# Quality Assurance Project Plan

# Squalicum Harbor
# Tributyltin (TBT) Investigation

by
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November 2005

Publication Number 05-03-117

This plan is available on the Department of Ecology home page on the
World Wide Web at *www.ecy.wa.gov/biblio/0503117.html*.

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# Squalicum Harbor
# Tributyltin (TBT) Investigation

November 2005

**303(d) Listings Addressed in this Study: None**

Waterbody Number: WA 01-0050

Project Code: 06-069

**Approvals**

| | |
|---|---|
| Approved by: | November 17, 2005 |
| Mary O'Herron, Client, BFO, NWRO | Date |
| Approved by: | November 22, 2005 |
| Steven Alexander, Section Manager, TCP, NWRO | Date |
| Approved by: | November 15, 2005 |
| Nigel Blakley, Project Manager, Toxic Studies Unit | Date |
| Approved by: | November 15, 2005 |
| Dale Norton, Unit Supervisor, Toxic Studies Unit | Date |
| Approved by: | November 16, 2005 |
| Will Kendra, Section Manager, Watershed Ecology Section | Date |
| Approved by: | November 16, 2005 |
| Stuart Magoon, Director, Manchester Environmental Laboratory | Date |
| Approved by: | November 16, 2005 |
| Stewart Lombard, EAP QA Coordinator | Date |

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# Abstract

Six sampling stations in Inner Squalicum Harbor will be evaluated for tributyltin (TBT) concentrations in bulk sediment and pore water. Squalicum Harbor is a small-boat marina on the Bellingham city waterfront in northwestern Washington. Previous sampling showed elevated TBT concentrations in sediment near an area used for boat maintenance activities, including bottom scraping and repainting. TBT was used as an additive in antifouling paints from the 1960s until 1990.

Effects on benthic invertebrates will be assessed at each station with respect to TBT concentrations in sediment and pore water. Invertebrate abundance and diversity in sediment samples will be evaluated and TBT tissue concentrations will be measured at stations where sufficient invertebrate biomass is collected. If insufficient biomass is collected at three or more stations, TBT concentrations will be measured in tissue of clams exposed under laboratory conditions to sediment samples collected from these stations.

Results from this study will be used by the Washington State Department of Ecology (Ecology) Sediment Management Unit, Northwest Regional Office Toxics Cleanup Program and Bellingham Field Office to make recommendations for cleanup of sediments in Inner Squalicum Harbor. The results may also be used by Ecology in developing methods for regulating TBT sediment contamination under the Washington Sediment Management Standards regulation (WAC 173-340).

# Background

Sediments within Squalicum Harbor, in Bellingham Bay, Washington (Figure 1), have been contaminated with tributyltin (TBT). A Remedial Investigation and Feasibility Study (RI/FS) for the harbor's inner boat basin reported up to 10.68 mg TBT ion/Kg dry sediment and pore water concentrations up to 1.1 ug TBT ion/L (ThermoRetec, 2001). The area of contamination surrounds two boat lifts operated by Marine Services Northwest (MSNW) for the maintenance of small boats, including bottom scraping and repainting (Figure 2).

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

Criteria based on pore water, rather than bulk sediment, concentrations were recommended in a review of issues relating to the development of SMS standards for TBT (Michelsen et al., 1996) (Appendix A). The report recommended a value 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 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.

Three stations sampled in the MSNW investigation had pore water TBT concentrations exceeding the recommended TBT SQS and CSL (Figure 3). These stations had the highest bulk sediment TBT concentrations and are near the MSNW boat lifts.

Biological testing of these sediments has not been conducted; although, 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 biological effects exceedances. Recommendations for biological testing in the TBT issue paper include bioaccumulation studies and longer-term, rather than acute, bioassay tests (Michelsen et al., 1996).

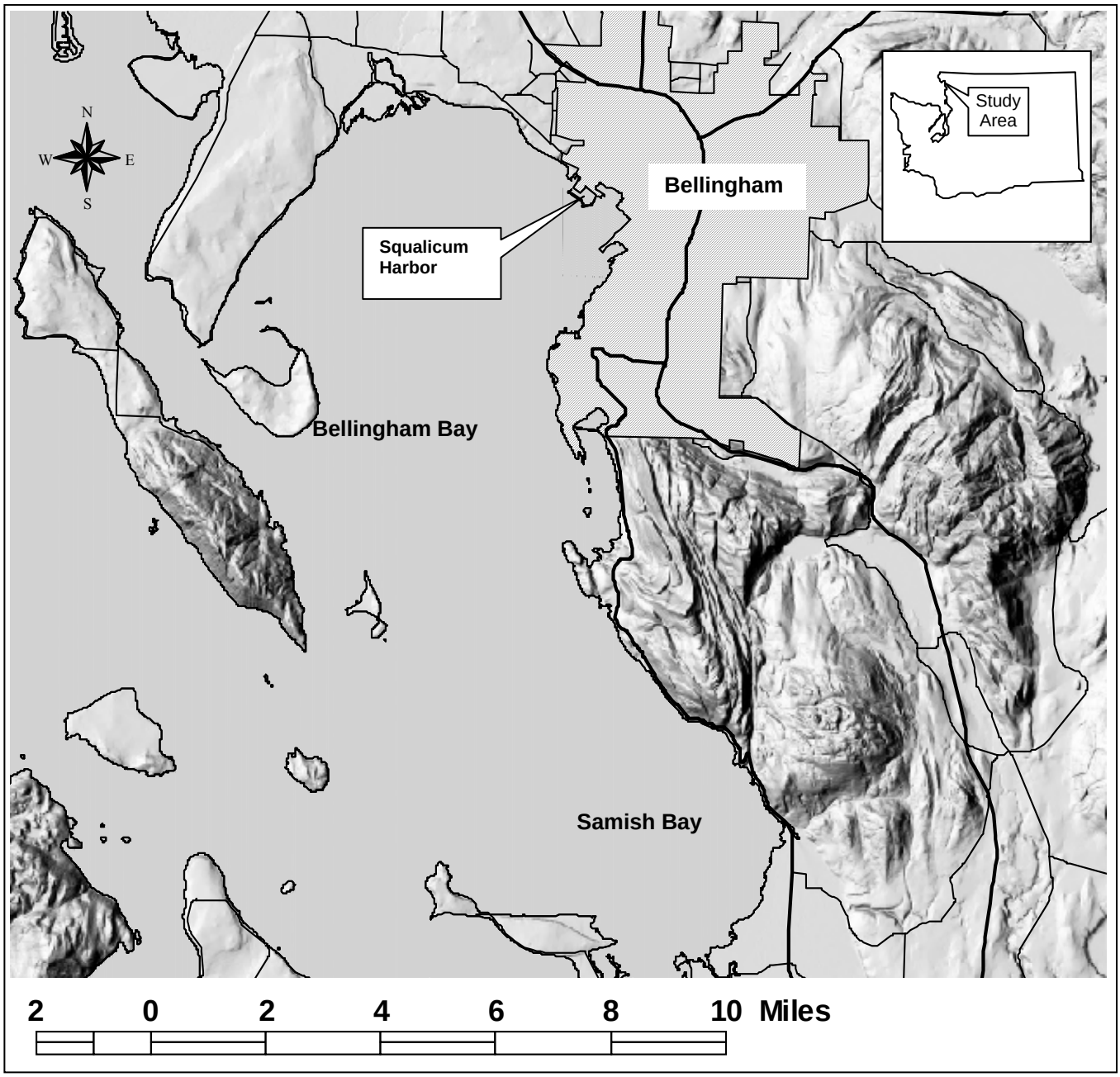


Figure 1: Location Map for Squalicum Harbor.

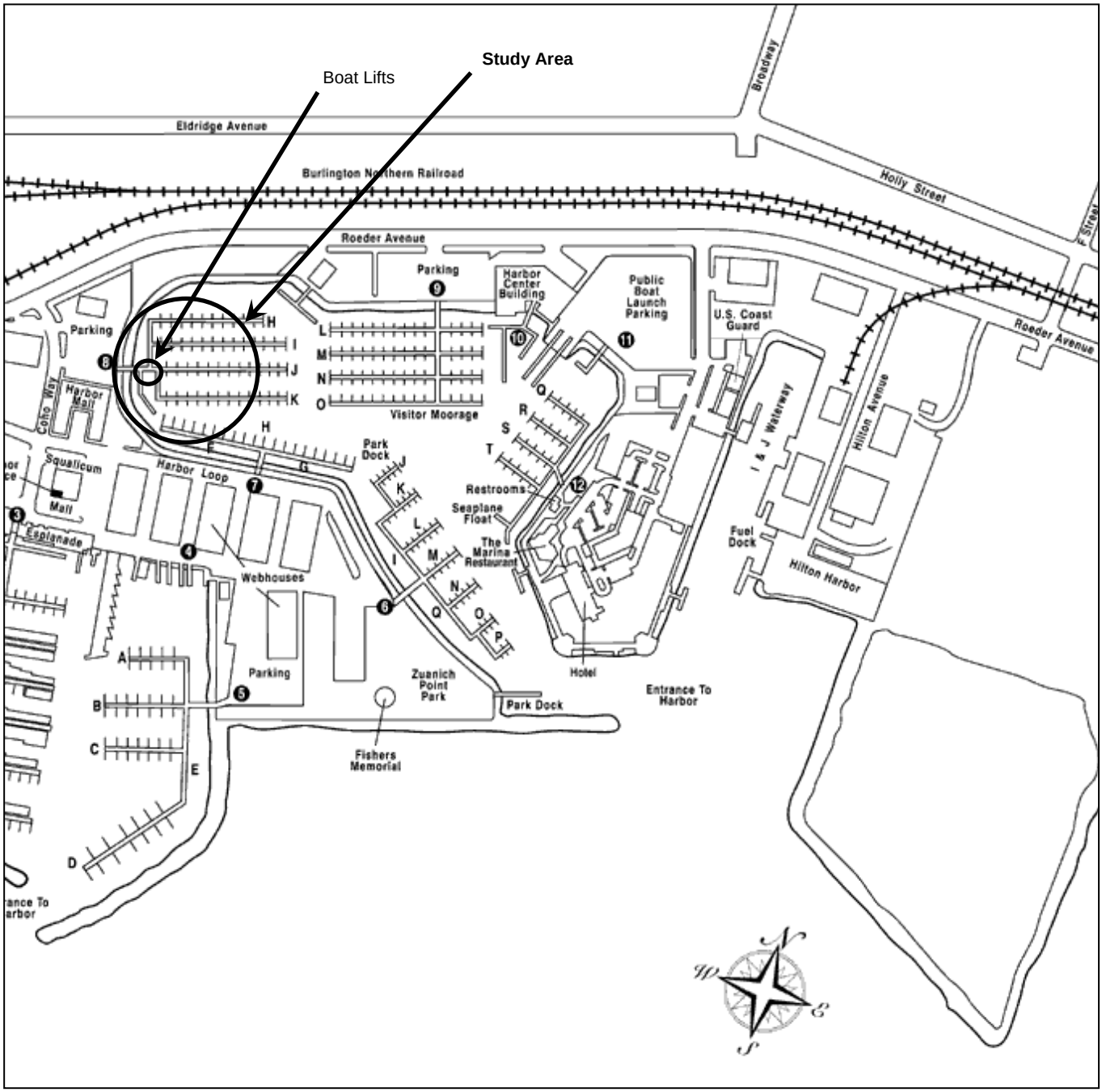


Figure 2: Inner Squalicum Harbor Study Area.

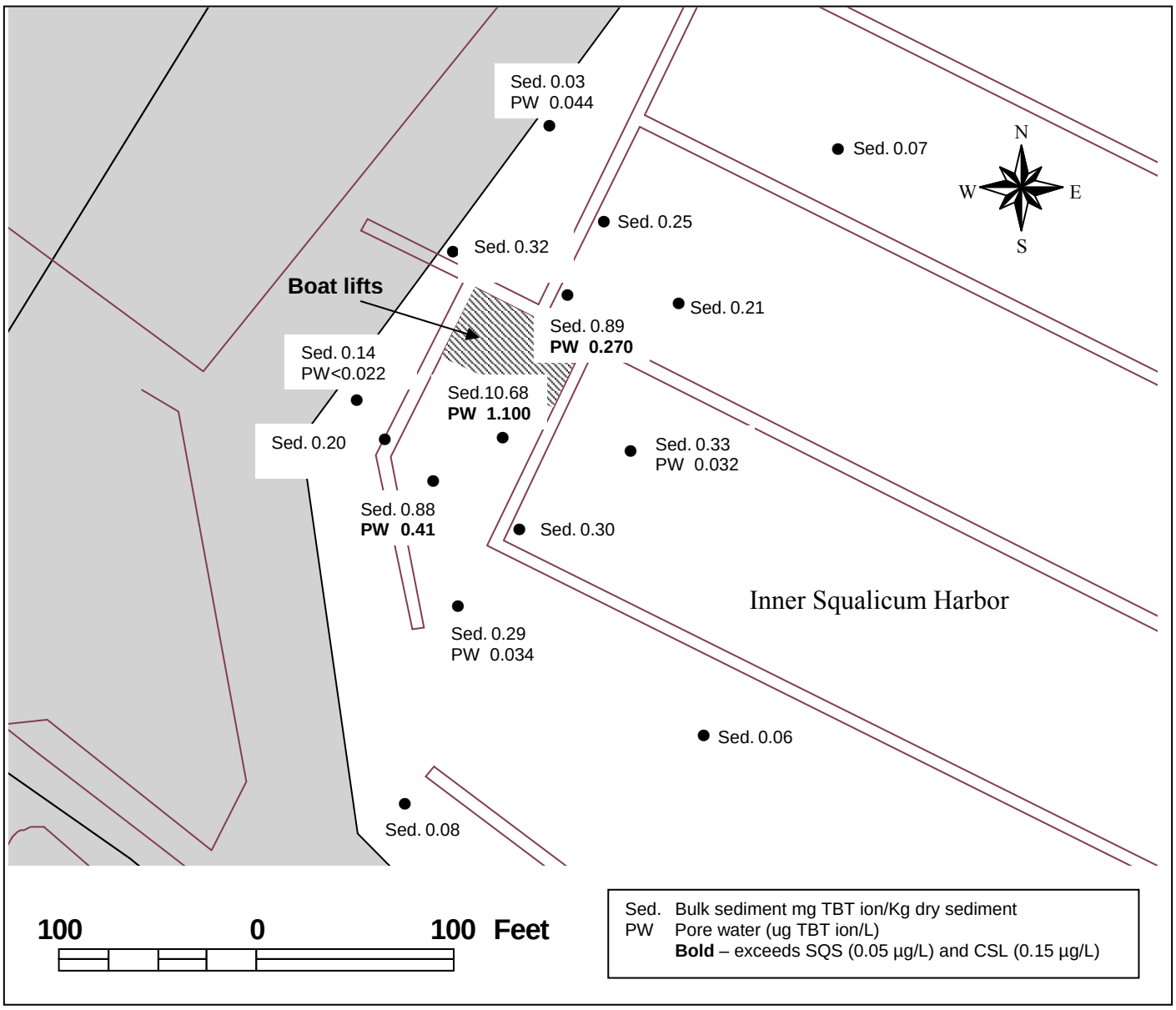


Figure 3: TBT Concentrations in Bulk Sediment and Pore water. Data from Marine Services Northwest Remedial Investigation and Feasability Study (ThermoRetec, 2001).

# Project Description

This project was requested by the Ecology Bellingham Field Office for use in making sediment cleanup decisions. The project will supplement data from the MSNW RI/FS sampling conducted in 2000 (ThermoRetec, 2001).

The primary objectives of this project are to supplement the limited data available on TBT bulk and pore water concentrations in the sediments and to evaluate the benthic effects of the TBT contaminated sediment.

A secondary objective is to obtain toxicity and tissue bioaccumulation information that may be useful to Ecology in developing appropriate methods for regulating TBT contamination under the SMS regulation and to understand the relationship between benthic effects, standard toxicity test results, 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).

Sampling will be conducted on a transect (Figure 4) across a TBT concentration gradient (Figure 3) documented in the MSNW RI/FS report (ThermoRetec, 2001). The transect sediment samples will be obtained using a double compartment Van Veen grab sampler operated from Ecology's R.V. *Skookum*. At each sampling location, sediment from four grabs will be collected for use in biological testing, chemistry analysis, and characterization of sediment physical properties, such as grain size.

# Organization and Schedule

## Roles and Responsibilities

| | | |
|---|---|---|
| Project Manager | Nigel Blakley (360) 407-6770 | Project management, report preparation. |
| Project Assistant | Peter Adolphson (360) 407-7557 | Assist with project planning, Quality Assurance (QA) Project Plan, and data analysis. |
| Project Assistant | Randy Coots (360) 407-76690 | Boat operator, station positioning, boat safety. |
| Project Assistant | Dave Serdar (360) 407-6772 | Assist with boat operations, boat safety. |
| Project Assistant | Maggie Dutch (360) 407-6021 | Assist with sampling, advise on project planning. |
| Client (TCP-NWRO/BFO) | Mary O'Herron (360) 738-6246 | Review QA Project Plan and report. |
| TSU Supervisor | Dale Norton (360) 407-6765 | Project review. |
| Lab Analyses | Pam Covey (360) 871-8827 | Laboratory contracts. |
| Laboratory Analyses | Bob Carrell (360) 871-8804 | TBT analyses. |
| Lab Quality Assurance | Karin Feddersen (360) 871-8829 | Assist in QA Project Plan preparation, data review. |
| EIM Data Entry | Carolyn Lee (360) 407-6430 | Data entry. |

## Schedule

| | |
|---|---|
| Field Sample Collection | September 2005 |
| Laboratory Analysis Complete | December 2005 |
| Benthic Infauna Analysis Complete | August 2006 |
| Data Transfer to SEDQUAL Templates | August 2006 |

## Schedule for EIM and Final Report

| Environmental Information System (EIM) Data Set (if applicable) | |
|---|---|
| EIM Data Engineer | Carolyn Lee |
| EIM User Study ID | NBLA0003 |
| EIM Study Name | Squalicum Harbor TBT |
| EIM Completion Due | October 2006 |
| **Final Report** | |
| Report Author Lead | Nigel Blakley |
| Schedule | |
| Report Supervisor Draft Due | October, 2006 |
| Report Client/Peer Draft Due | November 2006 |
| Report External Draft Due | NA |
| Report Final Due (original) | January 2007 |

## Summary of Estimated Laboratory Cost

| Analysis | No. of Samples | No. of QA Samples | Total | Unit Cost | Subtotal | Laboratory |
|---|---|---|---|---|---|---|
| Conventionals | | | | | | |
| Percent solids | 8 | | 8 | $10 | $80 | MEL |
| Grain size | 8 | 2 | 10 | $100 | $1,000 | Contract lab |
| Total Organic Carbon | 8 | 1 | 9 | $40 | $360 | MEL |
| Tributyltin (TBT) | | | | | | |
| Bulk sediment | 8 | 4 | 12 | $175 | $2,100 | MEL |
| Pore water | 8 | 4 | 12 | $225 | $2,700 | Contract lab* |
| Invertebrate tissue | 5* | 4 | 9 | $225 | $2,025 | MEL |
| Benthic infauna | 7 | | 7 | $600 | $4,200 | Contract lab |
| Laboratory TBT bioaccumulation | | | | | | |
| 45-day exposure | 3† | | 3 | | $20,000 | Contract lab |
| Tissue analysis | 23† | 4 | 27 | | $6,075 | MEL |
| | | | | Subtotal | $38,540 | |
| | | | | Contracting fee (25%) | $6,725 | |
| | | | | TOTAL | $45,265 | |

Estimate is based on 50% discount rate for analysis at Manchester Environmental Laboratory (MEL).

* Contract lab will perform pore water extraction and TBT analysis.

† Estimate assuming samples for tissue TBT analysis are not obtained at three stations. Sediment from those stations would then be used for the laboratory bioaccumulation study. If samples for tissue analysis are obtained at all stations and the laboratory bioaccumulation study is not conducted, the estimated total laboratory costs will be $20,840.

# Quality Objectives

Measurement Quality Objectives are shown in Table 1.

# Sampling Process Design

The sampling locations chosen for this investigation are shown in Figure 4, except for the reference station in Samish Bay (about 11 miles south of the study area). Sampling station coordinates are listed in Table 2. The objective in selecting the locations was to sample from a gradient of TBT sediment concentrations previously documented in the study area (Figure 3).

The Samish Bay reference station location was selected because it has previously been found to be uncontaminated and supporting a healthy benthic invertebrate community (Long et al., 1999).

Table 1: Quality Control Samples and Measurement Quality Objectives.

| Parameter | Lowest Measurement of Interest | QC Samples | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Field Duplicates[1] | | Method Blank | | Analytical Replicates[2] | | Laboratory Control Sample[3] | | Matrix Spike and Matrix Spike Duplicate | |
| | | Number | Evaluation | Number | Evaluation | Number | Evaluation | Number | Evaluation | Number | Evaluation |
| Grain size | 1% | 1 | See footnote 4 | -- | | 1 triplicate analysis | RSD ≤ 20 % | -- | | -- | |
| TOC | 0.1% | 1 | See footnote 4 | 1/batch | Analyte concentration < PQL | 1 triplicate analysis | RSD ≤ 20 % | -- | | -- | |
| TBT – bulk sediment | 5 µg/kg dry weight | 1 | See footnote 4 | 1 | Analyte concentration < PQL | 1 duplicate analysis | RPD ≤ 20 % | 1 | 50–150 % recovery, or performance based intralaboratory control limits, whichever is lower | 1 | 50–150 % recovery applied when the sample concentration is < 4 times the spiked concentration; RPD ≤ 20 % |
| TBT - pore water | 0.05 µg/L | 1 | See footnote 4 | 1 | Analyte concentration < PQL | 1 duplicate analysis | RPD ≤ 20 % | 1 | 50–150 % recovery, or performance based intralaboratory control limits, whichever is lower | 1 | 50–150 % recovery applied when the sample concentration is < 4 times the spiked concentration; RPD ≤ 20 % |
| TBT – tissue (field samples) | 5 µg/kg wet weight (25 µg/kg dry weight, assuming 80% moisture | 1 | See footnote 4 | 1 | Analyte concentration < PQL | 1 duplicate analysis | RPD ≤ 20 % | 1 | 50–150 % recovery, or performance based intralaboratory control limits, | 1 | 50–150 % recovery applied when the sample concentration is < 4 times |

| Parameter | Lowest Measurement of Interest | QC Samples | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Field Duplicates[1] | | Method Blank | | Analytical Replicates[2] | | Laboratory Control Sample[3] | | Matrix Spike and Matrix Spike Duplicate | |
| | | Number | Evaluation | Number | Evaluation | Number | Evaluation | Number | Evaluation | Number | Evaluation |
| | content) | | | | | | | | whichever is lower | | the spiked concentration; RPD ≤ 20 % |
| TBT – tissue (lab bioaccumulation study) | As above | 0 | | 1 | Analyte concentration < PQL | 1 duplicate analysis | RPD ≤ 20 % | 1 | 50–150 % recovery, or performance based intralaboratory control limits, whichever is lower | 1 | 50–150 % recovery applied when the sample concentration is < 4 times the spiked concentration; RPD ≤ 20 % |
| Benthic infauna | QA/QC procedures for the benthic infauna test are described in the Sample Handling Procedures section. | | | | | | | | | | |

Notes:
RPD Relative percent difference.
RSD Relative standard deviation.

[1] Field duplicates: Independent samples that are collected in separate casts as close as possible to the same point in space and time. They are two separate samples, stored in separate containers, and analyzed independently.

[2] Synonymous with Laboratory Replicates or, if applicable, Laboratory Duplicates.

[3] 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.

[4] Differences between field duplicate measurements should be less than among sampling stations.

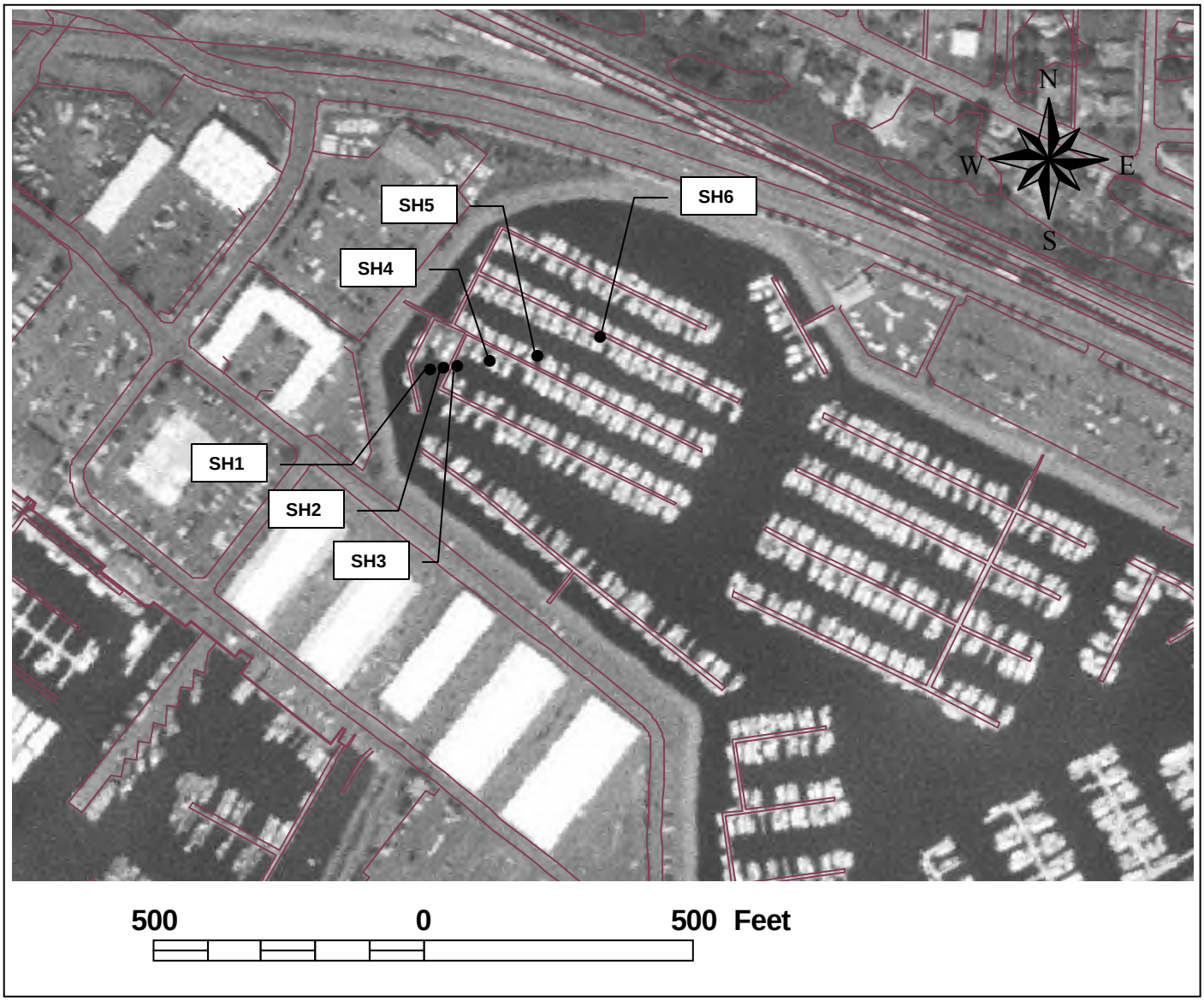


Figure 4: Proposed Sampling Locations in Inner Squalicum Harbor.

Table 2: Sediment Sampling Locations in Squalicum Harbor and Samish Bay Reference Area.

| Area | Station ID | Coordinates (NAD 1983) Degree and Decimal Minutes | | | |
|---|---|---|---|---|---|
| | | Latitude | | Longitude | |
| Squalicum Harbor | SH1 | 48 | 45.4854 | 122 | 30.1129 |
| | SH2 | 48 | 45.4860 | 122 | 30.1069 |
| | SH3 | 48 | 45.4866 | 122 | 30.1003 |
| | SH4 | 48 | 45.4884 | 122 | 30.0853 |
| | SH5 | 48 | 45.4902 | 122 | 30.0637 |
| | SH6 | 48 | 45.4962 | 122 | 30.0349 |
| | SH7 | Field duplicate* | | | |
| Samish Bay (Reference) | SH8 | 48 | 36.167 | 122 | 29.367 |

* Location of field duplicate will be decided during sampling.

# Sampling Procedures

## Navigation and Positioning

All sampling will be conducted from Ecology's 26-foot research vessel R.V. *Skookum*. The sampling stations will be located using a differentially corrected Global Positioning System. As a check, visual landmarks in the marina (such as dock pilings) will also be used to locate the approximate position of the sampling stations. Landmarks will be established using measurements from a scale map of the marina docks and other structures (available in GIS form at www/.cob.org/gis/metadata/index.htm). The landmarks will be selected during a presampling reconnaissance site visit.

At each station grab samples will taken from within a 30-ft. radius of the target coordinates listed in Table 2. Logistic details, such as the timing and sequence of station sampling, will be determined in consultation with the Squalicum Harbormaster to further minimize disruptions to marina activities.

## Sediment Sample Collection

All utensils used to manipulate the samples (stainless steel scoops and mixing bowls) will be precleaned by washing with Liquinox® detergent, followed by sequential rinses with tap water, deionized water, and pesticide-grade acetone. The equipment will then be air dried and wrapped in aluminum foil until used in the field. The grab sampler will be precleaned with Liquinox® detergent and deionized water.

A minimum of four double Van Veen grabs will collected at each station (Table 3). A log will be maintained for each station (Appendix B). Between stations, the grab sampler will be cleaned by thoroughly brushing with on-site seawater. If oil or visible contamination is encountered, the grab will be cleaned between samples with a detergent followed by a rinse with on-site seawater.

Table 3: Samples to be Collected.

| Station | Double Van Veen Grabs #1 - #3 | | | | Double Van Veen Grab #4 | | Bioaccumulation | |
|---|---|---|---|---|---|---|---|---|
| | Chemistry (1 Compartment/Grab) | | Benthic Infauna (1 Compartment/Grab) | | Tissue (Both Grab Compartments) | | | |
| | Samples | Containers | Samples | Containers | Samples | Containers | Grabs | Containers |
| SH1-SH6 | 1 sample per station (pooled from 3 grabs) | 2 oz glass jar (TOC)<br>8 oz plastic jar (grain size)<br>2 oz glass jar (percent solids)<br>8 oz glass jar (TBT bulk sediment)<br>4 1-L glass jars (sediment for pore water TBT analysis) | 1 sample per grab (3 replicate samples per station) | Sieved invertebrates from each grab into plastic bags with formalin | 1 sample per station | 8 oz glass jars (clams)<br>2 oz glass jar (polychaetes) | Conducted in a second round of sampling at three stations and only if tissue sampling is unsuccessful | |
| Field Duplicate (SH7) | 1 sample per station (pooled from 3 grabs) | 2 oz glass jar (TOC)<br>8 oz plastic jar (grain size)<br>2 oz glass jar (percent solids)<br>8 oz glass jar (TBT bulk sediment)<br>4 1-L glass jars (sediment for pore water TBT analysis) | 0 | None | 1 sample per station | 8 oz glass jars (clams)<br>2 oz glass jar (polychaetes) | 0 | None |
| Samish Bay Reference (SH8) | 1 sample per station (pooled from 3 grabs) | 2 oz glass jar (TOC)<br>8 oz plastic jar (grain size)<br>2 oz glass jar (percent solids)<br>8 oz glass jar (TBT bulk sediment)<br>4 1-L glass jars (sediment for pore water TBT analysis) | 1 sample per grab (3 replicate samples per station) | Sieved invertebrates from each grab into plastic bags with formalin | 1 sample per station | 8 oz glass jars (clams)<br>2 oz glass jar (polychaetes) | 0 | None |
| SHB1-SHB3 | 0 | None | 0 | None | 0 | None | Sufficient to fill one 5-gal bucket at each station | 1 5-gal HDPE buckets |

Location of stations SHB1-SHB3 will depend on outcome of sampling for TBT tissue concentrations – see discussion under *Sediment Sample Collection.*

## Sediment Chemistry and Conventionals

Samples will be collected using two 0.1 $m^2$ stainless steel Van Veen grabs yoked together to form a double grab with two compartments. To be considered acceptable, a grab should not be over-filled with sediment, there should be overlying water on the sediment that is not excessively turbid, and the sediment surface should be relatively flat.

Approximately 1.2 gal. of sediment is required to fill all the chemistry containers (Table 3). Sediment from one compartment of three successive grabs will be composited by mixing in a stainless steel bowl to obtain this volume. For each grab, the overlying water will be siphoned off. The top 10-cm layer of sediment, not in contact with the sidewalls of the grab, will then be removed with a stainless steel scoop, placed in a stainless steel bucket, and homogenized by stirring. Subsamples of the homogenized sediment will be transferred to glass jars cleaned to EPA QA/QC specifications (EPA, 1990). Containers and holding times are shown in Table 4.

Clams or large polychaetes found while processing the sediment will be removed and combined with collections for tissue analysis (Grab #4).

Table 4: Containers, Preservatives, and Holding Times for Sediment Samples.

| Analyte | Container | Preservation Techniques | Holding Time |
|---|---|---|---|
| TOC | 2 oz glass jar | Cool to 4°C | 14 days |
| Grain Size | 8 oz plastic jar | Cool to 4°C | 6 months |
| Percent Solids | 2 oz glass jar | Cool to 4°C | 7 days |
| TBT – bulk sediment | 8 oz glass jar | Cool to 4°C | 14 days |
| TBT – sediment for pore water | 4 1-L glass jars* | Cool to 4°C | 7 days (extracted pore water) |
| TBT – invertebrate tissue | 8 oz glass jars – clams<br>2 oz glass jar – polychaetes | Cool to 4°C<br>Freeze -18°C | 14 days<br>1 year |
| Benthic invertebrates – from sieved sediment | Plastic bags (quart and gallon sizes) | 70% ethanol | Samples fixed in formalin solution – transferred to 70% ethanol after 7-10 days (recommended), 1 month max. Indefinite holding time in 70% ethanol. |
| Sediment for bioaccumulation | 5 gal HDPE bucket | Cool to 4°C<br>Freeze | 14 days<br>1 year |

* Samples will be shipped in 4 jars as a precaution against loss due to breakage during transportation. Pore water will be prepared from the samples by the contract lab.

## Benthic Infauna

Where applicable, sampling methods will follow Puget Sound Estuary Protocols (PSEP, 1987) and requirements of Ecology's Sediment Management Standards (Chapter 173-204 WAC; Ecology, 2003).

Three grabs per station will be collected for benthic infaunal analysis. Each grab will be handled as a separate replicate. To be acceptable, the grab should have a minimum penetration depth of 10 cm.

For each grab, all of the contents from one compartment will be retained and sieved through a 1.0 mm screen, while rinsing with seawater from the boat deck hose. All organisms retained on the screen will be preserved in the field with a 10% aqueous solution of borax-buffered formalin.

## Tissue TBT Concentrations

Invertebrates for tissue analysis will be obtained from a single grab, together with any specimens collected in the sediment for chemistry analysis in Grabs #1-#3. To be acceptable, the grab should have a minimum penetration depth of 10 cm. The entire grab will be sieved through a 1-mm mesh screen.

Clams are the most likely invertebrate group to be collected which would provide sufficient tissue mass for TBT analysis. Any clams recovered will be placed in a ziplock plastic bag and stored on ice in a cooler. To be acceptable for use, a minimum volume (combined volume of live, intact clams) of 20 ml is required . Stations where this minimum is not obtained will be designated unsuccessful for tissue sampling.

If sufficient polychaetes are present (volume of at least 20 ml), these will be placed in a separate 2-oz-glass jar for analysis. Should any other invertebrate groups be collected in sufficient quantity for tissue analysis, they will be stored in ziplock plastic bags.

## *Macoma* Bioaccumulation Test

Following completion of the first round of sampling at all stations (Grabs #1-4), sediment will be collected for the bioaccumulation test in a second round if sampling for the TBT tissue concentration analysis was unsuccessful at three or more stations. If there are exactly three such stations, the sediment will be collected from each of these locations. If there are more than three such stations, three of them will be selected for the sediment collection. A goal in the selection will be to include a range of TBT concentrations, based on available data summarized in Figure 3.

At each sediment collection location, successive grab samples will be collected until sufficient volume is obtained to fill the 5-gal. HDPE sampling container. The upper 10 cm of sediment from the sampler will be placed in a stainless steel mixing bowl and stirred with a stainless steel spoon. The sediment will not be screened through a sieve since this will be done when the samples are processed in the laboratory. However, stones, wood, or other similar hard objects

will be removed, together with macroscopic plants and invertebrates, before the sediment is poured into the 5-gal. container. Additions from successive grabs will not be stirred in the container and the composite sample will need to be homogenized by the receiving laboratory.

## Sample Documentation

Recordkeeping for this project will include:

- Documentation of field activities.
- Documentation of all samples collected for analysis.
- Use of sample labels and chain-of-custody forms for all samples collected for analysis.

The Project Manager will maintain the field logbook. The logbook will provide a description of sampling activities (primarily in the field log sheets--Appendix B), sampling personnel, weather conditions, and a record of all modifications to the sampling plan.

## Sample Handling Procedures

### Clam Tissue Preparation

Field-collected clams will be rinsed thoroughly with sea water to remove any adhering mud or sand, then placed in ziplock plastic bags. The clams will not be depurated. Each bag will be labeled with date and location of collection, and placed in a cooler with ice. The samples will be transported to the Ecology Headquarters chain-of-custody room within three days of collection and frozen in a secure freezer.

Tissues will be removed using techniques to minimize potential for sample contamination. Only non-corrosive stainless steel instruments will be used. Persons preparing the samples will wear non-talc polyethylene gloves and work on aluminum foil. The gloves and foil will be changed between samples.

Cleaning of resecting instruments and blender parts will be done by washing in tap water with Liquinox® detergent, followed by sequential rinses with tap water, 1% reagent-grade nitric acid, de-ionized water, and pesticide-grade acetone. The items will then be air dried on aluminum foil in a fume hood before use.

For each sampling station, all clams collected will be composited unless the volume exceeds one gallon. The species and shell width of each clam being included in each composite will be recorded. If it is not possible to composite the entire collection, larger individuals will be preferentially included.

After rinsing the clams with tap water and deionized water to remove any remaining debris, the entire soft parts will be removed, combined in a blender, and homogenized to uniform color and consistency with stainless steel implements.

A subsample of the homogenate will be placed in an 8-oz. glass containers with Teflon lid-liners, cleaned to EPA specifications. Each container will be labeled with sampling station ID, sample number, and analysis requested. The samples will be refrozen and taken by courier to Manchester Laboratory. The samples will be stored frozen at Manchester until analyzed.

## Tissue Preparation for Other Invertebrates

In the event that sufficient material is collected (composite homogenated tissue volume >10 ml), other invertebrate taxa will be processed for tissue analysis following the same procedures used for clams. Errant polychaetes are the most likely additional group and would be composited in a blender without dissection.

## Benthic Infauna

Benthic infauna samples will be transferred from formalin into glass jars containing 70% ethanol at least 24 hours following collection and fixation.

# Measurement Procedures

A Sediment Sample Log (Appendix B) will be maintained during sampling to record information for each location including GPS coordinates. For each grab sample judged acceptable, the following observations will be entered in the field log:

- Date and time.
- Station location at the time of bottom contact.
- Station depth.
- Penetration depth of sampler.
- Gross characteristics of the surficial sediment.
  - Texture.
  - Color.
  - Biological structures (e.g., shells, tubes, macrophytes, clams, polychaetes, and other macroinvertebrates).
  - Presence of debris (e.g., wood chips, wood fibers, and human artifacts).
  - Presence of oily sheen.
  - Obvious odor (e.g., hydrogen sulfide, oil, and creosote).
- Gross characteristics of the vertical profile (chemistry grabs only). Vertical changes in sediment texture and color.

Laboratory measurement methods to be used are listed in Table 5.

Table 5: Measurement Methods.

| Parameter | Sample Matrix | Analytical Method |
|---|---|---|
| TOC | Sediment | PSEP-TOCM (reported on a dry weight basis at 70°C) |
| Grain Size | | Plumb (1981) |
| Percent Solids | | EPA Method 160.3 |
| Benthic Macroinvertebrate Abundance | | PSEP 1987; Ecology 2003 |
| Tributyltin | | Jiang et al. 1996 modified GC/MS |
| Tributyltin | Pore water | GC/MS (Ikonomou et al., 2002); pore water obtained by centrifugation and filtration. |
| Tributyltin | Tissue | Jiang et al. 1996 modified GC/MS |

For TBT, Manchester uses the extraction and derivatization method of Jiang et al. (1996) for sediment and modifies the extraction for tissue. Analyses will be performed using a GC/MS.

Clams to be analyzed for tissue concentrations after laboratory exposure to sediment samples will be held in aquaria with test sediment for 45 days. The laboratory bioaccumulation test procedure is described in Appendix C.

Pore water will be obtained by centrifugation of the sediment samples, followed by filtration of the supernatant on a 0.4 μ polycarbonate filter (Appendix D). Extraction will be with tropolone, and derivatization with sodium tetraethyl borate for GC/MS analysis (Ikonomou et al., 2002).

A contract vendor will sort the benthic infauna samples. All macroinfaunal invertebrates will be removed and sorted into the following major taxonomic groups: Annelida, Arthropoda, Mollusca, Echinodermata, and miscellaneous taxa. Meiofaunal organisms such as nematodes and foraminiferans will not be removed from samples, although their presence and relative abundance will be recorded.

Sorting QA/QC procedures consist of resorting 25% of each sample by a second sorter to determine whether a sample sorting efficiency of 95% removal was met. If the 95% removal criterion is not met, the entire sample will be resorted.

Upon completion of sorting and sorting QA/QC, each group will be examined by a taxonomist with specialized expertise for that group. The taxonomist will identify each organism to the lowest possible taxonomic level, generally species. In general, anterior ends of organisms will be counted, except for bivalves (hinges), gastropods (opercula), and ophiuroids (oral disks). When possible, at least two pieces of literature (preferably including original descriptions) should be used for each species identification.

A maximum of three representative organisms of each species or taxon will be removed from the samples and placed in a voucher collection. Taxonomic identification quality control for all taxonomists will require reidentification of 5% of all samples identified by the primary taxonomist and verification of voucher specimens generated by another qualified taxonomist.

# Quality Control Procedures

Table 1 lists the quality control samples for this project and shows how the information from these samples will be used. For other laboratory analyses, quality control procedures are provided in the method protocol and laboratory Standard Operating Procedures.

# Data Management Procedures

Prior to completion of the project, all project data will be entered into Ecology's Environmental Information Management System (EIM). The sediment data will also be processed for entry into validated electronic SEDQUAL templates for inclusion into Ecology's SEDQUAL database.

# Audits and Reports

MEL participates in performance and system audits of their routine procedures. Results of these audits are available on request. The EA Program Quality Assurance Unit must accredit all contract laboratories performing work for Ecology. The accreditation process includes performance and system audits.

A draft report of project findings will be prepared. The tentative date for this report is January 2007. The report will contain all relevant information from this QA Project Plan and the presentation and evaluation of the results will include, but not necessarily be limited to, the following:

1. Map of the study area showing sampling sites.
2. Description of sampling and measurement procedures.
3. Assessment of data quality.
4. Summary tables of all TBT analyses results and benthic infauna data.
5. Assessment of statistical associations among TBT concentrations in bulk sediment, pore water, and benthic invertebrate tissue.
6. Assessment of statistical association between benthic infauna data and TBT concentrations in bulk sediment, pore water, and benthic invertebrate tissue.
7. Comparison of benthic infauna results with regulatory criteria in the Sediment Management Standards.

# Data Verification and Validation

Formal validation of the results will not be necessary for this project. The Manchester Environmental Laboratory will conduct a review of all laboratory analysis for the project including contract laboratory's data and case narratives. MEL will verify that the methods and protocols specified in the QA Project Plan were followed; that all calibrations, checks on quality control, and intermediate calculations were performed; and that the data are consistent, correct, and complete, with no errors or omissions. Evaluation criteria will include the acceptability of instrument calibration, procedural blanks, spike samples' analysis, precision data, laboratory control sample analysis, and appropriateness of the data qualifiers assigned. MEL will prepare a written report on the results of their data review.

The project manager will review the contract laboratory's data package and MEL's data QA report and verify that MQOs were met. The project manager will check these data and reports for completeness and reasonableness. Based on these assessments, the data will either be accepted, accepted with appropriate qualifications, or rejected.

# Data Quality (Usability) Assessment

Once the data have been verified and validated, the project lead will determine if the data can be used to make the calculations, determinations, and decisions for which the project was conducted. If the results are satisfactory, data analysis will proceed.

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# Appendices

# Appendix A

# PSDDA Issue Paper/SMS Technical Information Memorandum October 1996

**PSDDA ISSUE PAPER 10/96**

**SMS TECHNICAL INFORMATION MEMORANDUM**

# TESTING, REPORTING, AND EVALUATION OF TRIBUTYLTIN DATA IN PSDDA AND SMS PROGRAMS

Dr. Teresa Michelsen (Washington Department of Ecology), Travis C. Shaw (Corps of Engineers) and Stephanie Stirling (Corps of Engineers) for the PSDDA/SMS agencies.

## INTRODUCTION

Tributyltin (TBT) is a special chemical of concern under the PSDDA program and is classified as a deleterious substance under the SMS rule. Testing for this chemical in areas where it is likely to be found (e.g., marinas, ship repair facilities, shipping lanes) may be required under both programs. In 1988, the PSDDA agencies conducted a study on the presence of TBT in marinas in Puget Sound, and funded a risk assessment of TBT (Cardwell, 1989). In 1988, the PSDDA agencies developed a screening level (SL) and bioaccumulation trigger (BT) for use in the PSDDA program, based on the best available knowledge of this chemical and its properties.

In the past year, additional information has come to fight on TBT, its distribution in Puget Sound, and its effects on the environment that support a change in the way the agencies approach evaluation of TBT in sediments. Most recently, an interagency work group was convened by EPA to develop a site-specific screening value for the Commencement Bay Nearshore/Tideflats and Harbor Island Superfund sites (see EPA, 1996). This paper discusses some of the issues raised by this new information and modifications to the PSDDA and SMS programs to address these issues.

Authority to develop testing programs, interpretation guidelines, and regulatory levels for deleterious substances (substances that currently do not have standards) under SMS is provided by WAC 173-204-110(6) and WAC 173-204-310(3). This technical memorandum was circulated for public review and comment in conjunction with the 1996 Sediment Management Annual Review Meeting. Many comments were received and a substantial number of revisions and additions to this memorandum have been made.

## PROBLEM IDENTIFICATION

Worldwide information documenting TBT's adverse impact on the aquatic environment is extensive. In addition to direct mortality, adverse impacts on a wide variety of aquatic organisms include reduced larval growth, sexual abnormalities, reproductive failure, gross morphological abnormalities, immune system dysfunction, nervous system disorders, and skin and eye disorders. TBT has a strong inhibitory effect on the cytochrome P450 system, reducing the ability of the organism to metabolize and detoxify environmental pollutants, and on ATP synthesis, reducing the ability of the organism to produce energy. These effects are generalized enough to occur in many organisms

(including invertebrates, fish and mammals). Available evidence indicates that serious chronic effects resulting in population declines occur at water concentrations in the parts per trillion (ng/L) to parts per billion (ug/L**)** range, depending on the species (Fent, 1996; EPA, 1991).

The available literature indicates that the toxicity and bioaccumulation of TBT are affected by a variety of factors, including organic carbon in sediment and water, pH, salinity, clay fraction, and the presence of inorganic constituents such as iron oxides. TBT partitioning is further complicated by the fact that it occurs in several forms, including TBT+, TBTCl, and TBTOH and may interconvert among these forms with fluctuations in salinity and pH (Fent, 1996; EPA, 1991). Finally, TBT has been released into the environment in a variety of forms, including leaching directly from vessel hull paints (the most toxic and bioavailable form) and in the form of paint wastes from sandblasting (which may be less bioavailable but may represent a long-term source of the contaminant).

Sediment sampling in Puget Sound and elsewhere indicates that sediments in areas with vessel activity (e.g., marinas, harbors, boatyards, shipyards) are a significant reservoir of TBT (Parametrix, 1995). Worldwide, TBT-contaminated sediments adversely impact benthic organisms and contribute to water column concentrations that continue to be toxic to aquatic life (Fent, 1996). Very high, widespread TBT sediment concentrations have been found in the waterways of Commencement Bay, Elliott Bay (Harbor Island), and the Salmon Bay/Ship Canal area. Additional ongoing sources include domestic vessels that are still allowed to use TBT paints and shipping traffic from countries without TBT regulations.

Efforts to interpret environmental data in Puget Sound have been frustrated by the complexity of TBT partitioning in the environment and uncertainty over appropriate effects levels, testing strategies, and interpretive criteria. Recent data provided by NOAA suggest that the bioassay tests routinely used in the PSDDA and SMS program may not be of long enough duration to accurately reflect *in situ* effects due to TBT, and that other approaches may be more appropriate to the types of toxicity exhibited by this chemical (Meador *et al.*,1996 in press).

## TECHNICAL BACKGROUND AND DISCUSSION

### Analytical Methods

Analytical methods and detection limits for TBT are provided in the 1996 PSEP Organics Protocol, Appendix A (PSWQA, 1996). The recommended method involves reaction with sodium borohydride, methylene chloride extraction and analysis by GC/MS (Matthias *et al.*, 1986). However, this method is somewhat experimental and is not available at most commercial laboratories. Alternative methods involve methylene chloride extraction, followed by Grignard derivatization and analyzed by GC/MS (Krone et al., 1989) or GC/FPD (Unger et al., 1986).

**Reporting Conventions**

TBT data have historically been reported in a number of different ways. For example, in the literature TBT may be reported as Sn, TBT, TBTCl, or TBTO. For the same environmental concentration, these reporting conventions result in different numerical values because each of these forms has a different molecular weight. This has resulted in some confusion interpreting the data and in setting standards.

It is important that all data be reported in comparable units, and that any standards or guidance levels also be in those same units. The PSDDA program has used Sn in the past and the existing SL and BT are based in units of Sn. However, much of the analytical and research community recommends reporting TBT as the TBT ion (TBT+).

A simple conversion based on the ratio of molecular weights can be used to convert older data into these units for comparison with newer data:

| To convert TBT reported as: | To: | Multiply By: |
|---|---|---|
| mg Sn/kg | mg TBT/kg | 2.44 |
| mg TBTC1/kg | mg TBT/kg | 0.89 |
| mg TBTO/kg | mg TBT/kg | 0.95 |

The existing PSDDA SL for sediments (30 ug Sn/kg) corresponds to 73 ug TBT/kg.

**TBT and Apparent Effects Threshold Values**

The interagency work group followed the traditional approach in establishing regulatory thresholds for Puget Sound sediments by attempting to establish apparent effects threshold (AET) values for TBT. This effort was unsuccessful because of the widely varying responses in the bioassay and benthic data reviewed over a wide range of TBT concentrations (EPA, 1996). In some cases, despite extremely high TBT concentrations in sediments, no acute toxicity was exhibited by the standard suite of bioassay organisms. Current research shows that TBT partitioning is highly complex, and the relationship between concentrations and observed effects data is much stronger for interstitial water and tissue concentrations. Therefore, the work group discontinued efforts to develop AET values and instead focused its attention on using effects data associated with interstitial water and tissue concentrations as regulatory endpoints. However, Ecology will evaluate any additional synoptic data that are collected to further explore whether a reliable AET value can be calculated.

**Interstitial Water Concentrations**

As part of the TBT work group's efforts, an extensive literature review and compilation of effects levels in marine waters was developed for use in setting a site-specific screening value for the Commencement Bay Nearshore/Tideflats Superfund site

(EPA,1996). The reader is referred to this report, which received substantial public and technical review, for a detailed presentation of effects levels in water. TBT water concentrations that result in acute and chronic adverse effects to a wide range of marine species have been reported in the literature (Fent, 1996, EPA, 1991; EPA, 1996). Chronic effects to aquatic organisms have been reported at concentrations ranging from 0.002 - 74 ug TBT/L, with the majority of species responding below 0.5 ug TBT/L. Acute effects have been reported at concentrations ranging from 0.3 - 200 ug TBT/L.

The consensus of the TBT work group was that an interstitial water concentration of 0.05 ug TBT/L corresponds to a no adverse effects level that would protect most (approximately 95%) of the Puget Sound species that have been tested. This level is conceptually equivalent to the SQS under the Sediment Management Standards, and is consistent with the EPA approach to developing water quality and sediment criteria. For comparison, the EPA proposed draft marine chronic water quality criterion has been set at 0.01 ug TBT/L (EPA, 1991).

A higher adverse effects level was also evaluated by the TBT work group; however, less consensus was achieved on an upper or maximum allowable regulatory level. As one possibility, the work group discussed a value of 0.7 ug TBT/L. This concentration is lower than most of the acute effects levels reported in the literature. However, significant chronic effects are likely at this concentration, particularly to bivalve species present in Puget Sound. On the basis of the work group discussion and an associated report (EPA, 1996), an interstitial water concentration of 0.7 ug TBT/L was selected by EPA as the basis for a site-specific sediment trigger level for cleanup in the Hylebos Waterway (Commencement Bay Nearshore/Tideflats Superfund site). ***This value*** is ***not currently proposed as an upper regulatory level for either*** *the PSDDA* ***or SMS programs.*** For comparison, the EPA proposed draft marine acute water quality criterion has been set at 0.36 ug TBT/L (EPA, 1991).

**Tissue Concentrations**

In contrast to toxicity levels based on TBT water concentrations, which range over several orders of magnitude for various species, recent studies on tissue concentrations in Puget Sound organisms indicate that a much narrower range of tissue concentrations is associated with adverse effects to these organisms (see citations below). Different species have widely varying uptake, metabolic, and elimination rates for TBT, in part explaining the widely varying sediment and water concentrations that yield similar tissue concentrations and associated effects.

This finding provides an opportunity to develop tissue TBT concentrations that are directly correlated with observed effects in a wide range of ecologically relevant species. Meador *et al.* (1993; 1996 in press) have reported acute toxicity (LD50s) for *Rhepoxynius abronius, Eohaustorius washingtonianus* and *Armandia brevis* at concentrations ranging from 34 - 89 mg TBT/kg body weight (dry weight). Tissue concentrations within or above this range would represent a severe adverse effect and sediments associated with

these levels would exceed the level at which cleanup would be required, and would also be inappropriate for open-water disposal.

However, PSDDA and SMS require consideration of both acute and chronic effects. Chronic effects levels for species of concern in Puget Sound can be found in the literature (Salazar and Salazar, 1992, 1995; Moore et al, 1991; Davies et al., 1987, 1988; Page and Widdows, 1991; Widdows and Page, 1993; Thain et al., 1987; Waldock et al., 1992; Waldock and Thain, 1983; Meador *et al.,* in press; Minchin et al., 1987; Alzieu and Heral; these values typically fall within a range of 2-12 mg TBT/kg body weight (dry weight), with a median value of about 4.

Direct measurements of TBT in tissues of biota collected from the site and *in situ* bioaccumulation studies are considered promising methods for assessing TBT toxicity, and may be recommended by the agencies to support sediment management decisions. The ranges discussed above provide a starting point for interpretation of bioaccumulation data from dredging projects or cleanup sites,

**PSDDA Screening Level for TBT**

A review of the existing SL was conducted to evaluate its relationship to known effects levels in water. Butyltins were added to the list of chemicals-of-concern for limited areas in the PSDDA Management Plan Report - Phase II (PSDDA, 1989). At the time of the listing, an interim SL for TBT was established at 30 ug/kg (as Sn). This SL was established using the available information on TBT contamination in Puget Sound and an equilibrium partitioning model that estimated interstitial water concentrations of TBT based on TBT sediment concentrations. In addition, the professional judgment of dredged material decision-makers in other regions of the country was sought in selecting the interim SL.

The interstitial water TBT concentration corresponding to the SL can be calculated using an equilibrium partitioning approach and a representative partitioning coefficient of 25,000 (sd = 5,500) derived from Meador *et al.* (1996 in press). Assuming a sediment organic carbon content of 2%, the SL of 30 ug/kg TBT (as Sn) corresponds to an interstitial water concentration of 0.06 ug/L TBT (as Sn) or 0.15 ug/L TBT (as TBT). Because there are many uncertainties associated with the original PSDDA SL and with the partitioning approach described above, this proposed interstitial water level was further evaluated based on a comparison to acute and chronic adverse effects levels compiled by EPA (1996).

This concentration is below approximately 2/3 of the chronic effects levels reported in the literature, and is below the entire range of acute effects levels reported in the literature. PSDDA disposal sites have been carefully sited to avoid sensitive habitat areas (such as shellfish growing areas) and most are sited in deep water. For these reasons, many of the chronic impacts to bivalves and other species that would be predicted at lower concentrations are not expected to occur at the disposal sites. This interstitial water level is therefore expected to be protective of acute and most chronic effects, without

being overconservative. Thus, an interstitial water concentration of 0.15 ug/L TBT is appropriate for use as an SL for the PSDDA open-water disposal sites.

**Bioassay Testing**

Exceedances of the SL for TBT currently trigger the requirement to conduct bioassay testing. The PSDDA bioassays include a 10-day amphipod mortality test, a sediment larval bioassay and the 20-day *Neanthes* biomass test. Bioassay testing under SMS includes these same bioassays, although Microtox or benthic analysis can be substituted for the biomass test. However, recent project data and evidence from the scientific literature indicate that most or all of the bioassay tests typically used under SMS and PSDDA may not be appropriate for evaluation of TBT toxicity, particularly with the short testing durations routinely used (Meador *et al.,* in press; Moore *et al*., 1991; Langston and Burt, 1991; Fent, 1996). Most of the bioassay organisms currently used have been demonstrated to show serious acute and chronic toxicity associated with TBT in sediments, but at much longer exposure periods than employed in the standard PSEP bioassay protocols (EPA, 1996; Salazar and Salazar, 1991, 1996).

Results from recent projects (e.g., Puget Sound Naval Shipyard, Commencement Bay, Coos Bay, Harbor Island) would seem to bear out this prediction. Several sites have shown adverse benthic effects in areas with high TBT sediment concentrations, even when acute and/or chronic bioassays did not show adverse effects. In addition, bioaccumulation of TBT and associated adverse effects have been demonstrated at a number of these sites when short-term laboratory bioassays did not show a response. This may be because the longer-term bioaccumulation studies and *in situ* benthic assemblages better reflect the chronic endpoints with which TBT is associated and include long enough exposure durations for TBT in sediments and water to come into equilibrium with the organisms.

**PSDDA Bioaccumulation Testing for TBT**

The TBT bioaccumulation trigger was established at 219 ug/kg (as Sn), based on a multiple of the SL (PSDDA, 1989). Bioaccumulation testing is required when this threshold is exceeded. However, using the method described above for the SL, the existing BT corresponds to an interstitial water concentration of 1.07 ug/1 (as TBT). This concentration is well above a level considered protective by the PSDDA agencies and the EPA Superfund work group. Based on the evidence provided above, significant bioaccumulation and adverse effects may occur at much lower concentrations. The interstitial water SL (0.15 ug/L TBT) corresponds to a level above which adverse reproductive and population-level effects due to bioaccumulation of TBT have been observed, and will also be used as the BT.

**PROPOSED ACTIONS/MODIFICATIONS**

**Testing Locations**

The SMS program and PSDDA agencies have required testing for TBT in marinas, boat maintenance areas, and other locations where TBT is likely to be present. Sediment testing in Commencement Bay (Thea Foss and Hylebos Waterways), in the Duwamish River, and in Salmon Bay and Lake Union Ship Canal have shown TBT to be present throughout the waterways and at levels substantially above the existing sediment SL. These studies show that TBT is more widely distributed, and at higher levels**,** than previously thought. For this reason, the SMS and PSDDA agencies will require testing for TBT in areas where past data have demonstrated its presence (particularly urban bays), and at other appropriate project locations where it would be likely to be present, such as marinas shipyards, boatyards, and in the vicinity of large CSOs or treatment plant outfalls. Persons who have evidence that TBT is not present at their project location can ask to have this requirement waived.

**TBT Testing Strategy for PSDDA Projects**

***The available evidence indicates that neither sediment chemistry screening levels nor the existing PSEP bioassay protocols may be as useful in predicting actual environmental effects as measurement of TBT concentrations in interstitial water and tissues.*** Therefore, the current tiered testing protocol utilizing bulk sediment chemistry and short-term bioassays is not considered appropriate for evaluating the potential adverse effects of TBT. Because of the complexity of TBT speciation in the aquatic environment (including ionic forms) and because other factors may strongly affect its bioavailability, an alternative testing strategy is proposed.

Measurement of TBT in interstitial water provides a more direct measure of potential bioavailability, and hence toxicity, than bulk sediment concentrations. This approach also avoids the difficulties inherent in extrapolating to a sediment cleanup level, particularly where paint wastes or other less bioavailable forms may be present. Therefore, ***the agencies propose that interstitial water analysis replace bulk sediment analysis*** as the initial step in a tiered assessment of TBT toxicity for PSDDA projects.

TBT should be analyzed using approved methods as described above, and reported as TBT. A standard method for collection of interstitial water has not yet been determined though several techniques are available. Recommendations for a standardized method will be developed over the next year and discussed at the 1997 SMARM.

If the TBT concentration in the interstitial water is above 0.15 ug TBT/L, bioaccumulation testing of project sediments must be conducted using the PSDDA bioaccumulation guidelines in effect at the time of testing. Acute bioassay testing will not be required (other chemicals of concern may trigger acute toxicity testing). If unacceptable tissue concentrations are measured at the end of the bioaccumulation test, the sediment will be found unsuitable for open-water disposal.

**TBT Testing for SMS Cleanup Sites**

Although specific regulatory levels corresponding to the SQS and CSL have not yet been promulgated, a similar conceptual approach will be used for evaluation of TBT toxicity at SMS sites. As is typical of cleanup sites, a preponderance of evidence approach may be used rather than a strict tiered testing approach. However, interstitial water data and bioaccumulation (tissue) data will be given more weight in evaluating potential ecological effects than sediment concentrations or short-term bioassay results. Either laboratory or *in situ* bioaccumulation tests may be employed.

At many sites, bioassay testing will be conducted to evaluate the ecological effects of other chemicals in sediments. To evaluate ecological effects of TBT at these sites, longer-term bioassay/bioaccumulation studies could be considered as alternative chronic tests to those listed in SMS. Such alternative testing approaches may be particularly appropriate when other chemicals are also present that are slow to reach equilibrium in the laboratory, such as dioxins/furans and pesticides. Biological tests that measure both bioaccumulation and associated effects endpoints are recommended to assess the significance of measured tissue concentrations.

At sites where these alternative approaches are used to assess the effects of TBT, site-specific cleanup standards will need to be set based on the interstitial water and tissue effects ranges described in this paper. Consistent with the narrative standards set forth in WAC 173-204-100(3) and (7), site-specific cleanup standards shall include consideration of acute and chronic effects to aquatic organisms and human health, and shall range between no adverse effects and minor adverse effects levels. With respect to TBT, the presence of natural or cultured bivalve growing or collection areas shall be given special consideration in setting protective cleanup standards, since very low levels of TBT in water and sediments are known to adversely affect reproduction and growth of these culturally and economically important resources.

**Further Development of Bioassay/Bioaccumulation Tests**

Public comments recommended a wide variety of possible bioassay and bioaccumulation test strategies. Recommendations included side-by-side testing of amphipod species to determine relative sensitivity to TBT; use of a 60-day *Neanthes* bioassay with growth and reproduction endpoints; use of a 20-day *Macoma nasuta* test with bioaccumulation, tissue growth, and shell growth as endpoints; field-validation of laboratory bioaccumulation tests; use of longer-term larval tests with sensitive organisms such as oysters, mysids, and the copepod *Acartia tonsa;* and interstitial water bioassays. Although it is not currently within the PSDDA budget to conduct such studies, it may be possible to conduct some studies as part of large cleanup projects or through academic or agency research projects. The PSDDA agencies welcome and will carefully consider any information that is useful in better defining appropriate chronic tests for assessment of TBT and other compounds for which existing short-term bioassays may be inadequate to predict chronic effects.

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# Appendix B

# Field Log Sheets

**STATION** _____ DATE _____ TIME _____ Notes _____

GPS LAT _____ LON _____ (NAD83) _____

## CHEMISTRY

| Grab # | Time | Water Depth (ft) | Sediment Penetration (in) | Layer Depth (in) | Texture | Color | Debris | Odor | Sheen | Veg/Fauna |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | | | |
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| 2 | | | | | | | | | | |
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| 3 | | | | | | | | | | |
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## BENTHIC INFAUNA

| Grab # | Time | Water Depth (ft) | Sediment Penetration (in) | Texture | Color | Debris | Odor | Sheen | Veg/Fauna |
|---|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | | |
| 2 | | | | | | | | | |
| 3 | | | | | | | | | |

## TISSUE

| Grab # | Time | Water Depth (ft) | Sediment Penetration (in) | Layer Depth (in) | Texture | Color | Debris | Odor | Sheen | Veg/Fauna |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | | | |

Notes: _____

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TBT SQUALICUM HARBOR STUDY

**STATION** _____ DATE _____ TIME _____ Notes _____

GPS LAT _____ LON _____ NAD83 _____

**BIOACCUMULATION STUDY SEDIMENT**

| Grab # | Time | Water Depth (ft) | Sediment Penetration (in) | Layer Depth (in) | Texture | Color | Debris | Odor | Sheen | Veg/Fauna |
|---|---|---|---|---|---|---|---|---|---|---|
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Notes: _____

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| Stations | Target (NAD83) Latitude 48° | Target (NAD83) Longitude 122° | Actual (NAD83) Latitude 48° | Actual (NAD83) Longitude 122° | GPS Time | Date |
|---|---|---|---|---|---|---|
| SH1 | 0.75809 | 0.50188 | | | | |
| SH2 | 0.75810 | 0.50178 | | | | |
| SH3 | 0.75811 | 0.50167 | | | | |
| SH4 | 0.75814 | 0.50142 | | | | |
| SH5 | 0.75817 | 0.50106 | | | | |
| SH6 | 0.75827 | 0.50058 | | | | |
| SH7 | Duplicate at station: | | SH | | | |
| Samish Bay | | | | | | |
| SH8 | 0.60278 | 0.48945 | | | | |

# Appendix C

# Battelle 45-Day Bioaccumulation Test Procedure

Battelle
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**Marine Sciences Laboratory**

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Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

# 45-DAY SEDIMENT BIOACCUMILATION TESTING

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# 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 D

# Pore Water Extraction Procedure

*(David Hope, Pacific Rim Laboratories Inc.)*

# Extraction of Pore Water from Sediment

## 0. REFERENCE METHOD

None

## 1. INTRODUCTION

Sediment or mud collected from the seafloor has two parts. the tiny bits of rock and other solids and the water in between. Biota live in the pore water and are constantly filtering it with their gills, therefore it is important to know the toxic constituents of that pore water.

Sediments are shipped to the lab for processing and analysis. A portion of the sample will be analyzed as received. In addition, porewater can be extracted (separated) from the solids and analyzed. Therefore each sediment sample will therefore have two data points, one for solids and one for liquids.

## 2. SCOPE

This method is applicable to the pore water from sediment samples.

## 3. PRINCIPLE

Sediment samples can have a moisture content of 30-80%. Extracting the water from the sediment will first involve centrifuging the sample and decanting the supernatant. As organic contaminants are bound to particulate, it is important to filter the supernatant to ensure no sediment remains. This will be done under vacuum with a 0.4 μ polycarbonate filter. Approximately 50 mL of porewater should be extracted for analysis from each sediment sample.

## 4. METHOD VALIDATON

Not applicable

## 5. SUPPORTING DOCUMENTS

SOP LAB04, Determination of Tributyltins in Soil, Water and Tissue. Rev. 3.0

## 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.

## 7. SAMPLE REQUIREMENTS

7.1 Sediment

7.1.1 A minimum of 500 g of sediment should be collected in a 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 samples should be extracted within 14 days after sampling.

## 8. EQUIPMENT

8.1 Balance, top loading, capable of accurately weighing 100.00 g.
8.2 Centrifuge
8.3 Vacuum pump or aspirator
8.4 Refrigerator at 4°C

## 9. REAGENTS

9.1 *Dichloromethane (DCM), hexane, acetone, methanol* - distilled in glass or equivalent.
9.2 *Sodium Acetate* (NaOAc, 99.995%)

9.2.1 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.

## 10. SUPPLIES

10.1 Metal spatula.
10.2 Polycarbonate filter, 47 mm x 0.4 µ
10.3 60 mL vials with Teflon lined septum cap
10.4 500 mL amber glass bottles with Teflon lined cap.
10.5 Graduated cylinders, 100 mL
10.6 250 mL beaker
10.7 *Filtering Apparatus*

10.7.1 1 L Erlenmeyer side arm flask
10.7.2 Filter support, 47 mm with sintered glass
10.7.3 100 mL reservoir

10.8 *Gases*-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 Ensure that safety procedures are strictly adhered to at all times and instrumentation is operating at optimum conditions.

## 13. TEST PROCEDURE

13.1 Remove the sediment container from cool storage and open the lid. Decant off any free standing water into a beaker and save for later.
13.2 Using a metal spatula, transfer sediment to a 60 mL vial until it is 80% full.
13.3 Pass a stream of nitrogen into the vial to flush out oxygen.
13.4 Quickly cap the vial with a Teflon lined cap.
13.5 Place four or eight vials into the centrifuge and turn on. Centrifuge the sample for 5 minutes at 1800 rpm.
13.6 When the centrifuge has stopped, remove the samples. Decant the liquid into the same beaker used in 13.1.
13.7 Prepare a glass filtration unit and filter sample under vacuum through 0.4 μm polycarbonate filter.
13.8 Measure the volume of filtrate and transfer to a 500 mL amber glass bottle.
13.9 Add 1 mL of 1 M sodium acetate buffer and store sample in fridge until further analysis required.

### 13.5 Quality Assurance/Quality Control

A method blank, consisting of 2 x 50 mL of water should be carried through the procedure.

Duplicate extractions should be undertaken to understand the variability of the extraction procedure.

**Author**: David Hope

**Approved by**: _____, CEO

Date Implemented: August 15, 2005