat lifts (Figure 5).

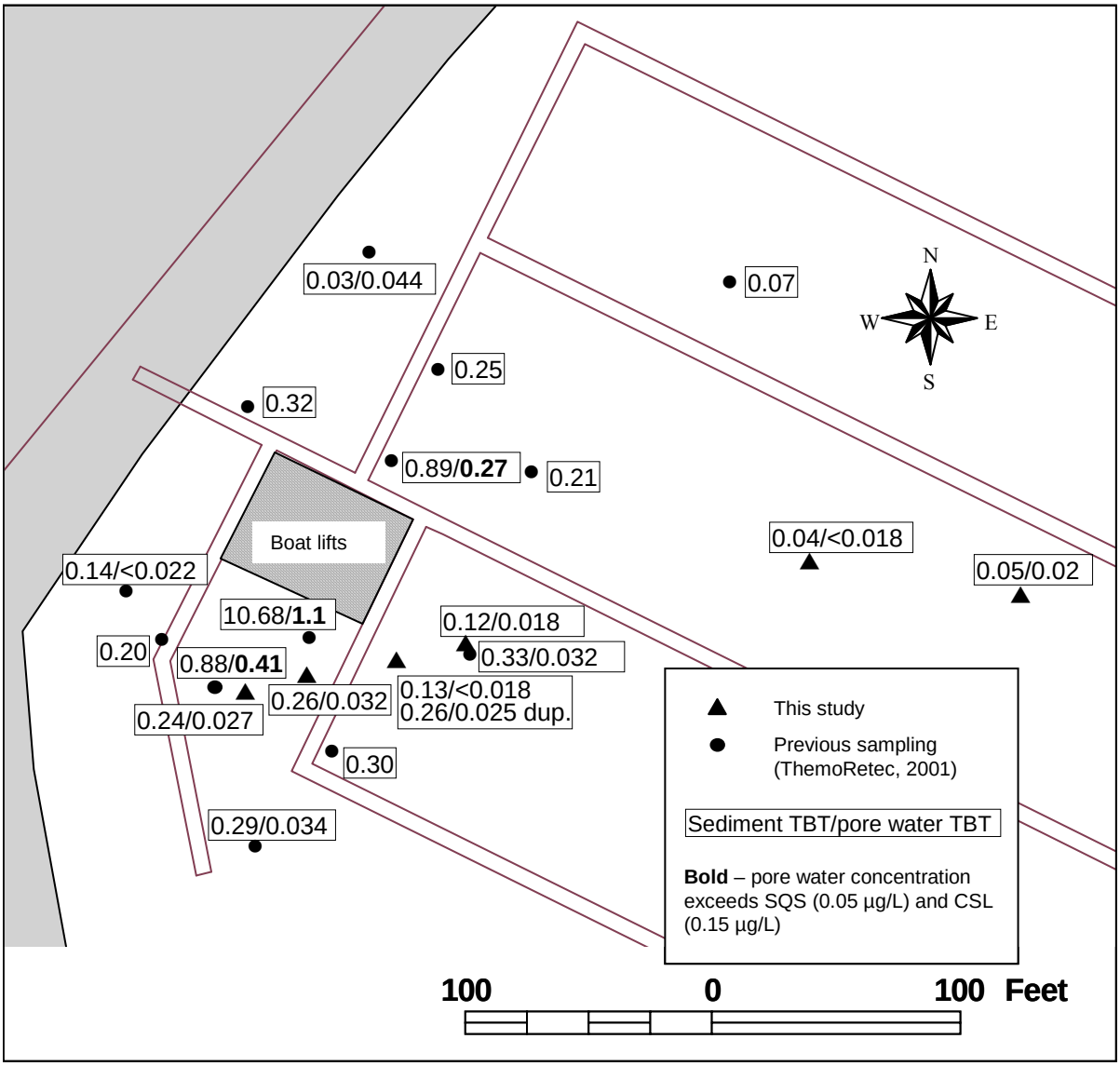

Figure 5. Comparison of TBT Sediment Concentrations (mg/Kg dw) and Pore Water Concentrations (µg/L) from this 2005 Study and Previous Sampling (ThermoRetec, 2001). Pore water data are not available for all stations.

As noted earlier, criteria based on concentrations in sediment pore water, rather than bulk sediment, have been recommended in a review of issues relating to the development of regulatory standards for TBT (Michelsen et al., 1996). The report recommended a value of 0.05 µg/L as conceptually equivalent to the Sediment Quality Standard (SQS). Under the Sediment Management Standards (SMS) regulation, SQSs are *no adverse biological effects* levels used as a sediment quality goal for Washington State sediments. The regulation also establishes Cleanup Screening Levels (CSLs), which are *minor adverse effects* levels used as an upper regulatory level for source control and as minimum cleanup levels. The recommended TBT equivalent for the CSL is 0.15 µg/L.

None of the stations sampled exceeded either the proposed CSL or SQS values for TBT. However, three stations near the boat lifts have previously been reported to have pore water TBT concentrations exceeding both the SQS and CSL values (Figure 5). These stations also had higher sediment TBT concentrations than found in this investigation, possibly due to a patchy distribution of particulates such as anti-fouling paint chip fragments.

Tissue concentrations in clams exposed to marina sediments under laboratory conditions were also below levels of concern. Chronic effects levels have been reported in the literature at 2-12 mg TBT/kg body weight on a dry-weight basis (Michelsen et al., 1996), while the maximum clam tissue concentration during this investigation was 0.61 mg/kg dw (Table 4).

In contrast, at stations with TBT concentrations in sediment of 0.12-0.26 mg/kg, there was evidence for adverse biological effects on benthic invertebrate communities. Both invertebrate abundance and diversity were reduced at these stations relative to those with less TBT in sediments (0.04-0.05 mg/kg). Station SH8 had no detectable TBT in sediment and even higher invertebrate diversity and abundance. However, the benthic community characteristics at this station were likely due at least partly to the sandy substrate, which differed considerably from the silty clay found at all the marina stations.

Differences between stations in physical characteristics of marina sediments were minor (Table 2) and therefore do not account for the variability in invertebrate community characteristics. However, the possibility of other confounding effects on the invertebrate communities, such as toxicity from chemical pollutants co-occurring with TBT, cannot be discounted. For example, elevated concentrations of copper and other metals have been found at some locations close to the Marine Services Northwest boat lifts. At one location near the lifts, the Sediment Management Standards were exceeded for copper, mercury, and zinc (Appendix G).

Although this report focuses on comparisons of benthic community data with proposed SMS standards for TBT, it should be noted that the SMS regulation also includes SQS and CSL criteria for benthic abundance. These effects-based criteria are not chemical-specific and are based on comparison of test and reference sediment samples.

Analyses of benthic abundances using the effects-based SQS and CSL criteria are presented in Table 6. Stations SH1, SH2, and SH3 exceeded both the SQS and CSL criteria (described in the table footnotes).

Table 6. Statistical Analysis of Benthic Invertebrate Abundance from Replicate Grabs at each Station (not available at SH6 and SH8).

Taxon	Grab	Station						
		SH1	SH2	SH3	SH4	SH5	SH6	SH8
Crustacea	Replicate 1	4	3	3	1	3	--	--
	Replicate 2	0	3	7	5	316[a]	--	--
	Replicate 3	0	0	0	0	1	--	--
	Total	4	6	10	6	320	14	1,168
	Mean	1.3	2.0	3.3	2.0	106.7	--	--
Mollusca	Replicate 1	38	11	66	72	233	--	--
	Replicate 2	57	31	78	89	476	--	--
	Replicate 3	26	19	2	62	213	--	--
	Total	121	61	146	223	922	764	1,759
	Mean	40.3*	20.3*	48.7*	74.3	307.3	--	--
Polychaete	Replicate 1	804	1121	651	1684	2180	--	--
	Replicate 2	175	704	664	1188	1741	--	--
	Replicate 3	542	135	112	1280	2263	--	--
	Total	1521	1960	1427	4152	6184	6157	2,332
	Mean	507.0*	653.3*	475.7*	1384.0*	2061.3	--	--
Exceeds SQS criteria?		Yes	Yes	Yes	No	Not applicable		
Exceeds CSL criteria?		Yes	Yes	Yes	No	Not applicable		

[a] Sample contained a rock with 312 attached barnacles (*Balanus*).
* Mean abundance significantly lower than at SH5 ($p<0.05$, one-tailed t-test).
SQS and CSL evaluations are based on comparisons with SH5 as a reference station.

SQS criterion: The test sediment has less than 50% of the reference sediment mean abundance of any one of the following major taxa: Class Crustacea, Phylum Mollusca or Class Polychaeta, and the test sediment abundance is statistically different (t test, $p \leq 0.05$) from the reference sediment abundance. [WAC 173-204-320(3)(c)]

CSL criterion: The test sediment has less than 50% of the reference sediment mean abundance of any two of the following major taxa: Class Crustacea, Phylum Mollusca, or Class Polychaeta and the test sample abundances are statistically different (t test, $p<0.05$) from the reference abundances. [WAC 173-204-520(3)(d)(iii)]

Stations SH6 and SH8 could not be included in the analysis because replicate values are needed for a t-test comparison with the reference station. At these two stations, invertebrates from replicate grabs were inadvertently combined prior to taxonomic identification and enumeration. Of the remaining stations, SH5 was used as the reference for all comparisons because it was the most distant from the presumed center of the TBT contamination (Figure 2) and had low levels of TBT (Table 3).

Conclusions and Recommendations

Conclusions

Results from this 2005 investigation suggest that sediments containing elevated bulk concentrations of TBT (0.12 to 0.26 mg/kg) may exhibit adverse effects on benthic invertebrate communities even if TBT concentrations in pore water do not exceed 0.05 ug/L. However, the observed effects may have been due in part to other contaminants not measured in this chemical testing program.

TBT concentrations in sediment pore water and clam tissue show an association with TBT concentrations in bulk sediment. However, a larger sample size and improved precision for bulk sediment and pore water measurements would be needed to quantify these relationships.

Results from this investigation suggest that TBT in marine sediments may have adverse effects on benthic invertebrate communities, even at TBT concentrations below currently proposed regulatory criteria. Although these effects may be due in part to co-occurring stressors, including metals contamination, TBT contamination is unlikely to occur in isolation from other anthropogenic (human-caused) stressors because this chemical is typically associated with areas used for commercial and recreational maritime activities.

Recommendations

As a result of this study, the following recommendations are made:

- In sediments with elevated bulk concentrations of TBT, evaluation of potential toxicity using benthic infaunal abundance may be appropriate, even if TBT concentrations in sediment pore water are not above 0.05 ug/L.

- In future analyses of TBT in bulk sediment and pore water, both measurements should be made by the same laboratory on the same sediment material, if possible. This procedure should reduce variability in measurements resulting from separate handling and transportation of sediment and pore water samples.

- Sediment samples should be divided into laboratory replicates for TBT analyses. This precaution will ensure, for example, that a sample will not be mischaracterized on the basis of a single, small paint chip with a high TBT content.

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Appendices

A. Sampling Station Location Information (Field Log)

B. Battelle 45-Day Bioaccumulation Test Procedure

C. Pore Water Extraction Procedure

D. Data Quality Assessment

E. Case Narratives

F. Benthic Invertebrate Data

G. Remedial Investigation and Feasibility Study Metals Data

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Appendix A. Sampling Station Location Information

Table A-1. Field Log Notes.

Station	Latitude	Longitude	Grab #	Depth[b] (ft)	Date	Time	Grab Penetration (cm)	Comments
	Degrees decimal minutes[a]							
SH1	122º 30.1135	48º 45.4847	1	15.5	9/20/05	9:25	14	Black silty clay. Faint sulfur odor.
			2			10:00	16	
			3			10:40	15	
SH2	122º 30.1074	48º 45.4860	1	12	9/20/05	12:00	14.5	Black silty clay. Thin (~1 mm) overlying fluffy layer. Faint odor.
			2			12:30	15.5	
			3			13:05	16	
SH3	122º 30.0985	48º 45.4871	1	15.7	9/20/05	16:55	13.5	Black silty clay. Thin (~2 mm) overlying fluffy layer. Faint odor.
			2			17:55	14.5	
			3			18:50	13.0	
SH4	122º 30.0915	48º 45.4881	1	15.2	9/21/05	16:55	14.5	
			2			17:15	12	
			3			17:35	15	
SH5	122º 30.0572	48º 45.4944	1	15.3	9/21/05	9:15	12	Black silty clay. Fluff layer on top.
			2			9:35	11	
			3			9:50	13	
SH6	122º 30.0359	48º 45.4923	1	12.5	9/21/05	12:30	12	Heavy gray-black silty clay. 2 cm. fluff layer on top.
			2			13:30	10	
			3			13:45	10	
SH7	Field duplicate at SH3		1	17.2	9/20/05	19:05	7	
			2			19:35	11	
SH8 (Samish Bay Reference Station)	122º 29.3611	48º 36.2020	1	21	9/19/05	18:05	7	Sandy brown. Shells, worm tubes.
			2			18:20	6	
			3			18:30	4	

a NAD83
b Actual depths at sampling time.

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Appendix B. Battelle 45-Day Bioaccumulation Test Procedure

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45-DAY SEDIMENT BIOACCUMILATION TESTING

45-DAY SEDIMENT BIOACCUMULATION TESTING

1.0 SCOPE AND APPLICATION

This is a procedure for conducting a 45-day bioaccumulation test exposure with the bent-nose clam *Macoma nasuta* and the polychaete *Nephtys caecoides.* The purpose of this test is to determine the bioaccumulation potential of metals and organic contaminants in tissues by comparing uptake of selected contaminants in test sediments to that observed in reference sediments. This procedure is adapted from the document, *Evaluation of Dredged Material Proposed for Ocean Disposal Testing Manual* (EPA/USACE 1991) and the EPA Guidance Manual, *Bedded Sediment Bioaccumulation Tests* (EPA 1989).

2.0 RESPONSIBLE STAFF

MSL Staff
- MSL Manager
- Project Manager
- MSL Laboratory Supervisor
- Quality Assurance Representative

3.0 PROCEDURE

3.1 Source of Seawater

All seawater used for test organism acclimation and holding, as well as for the dilution water during exposures, is natural seawater pumped directly from Sequim Bay, Washington. Sequim Bay water is passed through a sand filter (2-mm pore size) prior to entering the test chambers. Sequim Bay seawater is routinely analyzed for a suite of potential contaminants and has been shown to be free of contaminants.

3.2 Test Organisms

3.2.1 Procurement and Acclimation. *Macoma* and *Nephtys* are obtained from commercial suppliers in Discovery Bay, Washington and Dillon Beach California, respectively. Procurement of test organisms should be coordinated to ensure that organisms are acclimated for a minimum of 48 hours prior to test initiation. The organisms and sediment should be shipped in clean coolers containing native sediment and seawater by overnight delivery service.

Record data pertaining to the receipt of organisms and native control sediment on a Test Organism Receipt and Acclimation Record form. Upon receipt of the test animals, perform water quality measurements [dissolved oxygen (DO), pH, temperature, and salinity] on the seawater in the shipping containers and record results on the Test Organism Receipt and Acclimation Record form. Either transfer the test organisms into a suitable holding tank with a flow-through system at the same temperature as the shipping seawater or keep in the same shipping container for acclimation. Gradually acclimate to testing conditions listed in 3.5.1. Temperature and salinity should increase or decrease at a rate of < 1°C/h and <0.5‰/h until the test parameters are reached

3.2.2 Care and Maintenance. After acclimating the organisms to test conditions, start the flow-through system and hold the test organisms in clean sediment at 12-15°C for a minimum of 48 hours prior to testing. Under these conditions, no additional feeding is necessary during holding. If mortality in excess of 10% occurs within 24 hours immediately prior to test initiation, do not use the stock for testing. In addition, the stock should not be used if the animals appear stressed, diseased, or exhibit other abnormal behavior.

3.3 Sediment Samples

Upon receipt, check all samples against the chain-of-custody forms. Note the temperature and condition at the time of receipt. All samples are held in the dark at 4°C ± 2°C until they are needed for testing. Sediment samples should be used for testing as soon as possible, but no later than 8 weeks after collection.

Press-sieve both the reference and control sediment through a 1.0-mm screen to remove large debris and any live organisms.

Each test, reference, or control sediment sample should be given a randomly allocated sediment-treatment code, which will be used for identification during toxicity testing and chemical analysis.

3.4 Preparation of Test System

3.4.1 Materials. Whenever possible, materials that contact the test water or sediment should be glass, fluoroplastics (Teflon), silicone (adhesive, tubing, and stoppers), or nylon. Use of these materials will minimize either adsorption of toxicants onto equipment or leaching of chemical compounds from the equipment into test water. Before use, clean all equipment that may have direct contact with the test material in accordance with MSL-C-011.

Condition other materials used in water delivery or in holding and acclimation (concrete, fiberglass, or PVC) by continuous flushing with seawater prior to use. Apply silicone adhesives in a manner that minimizes direct contact with test solutions.

3.4.2 Test Chambers. Test chambers are 10-gal glass aquaria. Five replicate containers are needed for each test, reference, and control sediment unless otherwise specified by the project plan. All test containers shall be positioned randomly. Be sure containers are labeled with their position numbers, sediment treatment code number (see section 3.3), and replicate number.

3.4.3 Sediment Preparation. Test sediment samples should be kept refrigerated (4°C ± 2°C) until needed for testing. Prior to testing, all test sediments should be homogenized in the original sample container or a $MeCl_2$-rinsed, large stainless-steel bowl using a $MeCl_2$-rinsed, stainless-steel spoon.

Before adding sediment treatments to containers, turn the dripper arms to stop water flow, and pull the standpipe to reduce the water volume in the tank to approximately 8 L. Once the volume is reduced, replace the standpipe, but do not allow water to flow into the tank.

Add sediment into each test chamber by measuring out approximately 1 gal into a clean glass container. Check the sediment treatment code number and the position number on the tank label before gently transferring the sediment into the aquarium. Using the seawater in the aquarium, wash and distribute the sediment evenly. The sediment in each test chamber should be approximately 3-cm deep. Take care to avoid contamination of neighboring test aquaria. Use clean transfer containers for each sediment treatment.

Once sediment has been added to each test chamber, start the seawater flow-through system into each test chamber at a flow rate of 125 mL/min ± 10 mL and leave overnight.

3.5 Test Initiation

3.5.1 Water Quality Parameters and Test Conditions. Prior to introducing the animals into the test, water quality measurements (DO, pH, salinity, and temperature) should be performed on each replicate test chamber. Record all results of measurements on Water Quality Monitoring forms. Water quality during testing should fall within the following ranges for these parameters:

DO	≥5.0 mg/L
pH	7.8 ± 0.5 units
Temperature	15.0 ± 2.0 C
Salinity	30.0 ± 2.0‰
Flow rate	125 mL/min ± 10 mL

Gentle aeration should be supplied to all test chambers; adjust the air flow to avoid sediment resuspension.

3.5.2 Addition of Test Animals. The bioaccumulation test is initiated when 30 *Macoma* and 45 to 50 *Nephtys* are placed randomly in each test chamber (30 individuals in each of 5 replicate test chambers for a total of 150 clams and 45 to 50 individuals in each of 5 replicate test chambers for a total of 220 to 250 worms per test treatment). *Macoma* and *Nephtys* can be placed in the same test chamber. Handle organisms as quickly and gently as possible. If any organisms are injured or damaged during transfer from the holding tanks, replace them immediately. **NOTE: Organisms that have not buried within 4 h may be replaced.**

3.5.3 Collection of Background Tissue Samples. Reserve 3 sets of 30 *Macoma* and 45 to 50 *Nephtys* from the unexposed test population for background tissue samples. Set up a flow-through seawater system in a 10-gal aquarium as a depuration tank. Allow the tank to equilibrate for 24 h before adding the animals. Clams can be placed in baking dishes above the depuration tank with the worms. Depurate the organisms for 24 h. Animals are not fed during depuration. After depuration, scrub the clam shells and dissect the tissue using a titanium scalpel. Worms are left as whole organisms. Divide the tissue among separate sample containers for analysis of trace metals and organic compounds. **NOTE: Sampling containers, instruments, and utensils used for samples to be analyzed for metals and organic compounds must be prepared according to established cleaning procedures**.

3.5.4 Initial Tissue Weight

If the change in biomass during the 45-d test is a programmatic requirement, record the wet weight of the background tissues prior to their placement in chemistry sample containers. Special care should be taken during shucking of the clams to retain as much of the adductor muscle as is possible. Also, record the general condition of the *Nephtys*, including any missing appendages or damage to soft body parts.

3.6 Test Monitoring and Maintenance

3.6.1 Biological Monitoring

Monitor organisms in each test chamber daily. Record all observations on a Daily Observation Form.

For clams, the observations include the number of dead (removed daily), the number of animals with gaping shells that are unable to close their shells when prodded (the adductor muscle is not functional), and the number of siphons exposed. For worms, the observations include the number of dead (removed daily), the number on the surface and the number of tails or heads protruding from the sediment. The following criteria are used for determination of death: absence of movement of respiratory and feeding appendages, white opaque coloration, and lack or response to gentle prodding. In addition, note the presence of turbidity, precipitates, or any other unusual condition. **NOTE: Test organisms are not fed during the test**.

3.6.2 Water Quality Monitoring.

After test initiation, measure DO, pH, salinity, temperature, and flow rate daily in at least one replicate of each test treatment. Rinse water quality instruments with clean seawater before placing them in the next test chamber to avoid contamination between chambers. Record all results of measurements on Water Quality Monitoring forms. Reporting units for these parameters will be as follows:

DO	0.1 mg/L
pH	0.01 Unit
Temperature	0.1 C
Salinity	0.5‰
Seawater flow rate	1 mL/min

Water quality parameters should stay within the ranges listed in Section 3.5.1. If any of the parameters listed in 3.5.1 are out of range, the Project Manager or Task Leader shall be notified. Staff members shall document the corrective action taken to correct the out-of-range exceedences and the duration that the test parameters were out of range.

3.6.3 Sediment Additions

Once every 7 d, add 175 mL of each respective test sediment to the appropriate test chamber. Prior to adding sediment supplements, stop flow to the aquaria. Sediment additions are performed by measuring 175 mL of sediment in 1-L container, then adding seawater (at test conditions) to create a slurry. This slurry is then poured over

the bedded sediment in the test container. Once any suspended material has settled, flow should be restored. Flow should not remain off for more than 4 to 6 h.

3.7 Test Termination

3.7.1 By Day 44 of the bioaccumulation test, set up a flow-though seawater system in a 10-gal aquarium and a baking dish as depuration containers for each replicate of each test chamber to be terminated. Allow each container to equilibrate for 24 h.

3.7.2 On Day 45 of the bioaccumulation test, take final water quality measurements in all test chambers.

3.7.3 Terminate the 45-d test by gently sieving the test sediment with a 0.5-mm screen in clean seawater. Sieving can be facilitated by gently spraying seawater over the sediment. Carefully transfer test organisms into glass dishes containing seawater. Special care should be taken with *Nepthys* to ensure that soft body parts or appendages are not damaged during the termination process. Note the number of live and dead *Macoma* and *Nepthys*. Also note general condition and whether any *Nepthys* have missing appendages or are damaged in any way.

Transfer all surviving organisms from the test treatments to their respective depuration tanks. For worms, set up a flow-through seawater system in a 10-gal aquarium as a depuration tank. Allow the tank to equilibrate for 24 h before adding the animals. Clams can be placed in baking dishes above the depuration tank with the worms. Allow the animals to depurate for 24 h. Animals are not fed during depuration. Note whether any animals have died during depuration.

3.7.4 After depuration, scrub the clam shells and dissect the tissue using a titanium scalpel. Divide the tissue among separate sample containers for analysis of trace metals and organic compounds. **NOTE: Sampling containers, instruments, and utensils used for samples to be analyzed for metals and organic compounds must be prepared according to established cleaning procedures.**

3.8 Final Tissue Weight

If the change in biomass during the 45-d test is a programmatic requirement, record the wet weight of ten individuals (*Macoma and Nephtys*) from each test container prior to their placement in chemistry sample containers. Individuals for final tissue weight should be randomly selected. Special care should be taken during shucking of the clams to retain as much of the adductor muscle as is possible. Also, record the general condition of the *Nephtys*, including any missing appendages or damage to soft body parts. It is important that the methods used for initial weights are used for the final weights (eg. the same microbalance, the same observer).

3.8 Test Validity

A test is considered unacceptable if insufficient tissue is available for analysis. If any one of the following occurs, an explanation (including determination of impact of the test) needs to be provided by the Project Manager.

- Organism mortality in the native sediment control exceeds 30%.
- Test was not randomized
- No reference (if required) or control sediment was used
- Water quality parameters are out of range or not measured

4.0 DATA ANALYSIS AND CALCULATIONS

4.1 Data Analysis

The results of the bioaccumulation test should be analyzed appropriately according to the following methods:

Analysis of Variance (ANOVA) comparing chemical concentrations in tissues exposed to each sediment treatment to those of the reference treatments
Dunnett's or Tukey's HSD - statistical comparison among sediment treatments as well as to reference(s) or control(s)
45-d change in individual weight (wet weight)

Report the range for the measurements of water-quality parameters (DO, pH, temperature, and salinity) and flow rates.

4.2 Documentation

Keep all laboratory records, test results, measurements, and other supporting documentation for each sediment test in a Laboratory Record Book or project file dedicated to that purpose.

4.3 Reporting

A report should be prepared including, but not limited to, the following information:

Sources of test organisms, sediment samples, and seawater
Description of test methods and test organisms
Summary of water quality conditions for each sediment treatment
Summary of ANOVA and Dunnet's or Tukey's HSD results for all sediment treatments
Summary of any deviations from the project test plan
Copies raw data, observations, or data forms generated during the test

5.0 QUALITY CONTROL

5.1 Sample Custody

Custody of sediment and tissue samples should be documented using chain-of-custody forms in accordance with MSL-A-002. These forms should be initiated at the time of sample collection and signed by testing laboratory personnel at the time of sample receipt. The chain-of-custody should continue to laboratories conducting chemical analyses of the samples.

5.2 Quality Assurance Verification Activities

Routine assessments should be conducted by the MSL's Quality Assurance Representative to ensure that all aspects of the testing accurately reflect the work that was planned and completed, and that all necessary information, as defined by regulations, SOPs, or program-specific plans, is included. Results of assessments shall become a part of the project files.

6.0 SAFETY

6.1 Personal Safety

Laboratory personnel should have access to data on potential risks of working with contaminated sediment and various hazardous substances, and procedures for minimizing accidents with these materials. Appropriate attire, including lab coats and protective gloves, should be worn when working in the vicinity of test materials, test solutions, or test apparatus.

6.2 Disposal of Test Sediment

Because of the likelihood that this bioassay procedure will be used to study contaminated sediments, any sediment remaining after testing should be disposed of properly. The client or funding agency should make arrangements with the testing laboratory to receive and/or dispose of surplus test sediment.

7.0 TRAINING REQUIREMENTS

All staff members who will be performing Sediment Bioaccumulation Tests shall first read this SOP prior to working on the test. Documentation of training shall be recorded on a training assignment form from SOP MSL-A-006.

8.0 REFERENCES

U.S. Environmental Protection Agency and U.S. Army Corps of Engineers. 1991. *Evaluation of Dredged Material Proposed for Ocean Disposal Testing Manual*. EPA-503/8-91/001.

U.S. Environmental Protection Agency. 1989. *Guidance Manual: Bedded Sediment Bioaccumulation Tests*. EPA-600/X-89/302.

MSL-Q-004 Quality of Testing Water and Feed
MSL-C-011 Glassware and Equipment Cleaning Procedure
MSL-M-055 Animal Receipt, Acclimation, and Holding
MSL-A-002 Sample Chain of Custody
MSL-A-006 Marine Sciences Laboratory Training

Appendix C. Pore Water Extraction Procedure

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DETERMINATION OF TRIBUTYLTINS IN SOIL, WATER AND TISSUE

0. REFERENCE METHOD

Ikonomou, M.G.; Fernandez, M.; He, T.; Cullon, D. A Gas Chromatography - High Resolution Mass Spectrometry (GC-HRMS) Based Method For The Simultaneous Determination Of Nine Organotin Compounds In Water, Sediment And Tissue. *J. Chrom. A*, **2002**, *975(2),* 319-333

Humphrey, B. & Hope, D. (1987) Analysis of water, sediments and biota for organotin compounds. In: *Proceedings of the Organotin Symposium, Oceans '87 Conference, Halifax, Nova Scotia, Canada, 28 September-1 October, 1987,* New York, The Institute of Electrical and Electronics Engineers, Inc., Vol. 4, pp. 1348-1351.

1. INTRODUCTION

Tributyltin (TBT) is the active ingredient of many products that act as biocides against a broad range of organisms. It is primarily used as an antifoulant paint additive on ship and boat hulls, docks, fishnets, and buoys to discourage the growth of marine organisms such as barnacles, bacteria, tubeworms, mussels and algae.

TBT by itself is unstable and will break down in the environment unless it is combined with an element such as oxygen. One of the most common TBT compounds is bis(tributyltin) oxide, or TBTO. This form has been the subject of most TBT testing.

TBTO and eight additional TBT compounds are registered for use as marine antifoulants. Other TBT compounds are used as disinfectants, fungicidal wood preservatives, textile disinfectants, and stabilizers in PVC resin. Paper and pulp mills, cooling towers, breweries, textile mills and leather-processing facilities may also use some forms of TBT. Collectively these compounds are referred to as organotins.

2. SCOPE

This method is applicable to the quantitative determination of butyltins in soil, water and tissue.

Applicable Matrices:

Liquids/Waters
Solids/Soils
Tissue

Analytes:

Tributyltin (TBT)
Dibutyltin (DBT)
Monobutyltin (MBT)

3. PRINCIPLE

Organotin compounds have a backbone of the tetravalent tin IV molecule. When fully alkylated, that is for tetrabutyltin and tributyltin oxide (TBT-O-TBT), this molecule acts

like a typical organic compound and is easy to extract into solvent. However, as the molecule becomes less organic, as in dibuyltin, it develops a stronger inorganic affinity for water and is difficult to extract. Monobutyltin exhibits very little organic tendencies and is not extractable from aqueous media with solvent alone. In this method, assistance is provided in the form of tropolone, $C_7H_60_2$ (2-hydroxy-2,4,6-cycloheptatriene-l-one) a ligand. The seven member ring uses the adjacent hydroxy-ketone functional groups like a set of jaws to "grab" onto the tin molecules (more chemically correct is that it uses hydrogen bonding).

Once extracted into solvent, the tins must be fully alkylated before they are chromatographable on a GC. The compounds are reacted with a sodium tetraethyl borate and derivatized to ethyl-butyltins. Finally, the tropolone and other polar compounds are removed by columning on silica gel. Final analysis is by gas chromatography - mass spectroscopy using selected ion monitoring (SIM) to separate the individual analytes for identification and quantitation.

In this method waters are liquid-liquid extracted with tropolone-spiked dichloromethane (DCM) and solids shaken with tropolone-spiked ether:hexane (80:20). Tissue is solublized with TMAH and subsequently extracted with tropolone spiked ether:hexane (80:20).

4. METHOD VALIDATON

4.1 *Method Detection Limit*

4.1.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero. The MDL concentrations were obtained using organic-free water and sand spiked with a Butyltin standards mix at a value 10 x the estimated MDL.

4.1.2 The method detection limits for the analytes are dependent upon the nature of interferences in the sample matrix and the boiling point range which it spans.

Table 1. Method Detection Levels of Analytes

Analyte	Water Detection Limit (µg/L)	Soil Detection Limit (mg/kg)	Tissue Detection Limit (mg/kg)
Monobutyltin	0.005	0.001	0.02
Dibutyltin	0.002	0.001	0.02
Tributyltin	0.001	0.001	0.02

4.2 *Linear Range*

For 1 µL injections, the response of the mass spectrometer has been demonstrated to be linear in the range of concentrations from 1 to 4000 ppb. Concentrations >4000 ppb require dilutions into the working range of calibration.

5. SUPPORTING DOCUMENTS

none

6. INTERFERENCES

6.1 *Glassware*

6.1.1 All glassware must be clean and free of potentially interfering contaminants. Glassware must be rinsed with appropriate solvents before and after use.

6.1.2 Wash glassware with soap and hot water and rinse well with tap water prior to use. If glassware is required before it can adequately dry, rinse with acetone, hexane and dichloromethane.

6.1.3 Clean dry glassware must be stored in a clean place.

6.2 *Solvents, Reagents, and other laboratory hardware*

6.2.1 Solvents must be 'Pesticide Grade' or equivalent. (i.e. distilled in glass - DIG)

6.2.2 Reagents must be >99% pure and kept free of contamination.

6.2.3 GC carrier gas, inlet parts, detectors, and column surfaces must be maintained with minimum contamination.

6.2.4 Other hardware including syringes, extract concentrators, sampling equipment, and transferring apparati must be free of contamination.

6.3 *Sample Co-extractive Interferences*

6.3.1 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences depends on the nature of the sample.

6.3.2 Components co-eluting with and having fragments with m/z same as target compounds are potential sources of interference.

7. SAMPLE REQUIREMENTS

7.1 *Solids*

7.1.1 A minimum of 100 g of soil sample should be collected in a 125 mL glass jar with a Teflon-lined screw cap and iced or refrigerated at approximately 4°C from time of collection to the time of sample preparation. Store samples away from direct sunlight.

7.1.2 All solid samples should be extracted within 14 days after sampling and analyzed within 40 days after extraction (refrigerated).

7.2 *Liquids*

7.2.1 A 1 L water sample should be collected in a 1L amber glass bottle with a Teflon-lined screw cap and iced or refrigerated at approximately 4°C from time of collection to the time of sample preparation. Preserve samples to pH <2 with 1 mL HCl.

7.2.2 All samples should be extracted within 14 days after sampling and analyzed within 28 days after extraction (refrigerated).

7.3 *Tissue*

7.3.1 A minimum of 25 g of tissue sample should be collected in a 125 mL glass jar with a Teflon-lined screw cap and frozen at <-10°C from time of collection to the time of sample preparation. Store samples away from direct sunlight.

7.3.2 Tissue samples should be extracted within 30 days after sampling and analyzed within 40 days after extraction (refrigerated).

8. EQUIPMENT

8.1 Balance, top loading, capable of accurately weighing 100.00 g.
8.2 Rotary evaporator.
8.3 Platform shaker
8.4 Refrigerator at 4°C.
8.5 Oven at 130 – 150 °C
8.6 GC/MS instrumentation - a gas chromatograph complete with a temperature programmable oven and a split/splitless injector system with consumables, parts and supplies.
- 8.6.1 Gas chromatograph (Hewlett Packard 5890 Series II).
- 8.6.2 Column: OV-5 (15 m, 0.25 mm ID., 0.25 µ film thickness) or equivalent.
- 8.6.3 Mass spectrometer (VG70 VSE).
- 8.6.4 Data collection system used to integrate the data.
- 8.6.5 Automatic sampler (CTC100S).

9. REAGENTS

9.1 *Dichloromethane (DCM), hexane, acetone, methanol, diethylether* - distilled in glass or equivalent.

9.2 *Sodium sulfate* - ACS granular, anhydrous.

9.3 *Silica Gel,* 40-120 mesh, 100% activated. Pour silica gel into beaker and place in an oven at 130-150 °C for 12 h to activate. Use warm from the oven.

9.4 *Sand, white fine* – VWR C4549V or equivalent.

9.5 Sodium tetraethylborate, 97%, obtained from Sigma (481483-1G)

9.5.1 1% $NaB(Et)_4$ – Transfer the contents of the 1 g container to a 100 mL volumetric flask containing 25 mL of methanol. Rinse container with methanol and add to volumetric. Make to the mark with methanol.

9.6 *Potassium Hydroxide*, ACS grade.

9.6.1 KOH, 2M. Add 11.22 g of reagent grade KOH to a 100 mL volumetric flask. Make to the mark with water and mix.

9.7 *Tropolone,* Aldrich Chemicals (T-8970-2)

9.8 *Tetramethylammonium hydroxide*, 25% in methanol, purchased from Sigma Chemicals (T0280)

9.9 *Acetic Acid,* ACS grade

9.10 *Sodium chloride* - ACS granular

9.10.1 30% w/v $NaCl_{(aq)}$ – In a 250 mL beaker, add 60 g of NaCl and 200 mL of water. Place on a hot plate and gently heat until all the salt is dissolved.

9.11 *Sodium Acetate* (NaOAc, 99.995%)

9.11.1 NaOAc buffer (1 M) – add 8.20 g of NaOAc to a 100 mL volumetric flask. Add 50 mL of water and shake until dissolved. Add 30 mL of acetic acid and make to the mark with water.

9.12 *TBT Standards* - 1.0 mL mix of TBT, DBT, MBT and Tetrabutyltin at 2000 µg/mL in DCM, purchased from AccuStandard (OTM-001).

9.13 *Tri-n-propyltin Chloride Surrogate* - 1 mL at 2000 µg/mL in DCM, purchased from AccuStandard (OTM-003).

9.14 *Tetra-n-propyltin Internal Standard* – 1 mL at 2000 µg/mL in DCM, purchased from AccuStandard (OTM-005).

9.15 *Standard Preparation*

9.15.1 Stock Solutions

9.15.1.1 Prepare 10 µg/mL TBT Stock Standard by diluting 125 µL of the 2000 µg/mL AccuStandard mix in 25.0 mL of DCM.

9.15.1.2 Prepare a 100 ng/mL TBT Spiking Standard by diluting 1.0 mL of 10 µg/mL Stock Standard in 100.0 mL of DCM.

9.15.1.3 Prepare 10 µg/mL Surrogate Std. by diluting 125 µL of 2000 µg/mL in 25.0 mL DCM.

9.15.1.4 Prepare 0.2 µg/mL Surrogate Std. by diluting 200 µL of 10 µg/mL in 10.0 mL DCM.

9.15.1.5 Prepare 10 µg/mL Internal Std. by diluting 125 µL of 2000 µg/ml in 25.0 mL DCM.

9.15.1.6 Prepare 1.0 µg/mL Internal Std. by diluting 1.0 mL of 10 µg/ml

Internal Standard in 10.0 mL hexane.

9.15.1.7 Store stock solution in Teflon-lined screw cap glass vials, in the refrigerator.

9.15.1.8 Stock standard solutions should be checked prior to preparing working standards for signs of evaporation, contamination or degradation.

9.15.1.9 Stock standard solutions must be checked after one year. This check must be documented. If QC indicates that the standards are still within the acceptable criteria, then the standards may be used after the one year term until routine QC indicates a problem. If QC fails, the standard(s) must be replaced.

9.15.2 *Dilutions*

9.15.2.1 Prepare 25.00 mL Calibration Solutions at 10, 40, 100, 200, 400, 1000 and 4000 ppb in hexane as follows:

	Calibration Level (ng/mL)	Volume of TBT mix (10 ppm) (mL)	Volume of Surrogate (10 ppm) (µL)	Volume of ISTD (10 ppm) (µL)
CS-L	10	0.01	250	250
CS-1	40	0.1	250	250
CS-2	100	0.25	250	250
CS-3	200	0.5	250	250
CS-4	400	1.0	250	250
CS-5	1000	2.5	250	250
CS-6	4000	10.0	250	250

9.15.2.1.1 Prepare seven graduated centrifuge tubes by labeling with appropriate calibration level.

9.15.2.1.2 Add the prescribed amounts of TBT mix and Surrogate as listed above, into appropriate centrifuge tube.

9.15.2.1.3 Concentrate the solution to 100 µL by passing a gentle stream of nitrogen over the solvent. Add 2 mL of 1:1 ether/hexane.

9.15.2.1.4 Add 1 mL of 1% $NaB(Et)_4$ and allow to sit for 1 hour.

9.15.2.1.5 Add 2 mL of 2M KOH and shake. Allow the layers to separate and transfer the top (hexane) to a 25 mL volumetric flask.

9.15.2.1.6 Add another 2 mL of Hexane and shake for 1 minute.

9.15.2.1.7 Allow the layers to separate and transfer the hexane layer to the volumetric flask

9.15.2.1.8 Add a further 2 mL of Hexane and shake for 1 minute. Transfer hexane layer to volumetric flask.

9.15.2.1.9 Add 250 µL of internal standard (10 ppm) and make up to the mark with hexane.

9.15.2.2 Transfer each calibration mix to a 20 mL amber glass vial with Teflon lined silicone septa closure and store at 4°C.

9.15.2.3 Label each vial with a name of the solution, the concentration, the date

prepared, and the initials of the analyst who prepared the solution.

9.15.2.4 Calibration standard solutions should be checked prior to analysis for signs of evaporation, contamination or degradation.

9.15.2.5 Calibration standard solutions should be checked after 6 months. This check must be documented. If QC indicates that the standards are still within the acceptable criteria, then the standards may be used after the 6-month term until routine QC indicates a problem. If QC fails, the standard(s) must be replaced.

10. SUPPLIES

10.1 Autosampler vials with Teflon lined septa in aluminium crimp caps, 0.7 mL graduated (VWR/Agilent CA5182-0714 or equivalent).

10.2 Disposable Pasteur pipettes (size 9").

10.3 Glass wool.

10.4 Metal spatula.

10.5 Various volumes of microlitre glass syringes capable of delivering organic solvents.

10.6 *Glassware*

10.6.1 Test tube with Teflon-lined screw caps, 15 mL (VWR #21020-684 or equivalent).

10.6.2 Erlenmeyers, 250 mL

10.6.3 Conical stem funnels.

10.6.4 Separatory funnels - 2 L, 1 L, 0.5 L with Teflon stopcock.

10.6.5 Volumetric flasks, Class A - 10, 25, 50, 100 and 1000 mL as required.

10.6.6 Graduated cylinders, 100 mL, 250 mL.

10.6.7 Boiling flasks, 250 mL, 500 mL.

10.6.8 Glass columns with drip tip, 45 cm x 22 mm i.d.

10.6.9 Glass pipets, disposable, 5 mL

10.7 *Gases*

10.7.1 Helium – Ultra high purified (UHP) or equivalent.

10.7.2 Nitrogen - Pre-purified or equivalent.

11. SAFETY

11.1 The toxicity and carcinogenicity of the reagents used in this method have not been precisely defined; however, all chemical compounds should be treated as potential health hazards.

11.2 From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available.

11.3 Refer to Material Safety Data Sheets.

12. SPECIAL PRECAUTIONS

12.1 This method is restricted in use by, or under the supervision of, analysts experienced in the use of a gas chromatograph/mass spectrometer and skilled in the interpretation of gas

chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method and have undergone analytical training.

12.2 Ensure that safety procedures are strictly adhered to at all times and instrumentation is operating at optimum conditions.

13. TEST PROCEDURE

13.1 Sample Extraction

13.1.1 *Soil/Sediment*

13.1.1.1 Accurately weigh out 10-15 g of wet soil into a 40 mL vial.

13.1.1.2 Dry Weight Determination

13.1.1.2.1 Accurately weigh a piece of aluminium foil or small beaker.

13.1.1.2.2 Add 10-15 g of soil/sediment to the container and weigh. Record the weight and calculate the wet weight (Wet Wt.)

13.1.1.2.3 Dry the sample in an oven set at 105-120 °C.

13.1.1.2.4 Weigh the container plus soil. Record the weight and calculate the dry weight (Dry Wt.)

13.1.1.2.5 % Dry Wt. = Dry Wt / Wet Wt.

13.1.1.3 If the sample is a matrix spike or matrix spike duplicate, add 25 µL of 10 ppm Spike Solution. Record the spike amount.

13.1.1.4 To each blank, sample and spike(s) add 250 µL of 0.2 ppm tri-n-propyltin surrogate solution.

13.1.1.5 Add 3 mL of glacial acetic acid, 2 mL of 1 M NaOAc buffer (pH 4.5) and 5 mL of 30% w/v $NaCl_{(aq)}$.

13.1.1.6 Add 5 mL of 8:2 Et_2O:Hexane containing 0.02% tropolone. Cap the vial and shake for 1 hour on mechanical shaker.

13.1.1.7 If necessary, centrifuge the sample at 1500 rpm for 3 minutes. Transfer the organic layer to a 15 mL test tube.

13.1.1.8 Repeat the extraction with a further 5 mL of 8:2 Et_2O:Hexane containing 0.02% tropolone.

13.1.1.9 Centrifuge the sample at 1500 rpm for 3 minutes. Transfer the organic layer to the same 15 mL test tube as in 13.1.1.7.

13.1.1.10 Reduce the volume to 2 mL by passing a gentle stream of nitrogen over the surface of the solvent.

13.1.1.11 Add 1 mL of 1% $NaB(Et)_4$ in methanol. Cap the vial and shake (vortex).

13.1.1.12 Add 0.5 mL of 1% $NaB(Et)_4$ in methanol. Shake sample.

13.1.1.13 Allow the samples to sit overnight.

13.1.1.14 Add 2 mL of 2M KOH. Add 5 mL of 2:8 Et_2O:Hexane and shake for 1 minute.

13.1.1.15 Allow the layers to separate and transfer the solvent layer to a second test tube.

13.1.1.16 Repeat the extraction with a second aliquot of 8:2 Et_2O:Hexane

13.1.1.17 Allow the layers to separate and transfer the solvent layer to a second test

tube.

13.1.1.18 Reduce the volume to 2 mL by passing a gentle stream of nitrogen over the surface of the solvent.

13.1.1.19 Prepare a silica gel column as follows:
Place a glass wool plug into the bottom of a 22 mm o.d. glass column. Add 10 g of 100 % activated silica gel and top with 1 cm of anhydrous sodium sulphate. Elute 50 mL of hexane through the column and discard.

13.1.1.20 Remove the hexane (top) layer of the sample and transfer onto the silica gel column. Add 2 x 2 mL of hexane to the test tube, shake gently transfer to the column. Elute column with 100 mL of hexane, collecting the hexane into a 250 mL boiling flask.

13.1.1.21 Concentrate extract to 2 mL on a rotary evaporator and transfer to a test tube. Rinse boiling flask with 2 x 2 mL hexane.

13.1.1.22 Concentrate the solution to 250 µL by passing a gentle stream of nitrogen over the solvent.

13.1.1.23 Transfer to 0.7 mL gc vial and make up to 500 µL. Add 50 µL of 1.0 µg/mL TBT internal standard to the extract and seal the vial with a Teflon-lined vial closure. Label vial with sample identification and any dilution factors.

13.1.1.24 Inject 1 µL of extract in the injection port of the GC-MS system.

13.1.2 *Liquids/Waters*

13.1.2.1 Pre-rinse all glassware and glass wool with the appropriate extraction solvent before use.

13.1.2.2 Remove sample from the refrigerator and equilibrate to room temperature.

13.1.2.3 Transfer the sample quantitatively from the bottle into a 2 L separatory funnel.

13.1.2.4 If the sample is a matrix spike or matrix spike duplicate, add 10 µL of 10 ppm Spike Solution. Record the spike amount on the sample batch sheet.

13.1.2.5 To each blank, sample and spike(s) add 250 µL of 0.2 ppm tri-n-propyltin surrogate solution.

13.1.2.6 Add 10 mL of 1M NaOAc buffer (adjusted to pH 4.5 by adding AcOH). Shake funnel to mix.

13.1.2.7 Add 100 mL of dichloromethane containing 100 ppm of Tropolone to the bottle and rinse any residue into the separatory funnel. Seal and shake the separatory funnel vigorously for 1 minute with periodic venting to release excess pressure. Allow the organic layer to separate from the aqueous phase for approximately 3 minutes. Collect the solvent extract by filtering through a bed of precleaned anhydrous sodium sulfate and glass wool into a 500 mL boiling flask.

13.1.2.8 If an emulsion occurs and it is more than one third the size of the solvent layer, the technician must employ techniques to complete the phase separation. The optimum technique depends on the sample and may include:

- Stirring,
- Filtration through sodium sulfate and glass wool,
- Centrifugation,
- Salting out with sodium chloride,
- Other physical methods compatible with hexane.

13.1.2.9 Repeat the extraction step 2 more times with additional 60 mL aliquots of 100 ppm tropolone in dichloromethane. Combine all three extracts in the 500 mL boiling flask and concentrate the entire sample extract to 2 mL using a rotary evaporator.

13.1.2.10 Transfer to a test tube and rinse boiling flask with 2 x 2 mL hexane.

13.1.2.11 Concentrate the solution to 250 µL by passing a gentle stream of nitrogen over the solvent. Add 2 mL ether and 1 mL of 1% $NaB(Et)_4$ in methanol. Cap the vial and shake (vortex). Let sit for 30 minutes.

13.1.2.12 Add 2 mL of 2M KOH. Add 5 mL of Hexane and shake for 1 minute.

13.1.2.13 Allow the layers to separate and transfer the solvent layer to a second test tube.

13.1.2.14 Repeat the extraction with a second aliquot of Hexane

13.1.2.15 Reduce the volume to 2 mL by passing a gentle stream of nitrogen over the surface of the solvent. You must remove all traces of ether.

13.1.2.16 Prepare a silica gel column as follows:
Place a glass wool plug into the bottom of a 22 mm o.d. glass column. Add 10 g of 100 % activated silica gel and top with 1 cm of anhydrous sodium sulphate. Elute 50 mL of hexane through the column and discard.

13.1.2.17 Remove the hexane (top) layer of the sample and transfer onto the silica gel column. Add 2 x 2 mL of hexane to the test tube, shake gently transfer to the column. Elute column with 100 mL of hexane, collecting the hexane into a 250 mL boiling flask.

13.1.2.18 Concentrate extract to 2 mL on a rotary evaporator and transfer to a test tube. Rinse boiling flask with 2 x 2 mL hexane.

13.1.2.19 Concentrate the solution to 250 µL by passing a gentle stream of nitrogen over the solvent.

13.1.2.20 Transfer to 0.7 mL gc vial and make up to 500 µL. Add 50 µL of 1.0 µg/mL TBT internal standard to the extract and seal the vial with a Teflon-lined vial closure. Label vial with sample identification and any dilution factors.

13.1.2.21 Inject 1 µL of extract in the injection port of the GC-MS system.

13.1.3 *Tissue*

13.1.3.1 Accurately weigh out 2-5 g of tissue into a 40 mL vial.

13.1.3.2 If the sample is a matrix spike or matrix spike duplicate, add 25 µL of 10 ppm Spike Solution. Record the spike amount.

13.1.3.3 To each blank, sample and spike(s) add 250 µL of 0.2 ppm tri-n-propyltin surrogate solution.

13.1.3.4 Add 10 mL of 25% TMAH solution. Cap the vial and sonicate for 1 h.

13.1.3.5 Add 3 mL of glacial acetic acid, 2 mL of 1 M NaOAc buffer (pH 4.5) and 5 mL of 30% w/v $NaCl_{(aq)}$.

13.1.3.6 Add 5 mL of 8:2 Et_2O:Hexane containing 0.02% tropolone. Cap the vial and shake for 1 hour on mechanical shaker.

13.1.3.7 Centrifuge the sample at 1500 rpm for 3 minutes. Transfer the organic layer to a 15 mL test tube.

13.1.3.8 Repeat the extraction with a further 5 mL of 8:2 Et_2O:Hexane containing 0.02% tropolone.

13.1.3.9 Centrifuge the sample at 1500 rpm for 3 minutes. Transfer the organic layer to the same 15 mL test tube as in 13.1.3.7.

13.1.3.10 Reduce the volume to 2 mL by passing a gentle stream of nitrogen over the surface of the solvent.

13.1.3.11 Add 1 mL of 1% $NaB(Et)_4$ in methanol. Cap the vial and shake (vortex).

13.1.3.12 Add 0.5 mL of 1% $NaB(Et)_4$ in methanol. Shake sample.

13.1.3.13 Allow the samples to sit overnight.

13.1.3.14 Add 2 mL of 2M KOH. Add 5 mL of 2:8 Et_2O:Hexane and shake for 1 minute.

13.1.3.15 Allow the layers to separate and transfer the solvent layer to a second test tube.

13.1.3.16 Repeat the extraction with a second aliquot of 8:2 Et_2O:Hexane

13.1.3.17 Allow the layers to separate and transfer the solvent layer to a second test tube.

13.1.3.18 Reduce the volume to 2 mL by passing a gentle stream of nitrogen over the surface of the solvent.

13.1.3.19 Prepare a silica gel column as follows:
Place a glass wool plug into the bottom of a 22 mm o.d. glass column. Add 10 g of 100 % activated silica gel and top with 1 cm of anhydrous sodium sulphate. Elute 50 mL of hexane through the column and discard.

13.1.3.20 Remove the hexane (top) layer of the sample and transfer onto the silica gel column. Add 2 x 2 mL of hexane to the test tube, shake gently transfer to the column. Elute column with 100 mL of hexane, collecting the hexane into a 250 mL boiling flask.

13.1.3.21 Concentrate extract to 2 mL on a rotary evaporator and transfer to a test tube. Rinse boiling flask with 2 x 2 mL hexane.

13.1.3.22 Concentrate the solution to 250 µL by passing a gentle stream of nitrogen over the solvent.

13.1.3.23 Transfer to 0.7 mL gc vial and make up to 500 µL. Add 50 µL of 1.0 µg/mL TBT internal standard to the extract and seal the vial with a Teflon-lined vial closure. Label vial with sample identification and any dilution factors.

13.1.3.24 Inject 1 µL of extract in the injection port of the GC-MS system.

13.2 **Instrumental Analysis**

13.2.1 Prior to analysis and calibration, ensure that the system is functioning in a satisfactory manner by tuning the MS.

13.2.2 Instrument Conditions

13.2.2.1 *Inlet*

Injection	Dual Split/Splitless
Split time	0.5 minutes
Total carrier flow	50 mL/min.
Carrier Gas	Helium
Temperature	300°C
Pressure	5 psi, initial
Inlet Liners	Duel gooseneck borosilicate liner

13.2.2.2 *Columns*

Analytical column(s)	15 m x 0.25 mm i.d. x 250 µm film OV-5 (fused silica). (Conditioned and ready for use)
Flow Rates	1.7 mL/min. @ 12 psi and 150°C.

13.2.2.3 *Oven*

Initial temp.	45°C.
Ramp 1	45°C to 120°C at 25°/min.
Ramp 2	120°C to 140°C at 5°/min., hold 2 min.
Ramp 2	140°C to 280°C at 30°/min., hold 5 min.
Total Run Time	21.00 minutes

13.2.2.4 *Detector(s)*

Temperature	265 °C
Scan mode	Selective ion monitoring (SIM)
Scan rate	1 scan/sec

13.2.3 Ions Monitored

Analyte	M1	M2
Monobutyltin	249.067	319.145
Dibutyltin	249.067	303.129
Tributyltin	249.067	303.129
Tetrabutyltin	291.114	289.153
Tri-n-propyltin	277.098	249.067
Tetra-n-propyltin	249.067	203.142

13.3 Calibration

13.4.1 Transfer ~0.5 mL of each calibration mix to GC injection vials.

14.4.2 Inject 1 µL of each calibration standard sequentially and tabulate the peak areas and the ppb concentration of each analyte for each injection.

13.4 Data Analysis

13.4.1 If sample interferences result in poor resolution of the internal standards and surrogates, dilution of the extract or extract cleanup may be required.

13.5 Quality Assurance/Quality Control

13.5.1 *Calibration Check*

13.5.1.1 Verification of the ratio of instrument response to analyte amount. The working calibration curve must be verified on each working day by measurement of a calibration standard (CS-3). If the response for any analyte varies from the predicted response by more than $\pm$ 25%, the test must be repeated using a fresh calibration standard. If the fresh calibration standard also fails, inspect the gas chromatographic system to determine the cause and perform whatever maintenance is necessary before verifying calibration and proceeding with sample analysis. If routine maintenance does not return the instrument performance to meet the QC requirements based on the last initial calibration, then a new initial calibration must be performed.

13.5.1.2 A calibration standard (CS-3) must also be injected after every 12 hours and at the end of the analysis sequence.

13.5.2 *Method Blanks*.

13.5.2.1 A method blank is the matrix minus the analytes of interest, carried through all the steps of the analytical procedure. It measures contamination (bias) introduced in the lab. Blank results are used to access contamination and/or provide background correction to analyte concentrations.

13.5.2.2 If a method blank result is above a Reported Detection Limit for a sample within a preparation batch, the data reported for that sample must be

qualified (it may be acceptable to increase the Reported Detection Limit of affected sample results to a level above that of the method blank result).

13.5.2.3 A method blank is required with every batch.

13.5.3 *Matrix Spikes/Matrix Spike Duplicates*

13.5.3.1 A matrix spike is a recovery test in which a predetermined quantity of the Calibration Standard is added to a sample matrix prior to sample extraction/digestion and analysis. Percent recoveries are calculated for each of the analytes detected to assess analytical accuracy. The relative percent difference between the samples is calculated and used to assess analytical precision.

13.5.3.2 A sample with each analytical batch is chosen to be used as a matrix for spike/spike duplicate recovery. An ideal matrix would have hydrocarbon levels that will not exceed the amount spiked into the sample. If a suitable matrix is not available, white sand may be used as a spike matrix. For water matrix, there is usually not enough sample to perform a matrix spike, so lab water is used.

13.5.3.3 A matrix spike is performed with each batch up to 10 samples. For batches of 11 or more samples, a matrix spike duplicate is required. For samples where detectable amounts of analytes are suspected, replicate samples may be more appropriate than spiked duplicates.

13.5.3.4 Calculate the spike recovery of each component of the mixture by quantitation against the appropriate component of the calibration standard. The recoveries are plotted on control charts and acceptance criteria are established to be within 2 standard deviations. The limits are valid for up to one year or until the method has been altered.

13.5.3.5 If the % recovery falls outside the range of $\pm$ 2SD (warning limits), the analysis is labeled as suspect and a recovery test is repeated within 5 days. If the % recovery falls outside the range of $\pm$ 3SD (control limits), then the recovery test is repeated immediately and action is taken to remedy the problem. Check for errors in calculations, surrogate solutions, internal standards and instrument performance. Recalculate the data and/or reanalyze the extract. If the repeated recovery is still outside the limits, the samples from the same batch must be repeated, or their data reports must be qualified.

13.5.3.6 Duplicate results should not vary by more than 30% RSD provided sample concentration is greater than 10 x MDL. If this criterion is exceeded, then extraction efficiency or sampling error is suspected and the duplicate analysis must be repeated.

13.5.4 *Duplicates*

13.5.4.1 A sample with each analytical batch (up to 20 samples) is chosen to be used as a duplicate. For water, unless the client has taken extra sample, there is usually not enough sample to perform a duplicate.

13.5.4.2 Duplicate results should not vary by more than 30% RSD provided sample concentration is greater than 10 x MDL. If this criterion is exceeded, then extraction efficiency or sampling error is suspected and the duplicate analysis must be repeated.

13.5.5 *Surrogates*

13.5.5.1 Surrogates are compounds similar to the analytes of interest in chemical composition, extraction efficiency, and chromatography, but which are not normally found in environmental samples.

13.5.5.2 Surrogates, where appropriate, are spiked to all blanks, standards, samples, matrix spikes and duplicates prior to extraction to monitor the extraction efficiency.

13.5.5.3 The % recovery of surrogate (tri-n-propyltin) must be between 30-130%. Surrogate recoveries are reported with each sample but the sample results are corrected for recoveries.

14. DATA ACCEPTANCE

14.1 Appropriate documentation of the laboratory performance is required in the form of control charts.

14.1.1 The measurements should consist of a minimum of 7 determinations numerous times over a defined period. Once these measurements have been obtained, a control chart should be established.

14.1.2 Results from daily calibration checks and recovery tests are used in the construction of control charts.

14.2 If the calibration check and/or % recovery of surrogates or matrix spikes falls outside the control limits, then the test is repeated immediately and action is taken to remedy the problem. Check for errors in calculations, surrogate solutions, internal standards and instrument performance. Recalculate the data and/or reanalyze the extract. If the repeated recovery is still outside the limits, then new limits must be established and/or the samples from the same batch must be repeated, or their data reports must be qualified.

15. CALCULATION OF RESULTS

15.1 *Internal Standard Calculation*

15.1.1 Results are obtained by comparison between the sample and the matrix working standards, and expressed in ppb (µg/L) for waters and ppm (mg/kg) for solids.

15.1.1.1 Calculate the relative response factors for each analyte from all calibration runs.

$$\mathbf{RRF}_x = \frac{A_x}{A_{IS}} \times \frac{C_{IS}}{C_x}$$

where
A_x = area of analyte
A_{IS} = area of Internal Standard
C_{IS} = concentration of Internal Standard
C_x = concentration of analyte
RRF_x = relative response factor for analyte

15.1.1.2 Calculate the average relative response factors for each analyte.

$$RRFAV = \frac{\Sigma\, RRF_{xi}}{n} \quad (i = 1,2,3,4,5)$$

where
RRFAV = average relative response factor
RRF_{xi} = relative response factor for analyte x, at level i
n = number of calibration levels

15.1.1.3 Calculate the analyte concentration:

$$C_x = \frac{A_x}{A_{IS}} \times \frac{C_{IS}}{RRFAV_x} \times \frac{1}{W}$$

where
A_x = area of analyte
A_{IS} = area of Internal Standard
C_{IS} = amount of internal standard added in µg
C_x = concentration of analyte
$RRFAV_x$ = average relative response factor for analyte
W = amount of sample extracted in g or L

15.1.2. The data are reported in mg/kg for soils, and in µg/L for waters, without blank correction or correction for recovery data. Spiked recovery samples are reported with the sample results.

15.1.3 It is typical to report results on a common basis, for example "mg/kg as Sn", or "mg/kg as TBTO." To convert data to these units, apply the following factors:

To convert	**To:**	**Multiply by:**
Tributyltin chloride	as Sn	0.3647
Tributyltin chloride	As TBTO	0.9760
Dibutyltin dichloride	as Sn	0.3907
Dibutyltin dichloride	As TBTO	0.9110
Monobutyltin trichloride	as Sn	0.4207
Monobutyltin trichloride	As TBTO	0.8461
As Sn	As TBTO	2.8097

15.2 *Spike Recovery Calculation*

$$\% \text{ Recovery} = ((C_x - C_s) \times W) / S_x \times 100\%$$

Where:

C_x = Concentration of analyte (µg/L or µg/g)
S_x = Spike Amount (µg)
C_s = Concentration of analyte in unspiked matrix
W = weight or volume of sample (L or g)

15.3 *Accuracy and Precision Calculations*

15.3.1 Accuracy and Precision are calculated using data generated from Matrix Spike (MS) and Matrix Spike Duplicate (MSD) recoveries.

15.3.2 Matrix Spike Recovery is calculated as follows:

$$\% \text{ Recovery}_{MS} = \frac{AMT_{MS} - AMT_{SAMP}}{SPK} \times 100\%$$

Where:

AMT_{MS} = Total amount of analyte in matrix spike sample (µg)

AMT_{SAMP} = Total amount of analyte in unspiked sample (µg)

SPK = Amount of analyte spiked to sample (µg)

15.3.3 % Accuracy is calculated as follows:

$$\% \text{ Accuracy} = \frac{\% \text{ Recovery}_{MS} + \% \text{ Recovery}_{MSD}}{2} \times 100\%$$

15.3.4 % Precision is calculated as follows:

$$\% \text{ Precision} = \frac{|\% \text{ Recovery}_{MS} - \% \text{ Recovery}_{MSD}|}{\% \text{ Accuracy}} \times 100\%$$

16. REFERENCED LITERATURE

16.1 VG70-VSE Operating Manual.

16.2 Opus User's Guide.

Author: David Hope

Approved by: _____, CEO

Date Implemented:	April 15, 2003		
Revision 1.1	July14, 2003	Added details on chemistry of analysis	DGH
		Documented the use of duplicates	DGH
Revision 1.2	September 15, 2003	Change soil extraction to shaker table	DGH
		Modify calibration standard concentrations	DGH
Revision 2	January 23, 2004	Add tissue method	DGH
Revision 3	October 5, 2005	Add ethylation procedure	DGH
Revision 3.1	August 15, 2005	Text corrections following WA DOE audit	DGH

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Appendix D. Data Quality Assessment

Table D-1: Quality Control Samples, Evaluation Criteria, and Assessment.

Parameter	Method Blank		Analytical Replicates[1]		Laboratory Control Sample[2]		Matrix Spike (MS) and Matrix Spike Duplicate (MSD)	
	Number	Evaluation	Number	Evaluation	Number	Evaluation	Number	Evaluation
Grain size	--		1 triplicate analysis	RSD ≤ 20% *Objective met for all grain sizes except gravel (RSD=173%)*	--		--	
Total organic carbon (TOC)	1/batch	Analyte concentration < PQL *Objective met*	1 triplicate analysis	RSD ≤ 20% *Objective met*	--		--	
TBT – bulk sediment	1	Analyte concentration < PQL *Objective met*	1 duplicate analysis	RPD ≤ 20% *Objective not met (RPD=72%)*	1	80–120% recovery, or performance-based intralaboratory control limits, whichever is lower. *Objective not met (78% recovery)*	1	75–125% recovery applied when the sample concentration is < 4 times the spiked concentration; RPD ≤ 20% *Recoveries were 49% (MS) and 59% (MSD) but sample concentration was not < 4 times the spiked concentration. RPD = 19%*
TBT – pore water	1	Analyte concentration < PQL *Objective met*	1 duplicate analysis	RPD ≤ 20% *Objective not met (RPD=33%)*	1	80–120% recovery, or performance-based intralaboratory control limits, whichever is lower *Objective not met (72% recovery)*	1	75–125% recovery applied when the sample concentration is < 4 times the spiked concentration; RPD ≤ 20% *Objective met (97% recovery for both MS and MSD)*
TBT – tissue (lab Bioaccumulation study)	1	Analyte concentration < PQL *Objective met*	1 duplicate analysis	RPD ≤ 20% *Objective met (RPD=7%)*	1	80–120% recovery, or performance-based intralaboratory control limits, whichever is lower *Objective not met (42% and 53% recovery, for two samples)*	1	75–125% recovery applied when the sample concentration is < 4 times the spiked concentration; RPD ≤ 20% *Objective met for two sets of MS and MSD (79% recovery MS and MSD; 77% recovery MS and 87% recovery MSD).*

RPD Relative percent difference.
RSD Relative standard deviation.
[1] Synonymous with Laboratory Replicates or, if applicable, Laboratory Duplicates.
[2] A known matrix spiked with analytes representative of the target analytes used to document laboratory performance. A Fortified Blank or a commercially available Certified Reference Material containing the analytes of interest may be used.

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Appendix E. Case Narratives

DATA QUALIFIER CODES

U	-	The analyte was not detected at or above the reported result.
J	-	The analyte was positively identified. The associated numerical result is an *estimate*.
UJ	-	The analyte was not detected at or above the reported estimated result.
REJ	-	The data are *unusable* for all purposes.
NAF	-	Not analyzed for.
N	-	For organic analytes there is evidence the analyte is present in this sample.
NJ	-	There is evidence that the analyte is present. The associated numerical result is an estimate.
NC	-	Not calculated
E	-	This qualifier is used when the concentration of the associated value exceeds the known calibration range.

State of Washington Department of Ecology
Manchester Environmental Laboratory
7411 Beach Drive East
Port Orchard WA. 98366

CASE SUMMARY

November 15, 2005

Project: Squalicum Harbor TBT

Samples: 38-4020-27

Laboratory: Analytical Resources, Inc.

By: Pam Covey

These eight (8) sediment samples required Grain Size analyses using Puget Sound Estuary Protocol (PSEP) method. The samples were received at the Manchester Environmental Laboratory and shipped to the contract lab on September 29, 2005 for Grain Size analyses.

The analyses were reviewed for qualitative and quantitative accuracy, validity and usefulness. One sample (38-4020) was analyzed in triplicate and was within QA requirements.

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Manchester Environmental Laboratory
7411 Beach Dr E, Port Orchard Washington 98366

CASE NARRATIVE

December 7, 2005

Subject: Squalicum Harbor TBT Project

Sample(s): 04384020-27 and 05384230-32

Officer(s): Nigel Blakely

By: Bob Carrell
Organics Analysis Unit

BUTYLTINS ANALYSIS

ANALYTICAL METHOD

These samples were extracted and derivatized following Manchester Laboratory's standard operating procedure for the extraction of butyltins using a 50:50 mixture of hexane and ethyl acetate containing 0.03% tropolone by weight. The extracts were transferred to 50 mL volumetric flasks and the solvent was evaporated to near dryness on the N-Evap. Two milliliters of hexane was added to the flask and the butyltins were derivatized using the sodium tetraethylborate reaction to the ethyl derivatives followed by a cleanup step utilizing silica gel. An internal standard was added to the extracts and the analyses were done by capillary gas chromatography using mass spectroscopy in the selected ion mode (GC/MS-SIM).

HOLDING TIMES

These samples were stored frozen, following the Puget Sound Estuary Program (PSEP), until extracted. All samples extracts were analyzed within the maximum recommended method holding time of 40 days from extraction.

CALIBRATION

The initial eight point calibration using a quadratic fit resulted in a correlation coefficient of 0.99 for all compounds and no standard (compound) varying from its true value by more than +/- 20%. The continuing calibration for subsequent day's analyses did not vary from their true values by more than +/- 20%.

BLANKS

No target analytes were detected in the laboratory method blanks at or above the method quantitation limits (MQL) demonstrating that the system was free from contamination.

SURROGATE

The in-house surrogate recovery limits are under review for the tripentyltin chloride surrogate and in the interim the recovery limits are set at 50% to 130%. Using these criteria, the surrogate recoveries were acceptable for all samples and QC.

LABORATORY DUPLICATES

The results of the sample duplicate indicated that the sample (05384023) may not have been homogeneous since the RPD for the sample and sample duplicate was 72%. This can not be confirmed given the relatively low concentration of the tributyltin in the sample and the possibility that this represents the normal random variability of the sample.

LABORATORY CONTROL SPIKES

The results of the laboratory control spike (OL05312T1) were acceptable.

MATRIX SPIKES

The in-house matrix spike recovery limits are under review and the interim limits have been set at 40% to 130%. Using these criteria the results of the matrix spike and matrix spike duplicate were acceptable.

COMMENTS

A certified Canadian sediment reference material known as PACS-2 was also extracted along with the batch and analyzed with the samples. This sample is identified as PAC05312T1, which has a certified tributyltin chloride value of 2687 +/- 356 ug/Kg dw. The recovery for this sample was acceptable for tributyltin chloride (74%).

It should be noted that none of the data for this project is recovery corrected.

Manchester Environmental Laboratory
7411 Beach Drive East, Port Orchard, Washington 98366

November 14, 2005

Subject:	Squalicum Harbor
Samples:	05-384070 through 384073
Project ID:	1681-05
Laboratory:	Pacific Rim laboratories, Inc. (PRLI)
Project Officer:	Nigel Blakely
By:	Karin Feddersen

Tributyltin

Summary

These samples were analyzed using PRLI's in-house SOPs. Routine QA/QC procedures were performed.
See the contract laboratory's case narrative for more details.

Blanks

A small amount of the target compounds were detected in the method blank, close to the amount detected in the samples. Since the blank levels were below the PQL and below the calculated MDL, they were not reported. However, they indicate there may be some contribution due to background contamination, resulting in a potential positive bias to the sample results. Detected sample results are therefore qualified as estimates (J).

Calibration

The %Relative Standard Deviations were less than 30% for all compounds in the initial calibration.

Laboratory Control Sample

A spiked blank water was carried through the derivatization and analysis procedures with the samples. Results were not included in the original data package. TBT recovery was 72.3%.

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October 27, 2005

Karin Feddersen
WA Dept. of Ecology
Manchester Laboratory
7411 Beach Drive East
Port Orchard WA 98366-8204
USA

Dear Karin,

RE: Preparation and analysis of porewater samples for Tributyltin
Project: Squalicum Harbor

On September 27th, 2005, Pacific Rim Laboratories Inc. (PRL) received four coolers with numerous 1 L sediment samples encased in glass jars. Porewater was extracted from the sediment and subsequently analysed for tributyltin. The analyses are now complete and the data is reported on the attached sheets.

If you have any questions about your data report, please do not hesitate to contact me.

Sincerely,
Pacific Rim Laboratories Inc.

David Hope
CEO

SAMPLE RECEIPT FORM / CHEMICAL ANALYSIS FORM

FILE #: PR50610

CLIENT: WA Dept. of Ecology
Manchester Laboratory
7411 Beach Drive East
Port Orchard WA 98366-8204
USA

Phone – 360-871-8829
Email:

RECEIVED BY: L. Haimovici **DATE/TIME:** Sep. 27, 2005 (1:30 p.m.)

CONDITION: several broken jars, temperature <4 °C

# of Containers	Sample Type	Sample (Client Codes)	Lab Codes	Test Requested
3	sediment	384070	PR50610	TBT
4	sediment	384071	PR50611	TBT
4	sediment	384072	PR50612	TBT
4	sediment	384073	PR50613	TBT
4	sediment	384074	PR50614	TBT
3	sediment	384075	PR50615	TBT
4	sediment	384076	PR50616	TBT
2	sediment	384077	PR50617	TBT

STORAGE: Stored at 4 C

ANALYTES: HRGC/HRMS analysis for TBT

SPECIAL INSTRUCTIONS: porewater to be extracted from sediment and analysed

COMMENTS:

METHODOLOGY
Reference Method: TBT: SOP LAB04
Porewater: SOP LAB10

Data summarized in Data Report Attached

Report sent to: Karin Feddersen Date: October 27, 2005

Case Narrative – TBT

Samples arrived in four coolers on September 27, 2005. Four of the jars were broken and their contents were disposed of. Instructions were received from Karin Feddersen (Manchester Lab) that the numbering system was incorrect on the samples. It was revised as follows:

Old Number		**New Number**
384020	→	384070
384021	→	384071
384022	→	384072
384023	→	384073
384024	→	384074
384025	→	384075
384026	→	384076
384027	→	384077

Sample Preparation

Porewater was extracted from the sediment samples following SOP LAB10. In summary, water was decanted from each container. Then approximately 25 g of sediment was placed in several 40 mL glass vials with Teflon lined septa. Nitrogen was passed over the sediment to reduce oxygen content, and the lid was sealed. Samples were centrifuged for 10 minutes at high rpm to settle out the solids. Water was removed from the top of the vial and combined with the decanted water. This process was repeated with multiple vials until enough water was collected (approx. 5 mL per vial). The water was filtered through a 0.4 µm polycarbonate filter, preserved with 1 mL of sodium acetate buffer (pH 4.5), and store at 4 °C until extraction.

This procedure was initially carried out on September 28th, with 200-300 mL of water collected. However, unanticipated problems with the subsequent derivatization made the data useless. A second round of porewater extractions began on October 6th with 70-100 mL collected.

Water was extracted in one batch commencing on October 7, 2005. Samples volumes were measured prior to transferring to a 500 mL separatory funnel. One sample (384073, PR50613) was split into three portions, two of which were spiked with 100 ng (1.0 mL of 100 ng/mL TBST-0149)) each of tributyltin chloride, dibutyltin dichloride and monobutyltin trichloride. All samples were spiked with 50 ng of tripropyltin chloride and 50 ng of tripentyltin chloride (250 µL of 0.2 µg/mL TBSS-0179)), then serial extracted with three portions of 0.01% tropolone in dichloromethane (100 mL, 60 mL, 60 mL). The solvent was collected in a 500 mL boiling flask, concentrated to 2 mL by rotary evaporator, and transferred with hexane washes to a 15 mL test tube. Samples were spit in half gravimetrically. One half was immediately derivatized with 1 mL of 1% sodium tetraethylborate and cleaned up on a neutral silica gel column. Blank levels for TBT were determined to be in the range of 60 ng/L so the data was not reported.

The underivatized half was stored in the dark (7 days) while awaiting arrival of a new source (Alfa Aesar) of derivatizing agent. The samples were then derivatized with 1 mL of 1% sodium tetraethylborate and cleaned up on a neutral silica gel column. The solvent was reduced in

volume by rotary evaporation, transferred to a test tube and further reduced to 0.5 mL by blowing a gentle stream of nitrogen over the surface of the solvent. Each sample was spiked with 50 ng (50 µL of 1.0 µg/mL TBIS-0049) of tetrapropyltin and transferred to a 700 µL gc vial for GC/HRMS analysis.

Instrument Calibration

All samples were analyzed on a VG-70VSE high resolution mass spectrometer coupled with an HP5890 Series II gas chromatograph. The column used was a 30 m OV-5, 0.25 µm, 0.25 mm i.d. An initial six point calibration (CS-LL (2 ng/mL), CS-LO (10 ng/mL), CS-1 to CS-4) containing all butyltins was run covering the range of 2 ng/mL to 400 ng/mL. Surrogate and internal standards are kept at a constant 100 ng/mL.

Calibration Verification
The calibration was verified at the beginning and ending of every run or every 12 hours with a mid-point standard (CS-3, 200 ng/mL). All CalVer's were acceptable, with butyltins <20% deviation and surrogates <50% deviation.

Quantitation
Data is quantified based on peak area using internal standard methods. All peak areas are the sum of two peak areas in the ion cluster (Sn has a number of abundant stable isotopes), as follows:

	M1	**M2**
TBT	263.082	261.082
DBT	263.082	261.082
MBT	235.051	233.050
TrPrT	249.066	247.066
TrPnT	333.160	331.160
TePrT	249.066	247.066

OPUSQUAN provides three sheets of data for each sample. The first page is a summary sheet, giving HRMS file names, sample IDs, etc. Sample PR50610 (instrument filename: VG002744 S:4) has been annotated for your reference.

Results

Tributyltin is sold commercially as bis-tributyltin oxide (TBT-O-TBT). In water, it can take a number of forms depending on the pH, including hydroxide, chloride and carbonate. In seawater, the three species remain in equilibrium. Data has been reported in µg/L as the butyltin chloride.

All data was quantified using OPUSQUAN software.
Detection limits are set at 0.02 µg/L.
All data <10x blank levels are flagged with a B.
All data <PQL are flagged with a J.
Any data that failed to meet acceptable ion ratios (1.35 ± 30%) has been flagged with an N.

PQL
Average sample size was 75 mL, however extracts were split in half. All samples were made up to a final volume of 0.5 mL. The low level calibration point is 2 ng/mL. Therefore the Practical Quantitation Limit is:

0.5 mL x 2 ng/mL / (0.068 L / 2) = 29.4 ng/L or 0.0294 µg/L

Surrogate Recoveries
All samples were spike with 50 ng of tripropyltin chloride and tripentyltin chloride as surrogates. Recoveries after taking into account the 50% split were within acceptable criteria of 30-130%. Typically, the tripropyltin gave a lower recovery, perhaps indicating increased loss due to volatility.

QC Samples

Blanks
One blank (100 mL of lab water) was carried through the porewater filtration steps and then subsequent TBT extraction and clean-up procedure. Although 100 mL was carried through the procedure, a nominal volume of 75 mL was used for calculations (average sample size). There were trace hits for most compounds, however all values were below the MDL. There is a background concentration of 1 ng of TBT in blank, which would correspond to a value of 0.013-0.015 µg/L in the samples.

Spikes
Porewater from sample 384073 (PR50613) was split into three portions, two of which were spiked with 100 ng each of TBT, DBT and MBT chlorides giving a level of fortification equivalent to 1.47 µg/L. The MS/MSD were treated like regular samples.

Recoveries for TBT were acceptable at 96.6 % in each sample. Recoveries were poorer and more variable for DBT (34.8-46.9%) and MBT (12.0-28.8%).

Duplicates
One porewater sample (384071, PR50611) was analysed in duplicate. The variability (diff / mean) at low levels was 33% for TBT and 4% for DBT. MBT was not detected in either sample.

Hold Times

Porewater samples were collected and preserved to pH 4.5 on October 6, 2005. Analysis commenced on October 7, 2005. Assuming samples were collected on September 22nd or earlier, this would put hold time at 15+ days. Hold times for water and sediment at 4 °C are set in our SOP at two weeks, although we did not see much effect on TBT after four weeks storage. A paper from Virginia Institute of Marine Science saw no degradation for waters in 13 weeks. http://www.vims.edu/env/projects/tbt_deq/manual/tbt_manual.pdf

_____
David Hope, CEO

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Manchester Environmental Laboratory
7411 Beach Dr E, Port Orchard Washington 98366

CASE NARRATIVE

February 2, 2006

Subject: Squalicum Harbor TBT Project

Sample(s): 05384233-55

Officer(s): Nigel Blakely

By: Bob Carrell
Organics Analysis Unit

BUTYLTINS ANALYSIS

ANALYTICAL METHOD

These samples were extracted and derivatized following Manchester Laboratory's standard operating procedure for the extraction of butyltins in tissue using a 99:1 mixture of hexane and acetic acid containing 0.1% tropolone by weight. After the initial extraction a 20% hydrochloric acid solution was added and the resultant solution was centrifuged for 30 minutes. The extracts were transferred to 50 mL volumetric flasks and the solvent was evaporated to dryness on an N-Evap concentrator. Two milliliters of hexane was added to the flask and the butyltins were derivatized using the sodium tetraethylborate reaction to the ethyl derivatives followed by a cleanup step utilizing silica gel. An internal standard was added to the extracts and the analyses were done by capillary gas chromatography using mass spectroscopy detection with selected ion monitoring of various quantitation and qualifier ions (GC/MS-SIM).

HOLDING TIMES

These samples were stored frozen, following the Puget Sound Estuary Program (PSEP), until extracted. All samples were analyzed within the maximum recommended method holding time of 40 days from extraction.

CALIBRATION

The initial nine point internal standard calibration using a quadratic fit resulted in a correlation coefficient of 0.99 for all compounds and no standard (compound) varying from its true value by more than +/- 20%. At the beginning of each analytical day a calibration check sample was run and no compound varied from it true value by more than +/- 20%.

BLANKS

No target analytes were detected in the laboratory method blanks at or above the practical quantitation limits (PQL) demonstrating that the system was free from contamination.

SURROGATE

The in-house surrogate recovery limits are under review for the tripentyltin chloride surrogate. In the interim the recovery limits are set at 50% to 130%. Using this criterion, the surrogate recoveries were acceptable for all samples.

LABORATORY DUPLICATES

The results of all samples and their duplicates were acceptable.

LABORATORY CONTROL SPIKES

No QC limits have been established for this method for LCS samples however our in-house limits are tentatively set at 40% to 130% for tributyltin chloride. The recoveries for the laboratory control spikes (LCS) were acceptable.

MATRIX SPIKES

As with the LCS above, no QC limits have been established, however our in-house limits are tentatively set at 40%-130% for tributyltin chloride. The recoveries for the tributyltin in the matrix spikes were acceptable. The relative percent difference recovery limit between compounds in the various matrix spike pairs is set at <40% and this limit was not exceeded.

COMMENTS

A certified reference material (mussel tissue) from the Commission of the European Communities, known as CRM477, was also extracted along with each batch and analyzed with these samples. These samples are identified as CRM06019T1 and CRM06023T1. The CRM477 has a certified tributlyltin chloride value of 2464 +/- 213 ug/Kg dw. The bias and precision for these samples were acceptable for tributyltin chloride at 67% and 69% recovery respectively of tributyltin.

It should be noted that none of the data for this project is recovery corrected.

PNWD-3633

Squalicum Harbor 45-Day Bioaccumulation Test with *Macoma nasuta*

Nancy P. Kohn

Battelle Marine Sciences Laboratory
Sequim, Washington

December 2005

Prepared for the
Washington State Department of Ecology

Battelle Memorial Institute
Pacific Northwest Division

Battelle
The Business of Innovation

Squalicum Harbor 45-Day Bioaccumulation Test with *Macoma nasuta*

Abstract

Battelle Marine Sciences Laboratory (MSL) conducted a 45-d sediment bioaccumulation assay to determine the bioavailability of tributyltin to the bentnose clam, *Macoma nasuta*. This assay was conducted in support of a Washington Department of Ecology (Ecology) evaluation of sediment from the Squalicum Harbor Marina in Bellingham, Washington. Three sediment samples were collected by Ecology and tested by MSL; sediment from Sequim Bay, Washington was also tested as a control treatment. Five replicates per sample were tested in 5-gal aquaria supplied with flowing seawater; 15 *M. nasuta* were exposed in each aquarium. During the 45-d exposure, the organisms were not fed, but 100 mL of the appropriate test or control sediment was added to each chamber once a week. Organisms were observed daily; water quality parameters were also monitored daily. After 45 days, the clams were sieved from the sediment and placed in clean flowing seawater to depurate for 24 h. Survival of *M. nasuta* was at least 87% in each replicate, yielding at least 40 g of tissue per sample for chemical analysis. Tissue samples were homogenized upon collection and then sent to Ecology's Manchester Environmental Laboratory for tributyltin analysis.

Introduction

Squalicum Harbor, a marina in Bellingham, Washington, is located on Bellingham Bay and is operated by the Port of Bellingham. The Washington Department of Ecology (Ecology) is concerned about sediment quality in Squalicum Harbor. The particular contaminant of concern is tributyltin (TBT), a bioactive component of some marine antifouling paints. Although use of TBT paint has been restricted since 1990 and is prohibited on hulls of vessels less than 25 m in length, TBT can persist in the environment where it has been associated with toxicity to nontarget organisms (fish, bivalves, crustaceans). To address concerns about the potential bioavailability of TBT in harbor sediments to marine biota, sediment sampling, analysis, and laboratory bioaccumulation testing were conducted by Ecology's Environmental Assessment Program. The laboratory bioaccumulation study was conducted by Battelle Marine Sciences Laboratory (MSL) in Sequim, Washington. MSL contributed to the development of the 45-d bioaccumulation test method now adopted by the Puget Sound contaminated sediment management program, and has conducted the test with sediments collected from a number of sites in the northwest.

Materials and Methods

Sediment Sample Receipt and Handling

Three sediment samples were collected from Squalicum Harbor, Bellingham, Washington by the Washington Department of Ecology. Samples were placed in 5-gal high density polyethylene pails with tight-fitting lids. Sample pails were kept cool on ice and were hand-delivered to the MSL by Mr. Nigel Blakley of Ecology. Upon receipt at the MSL, each Squalicum Harbor sediment sample was homogenized in its pail by using a stainless steel mixer blade attached to a drill motor. This mixing method avoids sample contamination and mass loss from multiple container transfers; the mixing blades were thoroughly cleaned and solvent -rinsed with methylene chloride between

samples to avoid cross contamination. Once the sediment was homogenized, a subsample was removed for chemical analysis before the headspace in the pails was purged with nitrogen and samples were stored in a walk-in cold room at 4ºC±2ºC until used in the 45-d test. Chemistry subsamples were hand-delivered to Ecology's Manchester Environmental Laboratory by Nigel Blakley.

Macoma nasuta control sediment was collected from Sequim Bay, Washington by Battelle MSL. Sequim Bay sediment was collected on September 22, 2005; it was sieved through 1-mm mesh to remove debris and live organisms, homogenized, and stored in MSL's walk-in cold room until used in the 45-d test.

45-Day Bioaccumulation Test

M. nasuta were supplied by Johnston and Gunstone, Port Townsend, Washington. The clams were transported in a cooler from the collection site in Discovery Bay to the Battelle MSL. Upon arrival at the MSL, the clams were placed in clean sediment covered by flowing unfiltered seawater at13.6ºC. Temperature and other water quality parameters were maintained at test conditions (listed below) for 10 days prior to test initiation. Test organisms were allowed to feed on sediment detritus during the holding period, but were not given supplemental food.

The 45-d bioaccumulation exposure with *M. nasuta* followed ASTM Method E1688 (bioaccumulation tests with benthic invertebrates), with modifications to the test duration and sediment renewal promulgated by the Puget Sound Dredge Disposal Analysis (PSDDA) Program in a series of issue and clarification papers (PSDDA 1994, 1996, 2000). Because the tissue mass needed for the one contaminant of concern was not great (10 g tissue per analysis), the test chamber size, number of clams, sediment volume, flow rate, and sediment renewal volume were reduced accordingly. The test is typically conducted in 10-gal aquaria containing 4 L sediment and 25 to 30 clams, whereas this test was conducted in 5-gal aquaria containing ~2 L sediment and 15 clams each. Test treatments included the three Squalicum Harbor samples SH 2, SH 4, and SH 6, and Sequim Bay (*M. nasuta* control) sediment. Five replicate chambers per treatment were assigned random positions on the same water table. The test chambers were 5-gal aquaria that received flowing seawater (65±5 mL/min) through dripper arms connected to a seawater distribution manifold. Each aquarium was fitted with a standpipe to maintain water depth. Prior to test initiation, 0.5 gal of sediment (~2 L) was placed in each chamber and allowed to settle overnight. The following day, water flow was initiated. Once the aquaria were filled, water quality parameters (temperature, pH, DO, and salinity) were measured in all replicates. Acceptable ranges for water quality parameters in the 45-d test were as follows:

Parameter	Acceptable Range
Temperature	12ºC – 16ºC
DO	>5 mg/L
pH	7.3 – 8.3
Salinity	28‰ – 32‰
Flow Rate	60 mL/min – 70 mL/min

The test was then initiated by introducing 15 *M. nasuta* to each chamber. On the day before the test was initiated, approximately 75 *M. nasuta* were placed in clean flowing seawater without sediment to depurate for 24 h. These organisms were used to create five samples from which initial tissue weights were obtained; three of these replicates were retained for pre-exposure (background) tissue analysis of tributyltin in *M. nasuta*. These tissue samples were collected in a precleaned glass jar with a teflon-lined lid and stored frozen until the end of the test. Background tissue samples were thawed, homogenized at the same time as the test tissue samples, frozen again and shipped to Ecology's Manchester Environmental Laboratory with the exposed tissue samples. Once the bioaccumulation test was initiated, the organisms in the test chambers were observed daily for any unusual behavior. Any dead organisms were removed promptly and disposed of. Water quality was measured in at least one replicate per treatment daily; flow rates to all aquaria were visually checked daily and quantitatively measured weekly. The 45-d bioaccumulation procedure developed for PSDDA calls for the appropriate (test or control) sediment to be added to each chamber once per week to maintain potential contaminant doses and to provide additional nutrients throughout the duration of the test. In keeping with the smaller chamber size, initial sediment volume, and number of clams, the sediment renewal volume was reduced from 175 mL to 100-mL weekly.

At the end of the 45-d exposure period, sediment from each aquarium was wet-sieved and test organisms were removed. Live and dead *M. nasuta* were counted, and all surviving organisms were placed in clean, flowing seawater for approximately 24 h for depuration. Following the depuration period, clams were shucked with solvent-rinsed titanium knives and the soft tissues transferred directly into preweighed glass sample jars. Excess water was drained and the jars reweighed to obtain tissue sample wet weights. The sample wet weight was divided by the number of surviving clams to obtain an average wet weight per clam in a replicate. Clam weights were compared by treatment by using analysis of variance (ANOVA) and Fisher's least significant difference post-hoc test (α=0.05). Tissue samples were hand delivered to MSL's organic chemistry prep lab where they were homogenized using a stainless steel tissuemizer (which was thoroughly cleaned and solvent-rinsed between samples to avoid cross-contamination). Homogenized tissue samples were frozen overnight and shipped to Ecology's Manchester Environmental Laboratory the following day.

RESULTS

The 45-d bioaccumulation exposure with Squalicum Harbor sediment was initiated on September 30, 2005 and terminated on November 14, 2005, with no significant problems encountered at any time during the test. Survival of *M. nasuta* was at least 87% in each replicate, averaging 97% in the Sequim Bay control and 95% to 96% in the Squalicum Harbor treatments (Table 1). Water quality monitoring indicated acceptable water quality throughout the test (Table 2). Although salinity reached 33‰ for one day (October 29) before the fall rains started, it was still well within the natural environmental conditions for *M. nasuta* and the test did not appear to be adversely affected. Total tissue mass in each sample was at least 40 g, generally 50 g and 60 g per sample, which was more than mass required for TBT analysis including quality control analyses. Average wet tissue mass at the end of the exposure ranged from 3.86 g/clam to 4.17 g/clam (Table 1); tissue mass at termination was not significantly different from the average wet tissue mass of 4.25 g/clam at test initiation (Figure 1).

CONCLUSIONS

Squalicum Harbor sediment was not acutely toxic to *Macoma nasuta* and did not significantly affect clam tissue mass after a 45-d laboratory exposure. The mass of tissue from surviving *M. nasuta* was more than adequate for TBT analysis. Frozen, homogenized tissue samples from pre-exposure (background) *M. nasuta* and *M. nasuta* exposed to control sediment for 45 d along with tissue samples of *M. nasuta* exposed to Squalicum Harbor test sediment were submitted to Ecology's Manchester Environmental Laboratory by Battelle Marine Sciences Laboratory. There were no quality assurance or quality control issues in the bioassay laboratory that would qualify the resulting bioavailability study data.

Table 1. Results of Squalicum Harbor 45-Day Bioaccumulation Test with *Macoma nasuta*

Sample ID	Rep	Number Alive	Number Dead or Missing	M. nasuta Survival		M. nasuta Sample Wet Weight		
				Replicate	Average	Replicate Total (g)	Replicate Average (g/clam)	Treatment Average (g/clam)
Sequim Bay	1	15	0	100%		63.68	4.25	
Sequim Bay	2	14	1	93%		62.90	4.49	
Sequim Bay	3	14	1	93%		54.55	3.90	
Sequim Bay	4	15	0	100%		54.87	3.66	
Sequim Bay	5	15	0	100%	97%	52.08	3.47	3.95
SH 2	1	14	1	93%		50.93	3.64	
SH 2	2	15	0	100%		57.24	3.82	
SH 2	3	14	1	93%		50.69	3.62	
SH 2	4	15	0	100%		67.42	4.49	
SH 2	5	14	1	93%	96%	59.40	4.24	3.96
SH 4	1	14	1	93%		62.16	4.44	
SH 4	2	13	2	87%		56.31	4.33	
SH 4	3	15	0	100%		64.49	4.30	
SH 4	4	15	0	100%		58.35	3.89	
SH 4	5	14	1	93%	95%	54.69	3.91	4.17
SH 6	1	14	1	93%		48.40	3.46	
SH 6	2	14	1	93%		60.70	4.34	
SH 6	3	15	0	100%		59.28	3.95	
SH 6	4	14	1	93%		55.60	3.97	
SH 6	5	15	0	100%	96%	53.59	3.57	3.86
Pre-Exposure Tissue	1	15	NA	NA		62.85	4.19	
Pre-Exposure Tissue	2	15	NA	NA		62.17	4.14	
Pre-Exposure Tissue	3	15	NA	NA		59.66	3.98	
Pre-Exposure Tissue	4[a]	15	NA	NA		74.81	4.99	
Pre-Exposure Tissue	5[a]	15	NA	NA	NA	59.62	3.97	4.25

a. Not submitted for TBT analysis.

Table 2. Summary of Water Quality during Squalicum Harbor 45-Day Bioaccumulation Test

Treatment	Temperature (°C)			Salinity (‰)			Dissolved Oxygen (mg/L)			pH (units)		
	Min	Average	Max	Min	Average	Max	Min	Average	Max	Min	Average	Max
Target Range	12	14	16	28	30	32	5	NA	NA	7.3	7.8	8.3
Sequim Bay	12	14	16	31	32	32	7.1	7.9	8.4	7.4	7.6	7.7
SH 2	12	14	16	31	32	33[a]	6.6	7.7	8.3	7.3	7.6	7.7
SH 4	12	14	16	31	32	33[a]	6.7	7.7	8.3	7.3	7.6	7.7
SH 6	12	14	16	31	32	32	7.0	7.8	8.4	7.4	7.6	7.7

a. Salinity reached 32.6‰ on October 29 only.

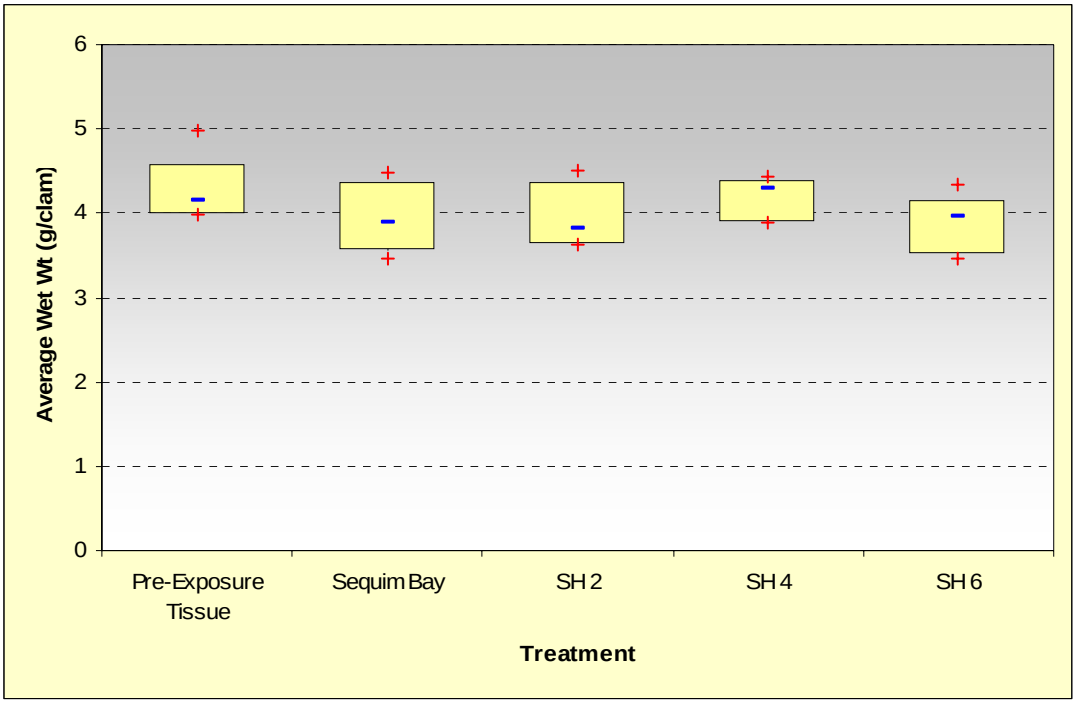

Figure 1. Median (dash), 25th and 75th Percentile (box), and range (+) of Tissue Wet Weights

References

ASTM (American Society for Testing and Materials). 2000. *Standard Guide for Determination of the Bioaccumulation of Sediment-Associated Contaminants by Benthic Invertebrates. Designation E 1688-00a.* American Society for Testing and Materials, West Conshohocken, PA.

PSDDA (Puget Sound Dredge Disposal Analysis). 1994. *Refinements to Bioaccumulation Testing Requirements: Adoption of a second test species for consistency with National Guidance.* Issue paper prepared by David Kendall, U. S. Army Corps of Engineers, Seattle District, Seattle, WA.

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PSDDA (Puget Sound Dredge Disposal Analysis). 2000. *Clarifications to the DMMP Bioaccumulation Protocol.* DMMP Clarification paper prepared by David Kendall, U. S. Army Corps of Engineers, Seattle District, Seattle, WA, and Russ McMillan, Washington Department of Ecology, Olympia, Washington.

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Appendix F. Benthic Invertebrate Data

Table F-1. Benthic invertebrate data.

SPECIES	STATIONS							Pollution Indicator[1]
	SH1	SH2	SH3	SH4	SH5	SH6	SH8	
CRUSTACEA								
Balanus sp.		1	3	5	312	5	49	
Bathyleberis sp.							3	
Euphilomedes carcharodonta		1					35	Slightly tolerant
Euphilomedes producta							3	Slightly tolerant
Nebalia sp.							6	
Leptochelia dubia	1			1	1		434	
Sinelobus stanfordi							1	
Diastylis santamariensis							4	
Ampelisca pugetica							8	Sensitive
Ampelisca agassizi							130	Sensitive to hypoxia
Byblis millsi							37	
Grandidierella japonica							1	
Argissa hamatipes							2	
Monocorophium acherusicum						4	2	
Monocorophium insidiosum					1			
Dexamonica reduncans							1	
Protomedeia prudens							22	
Anonyx lilljeborgi							1	
Orchomene pinguis							1	
Photis sp.							9	
Rhepoxynius boreovariatus							138	Sensitive
Dulichia sp.							1	
Caprella mendax	1						5	
Tritella pilimana							1	
Caridea - zoeae							2	
Crangon franciscorum					1			
Heptacarpus stimpsoni					1		1	
Cancer gracilis					2			
Hemigrapsus oregonensis						1		
Pinnotheridae - zoeae	2		1				88	
Scleroplax granulata		3	6					
Pinnixa schmitti					2	3	181	
Porcellanidae - zoeae							2	
Neotrypaea sp.						1		
Neotrypaea californiensis		1						
MOLLUSCA								
Acila castrensis						2		
Aeolidacea						1		
Alvania compacta	5				34	30	102	
Astyris gausapata							1	
Axinopsida serricata	2		31	18	24	48	259	Slightly tolerant
Clinocardium nuttalli					6	7	55	
Haminoea sp					3			
Haminoea vesicula					9	8	5	
Hiatella arctica					2			
Kurtziella plumbea					1	4	2	
Lucinoma annulatum							1	
Lyonsia californica							1	

SPECIES	STATIONS							Pollution Indicator[1]
	SH1	SH2	SH3	SH4	SH5	SH6	SH8	
Macoma balthica			1		11	12	3	Moderately tolerant
Macoma carlottensis					2			Moderately tolerant
Macoma inquinata					10	4		Moderately tolerant
Macoma nasuta	32	20	23	43	334	208	8	Moderately tolerant
Macoma sp					61	28	27	Moderately tolerant
Macoma yoldiformis							8	Moderately tolerant
Mya arenaria					2	11		
Mytilus sp					2			
Nassarius mendicus	14	21	10	4	10	8		
Nutricola lordi					19	9	334	
Odostomia sp	11		3		29	26	3	
Onchidoris bilamellata					1			
Parvilucina tenuisculpta							2	Moderately tolerant
Protothaca staminea				1	6	22	21	
Rochefortia tumida	57	20	78	157	317	296	772	
Saxidomus gigantea					7	3	2	
Solen sicarius						1	4	
Spiromoellaria quadrae					11	4	3	
Tellina modesta					7	20	134	
Tellina sp					9	5	10	
Tresus sp							1	
Turbonilla sp					5	7	1	
ANNELIDA								
Ampharete finmarchica							1	
Ampharetidae							1 juv	
Aphelochaeta glandaria	1379	1783	1295	3754	2220	1364		Tolerant
Aphelochaeta monilaris					149	609		Tolerant
Aphelochaeta sp	81	101	62	296	589	588		Tolerant
Armandia brevis							1	
Capitella capitata	2	1		1	165	131		Resistant to
Chone ecaudata					1			
Chone sp						4		
Cirratulus robustus	1		1	1	2354	2793	47	
Cossura pygodactylata						2		
Dipolydora cardalia							1	
Dipolydora socialis						1		
Dorvillea annulata		1			4	3		
Eteone sp					9	3	3	
Eteone spilotus						1	1	
Euclymeninae							122 juv	
Eudistylia sp						1		
Eumida longicornuta							20	
Exogone dwisula							14	
Exogone lourei							17	
Glycera nana							1	
Glycinde picta				2	61	43	47	
Harmothoe imbricata						1	2	
Heteromastus filobranchus		4	4	19	29	56		
Leitoscoloplos pugettensis						1	71	
Lumbrineris californiensis							48	
Magelona longicornis							3	Resistant to severe
Malmgreniella macginitiei							1	
Mediomastus californiensis				5	24	17	2	Slightly tolerant
Melinna elisabetheae							1	

SPECIES	STATIONS							Pollution Indicator[1]
	SH1	SH2	SH3	SH4	SH5	SH6	SH8	
Monticellina serratiseta				1	13	4		
Neanthes virens	1							
Nephtys caeca							9	
Nephtys caecoides							7	
Nephtys cornuta					5	1	1	Slightly tolerant
Nephtys ferruginea							1	
Nereis procera							3	Moderately tolerant
oligochaeta							7	Tolerant
Onuphis elegans						1	9	
Onuphis sp							88 juv	
Ophelina acuminata							1	
Ophiodromus pugettensis	48	66	56	62	62	35	7	
Owenia johnsoni		1	4	10	451	413	1652	
Paleanotus bellis					2			
Paraprionospio pinnata	2	2	3		2	3		Resistant to severe
Pectinaria californiensis				1				Slightly tolerant
Pectinaria granulata					1	2	15	Slightly tolerant
Pherusa plumosa						1		
Pholoe minuta						1	26	
Pholoe sp N1					5			
Phyllodoce hartmanae							2	
Pilargis maculata					1			
Pista wui						1		
Platynereis bicanaliculata					1		3	
Polycirrus sp						1		
Polydora limicola						1		
Prionospio lighti	7	1	2		11	3	23	Slightly tolerant
Prionospio steenstrupi					1	1	4	
Pseudopolydora paucibranchiata					7	36	9	
Scoletoma luti						30		
Scoloplos luti					15		38	
Sphaerosyllis californiensis							2	
Spiochaetopterus pottsi					1	4	5	
Spiophanes berkeleyorum							2	
Spiophanes kroeyeri					1			
Tenonia priops							14	
Terebellides sp						1		
CNIDARIA								
Anthozoa: Edwardsia sp G					20	17	7	
Anthozoa: Halcampidae							1 tiny juv	
ECHINODERMATA								
Holothuroidea:								
Pentamera rigida							5 adult 3 juv	
Pentamera sp							5 juv	
Ophiurida:								
Amphiodia sp					6 juv	3 juv	101 juv	
Amphiodia urtica/periercta					12	2	10	
Amphiuridae					1 juv		4 juv	
NEMERTEA								
Lineidae							2	
Micrura sp	2				4 adults 1 juv		7	
Paranemertes californica							1	
Tetrastemma nigrifrons							4	
Tubulanus polymorphus					1		2	

SPECIES	STATIONS							Pollution Indicator[1]
	SH1	SH2	SH3	SH4	SH5	SH6	SH8	
Tubulanus sp							15 juv	
PHORONIDA								
Phoronopsis harmeri				2		3		
PLATYHELMINTHES								
Leptoplanidae							4	
PRIAPULA								
Priapulus caudatus						1		
SIPUNCULA								
Thysanocardia nigra							6 adult 2 juv	

1. Source: Washington State Department of Ecology Marine Monitoring Program compilation (S. Aasen, personal communication).

Appendix G. Remedial Investigation and Feasibility Study Metals Data

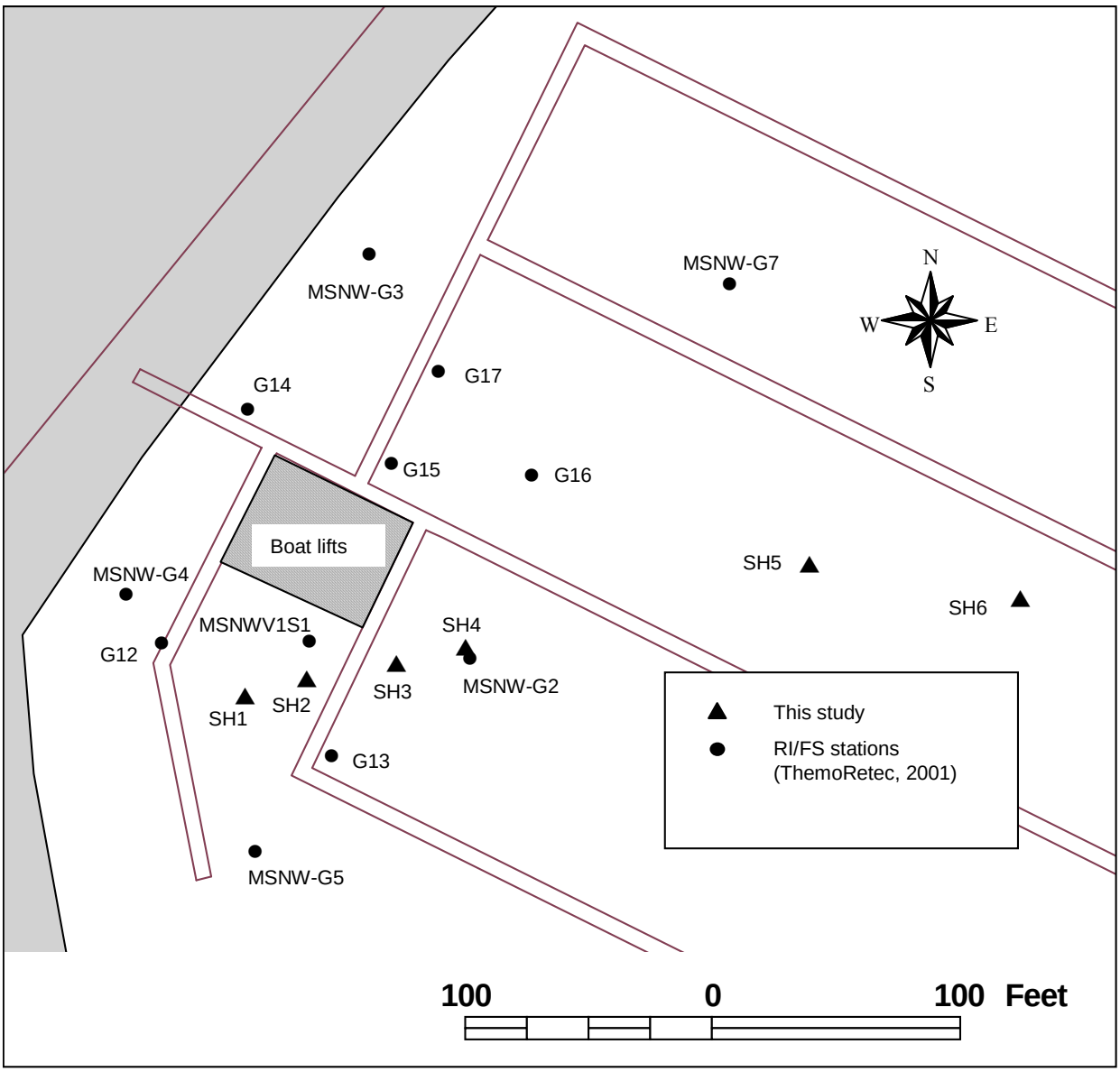

Figure G-1. Sediment Sampling Locations.

Table G-1. Metals Concentrations (mg/kg) from Marine Services Northwest (MSNW) RI/FS Investigation (ThermoRetec, 2001). Bold shaded values exceed SMS criteria. See Figure G-1 for station locations.

	Antimony	Arsenic	Cadmium	Chromium	Copper	Lead	Mercury	Nickel	Silver	Zinc
SMS Criteria (mg/kg)										
SQS	--	57	5.1	260	390	450	0.41	--	6.1	410
CSL	--	93	6.7	270	390	530	0.59	--	6.1	960
Station										
G12	<10	<10	0.7	76	111	16	0.3	105	<0.7	185
G13	20	<10	<0.5	69	116	16	0.3	96	<0.7	205
G14	<10	<10	<0.4	45	100	10	0.2	60	<0.6	125
G15	<10	<10	<0.5	71	283	19	0.3	98	<0.7	194
G16	<10	<10	<0.6	73	108	14	0.2	102	<0.9	145
G17	<10	<10	<0.5	77	114	16	0.3	105	<0.7	167
MSNW-G1	20	<10	0.5	83	112	17	0.3	115	<0.6	147
MSNW-G2	20	<10	0.6	77	92.5	18	0.26	108	<0.7	140
MSNW-G3	<9	<9	<0.3	37.3	45.5	7	0.17	46	<0.5	76
MSNW-G4	20	<10	0.5	59	83	14	0.2	80	<0.6	112
MSNW-G5	<10	<10	0.4	69	74	14	0.22	91	<0.6	122
MSNW-G6	20	<10	0.9	76	79.4	17	0.23	104	<0.6	136
MSNW-G7	20	<10	0.6	78	76.6	17	0.24	107	<0.7	130
MSNWV1S1	<10	<10	1.5	78	**665**	47	**0.5**	107	<0.7	**1170**