EAST WATERWAY OPERABLE UNIT
SUPPLEMENTAL REMEDIAL INVESTIGATION/ FEASIBILITY STUDY
ADDENDUM TO THE FINAL QUALITY ASSURANCE PROJECT PLAN
CLAM STUDIES

For submittal to:

The US Environmental Protection Agency
Region 10
Seattle, WA

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Prepared by:

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1 Introduction

This document is an addendum to the Final East Waterway Clam Survey Quality Assurance Project Plan (QAPP) (Windward 2008). A video survey of East Waterway identified probable geoduck siphons within East Waterway. This addendum provides detailed description for the proposed approach and methods for the collection of geoduck tissue samples and further survey of the EW for locations of geoducks. All sampling conducted will be consistent with sampling methods and the QA requirements set out in the Final East Waterway Clam Survey QAPP (Windward 2008) unless otherwise specified in this addendum. The purpose of this study is to collect geoduck tissue in the East Waterway for use in the Human Health Risk Assessment and for consideration with the ecological risk assessment. This study is not intended to characterize either the potential current or future distributions of geoducks or their habitat in EW.

2 Project Management

2.1 Problem Definition and Background

Several video surveys have been conducted in East Waterway for the purpose of determining whether or not geoducks were present. EPA conducted a remotely operated video survey on July 15th and 16th, 2008; Windward performed a towed-video survey on August 16th. The video survey tracks for the EPA survey and the Windward survey are shown in Figure 1. During both surveys potential geoduck siphons (see example in Photo 1) were observed at the Elliot Bay entrance to East Waterway in about 55 feet of water (Figure 2) west of the channel center during both surveys. One possible siphon "show" was also identified along the eastern shoreline near the mouth of the waterway in the vicinity of the Jack Perry Memorial public access point.

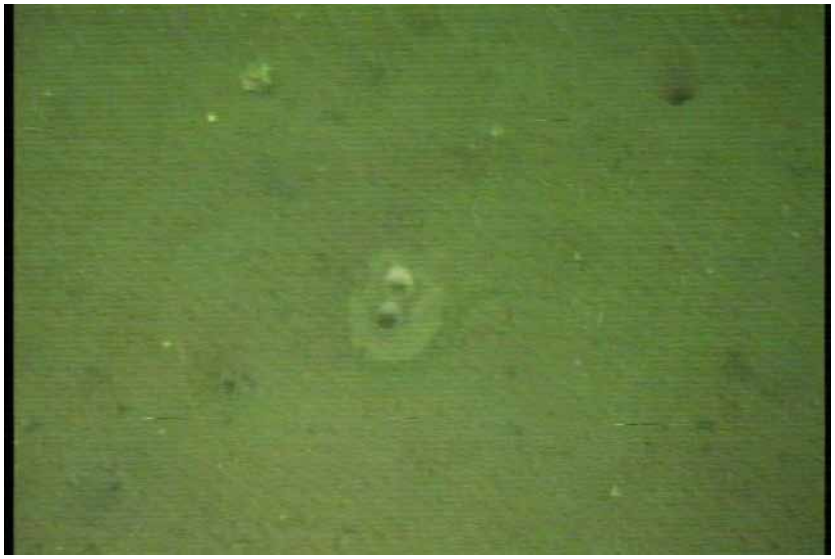
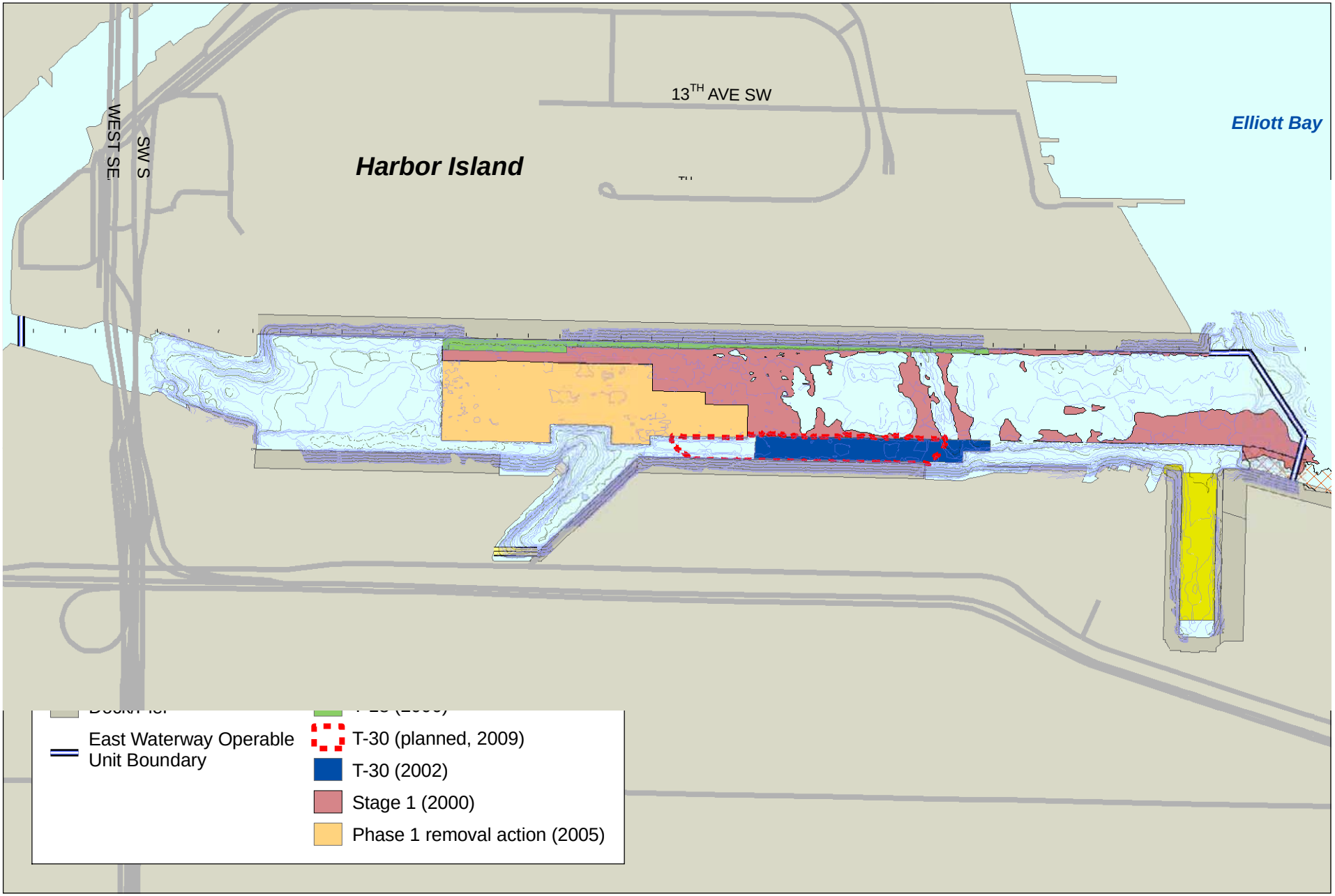


Photo 1. Geoduck siphons



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N

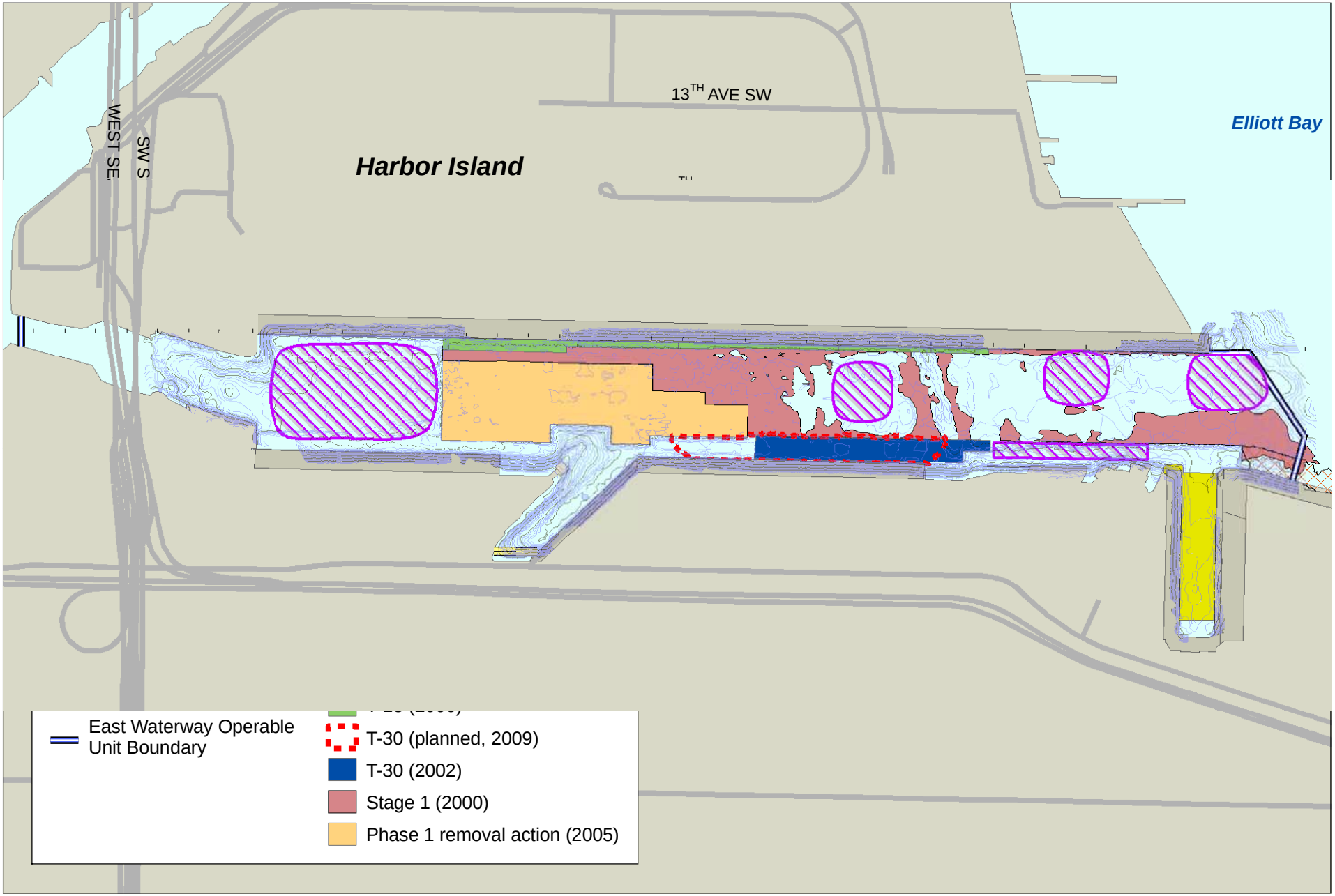
0 1,000 2,000 Feet

0 250 500 Meters

Figure 1. East Waterway video transects with geoduck observations

DRAFT

Prepared by CEH, 01/14/09; MAP #3455; W:\Projects\00-08-08 East Waterway\EW RI-FS\Data\GIS\Events\Clam sampling 09-08



Wind Ward environmental LLC

N

0 1,000 2,000 Feet

0 250 500 Meters

Figure 2. Diver observation/potential geoduck collection areas

Prepared by CEH, 01/14/09; MAP #3468; W:\Projects\00-08-08 East Waterway\EW RI-FS\Data\GIS\Events\Clam sampling 09-08

The QAPP prepared for clam tissue collection stated that the results of the video surveys would be used to select areas where divers would be deployed to confirm the presence of geoduck and collect individuals for tissue analysis in support of the human health risk assessment. Geoduck tissue chemistry will be a component of the Tribal shellfish consumption scenario.

2.2 TASK DESCRIPTION AND SCHEDULE

Geoducks will be collected from the area in which siphons were observed during the video surveys(Figure 1). Divers will locate siphons and select ten individual geoduck specimens for sampling based on their locations.

Following the geoduck collection, divers will survey the five subtidal areas that have been identified for additional characterization by divers. These areas have been identified based on the bathymetry, dredging history, sediment PCB concentrations and video survey coverage. Proposed areas for investigation are shown in Figure 2. If additional geoduck are located in these areas, then three individual geoduck will be collected from each survey area.

A draft schedule of sampling and analysis dates are provided in Table 1. Dates for analysis and reporting are estimated and subject to change based on analytical constraints or validation findings.

Table 1. Proposed schedule

ACTIVITY	PROPOSED START DATE	PROPOSED END DATE
Geoduck tissue collection and survey	October 22, 2008	October 24, 2008
Tissue analysis	October 27, 2008	December 1, 2008
Analysis for congeners and dioxins and furans	December 15, 2008	January 15, 2009
Initial validation	December 2, 2009	January 2, 2009
Congener and dioxin and furan data validation	January 16, 2009	February 16, 2009
Data report	January 2, 2209	March 16, 2009

3 Data Generation and Acquisition

3.1 SAMPLING DESIGN

The results of the video surveys and the approach to collecting geoducks were discussed with EPA on September 30, 2008. EPA and Tribal representatives requested that in addition to collecting geoduck at the mouth of the waterway, additional areas be evaluated for the presence of geoduck. Specifically, EPA and the Tribes were interested in deep areas of the waterway that had not been recently disturbed by dredging and were characterized by the video survey (Figure 1).

This sampling effort will include the collection of geoducks in conjunction with diver surveys throughout the waterway to identify any additional geoduck locations. Divers will be used to collect geoducks from the area in which potential siphons were observed. In addition, divers will survey the five areas where they may occur due to similarities in habitat and/or dredge history (i.e. areas with no recent dredge events) including additional survey of the area in which siphons have been observed. Based on historical information, the area of the waterway where geoducks were observed may not have been dredged since the original construction of the waterway in the early 1900's. The habitat in this area is characterized by coarse (< 50 percent fines), fairly consolidated sediments (>80 percent solids), with total organic carbon content less than 1.5% (typical of central Puget Sound). No other information about sediment quality exists for this area of the waterway, although nearby historical sediment sampling stations within the waterway do not have chemical concentrations exceeding the Washington State Sediment Quality Standards (Anchor and Windward, 2008).

Areas of proposed diver observations and potential geoduck collection are identified in Figure 2 and include:

- Area 1 : the western half of the channel at the mouth of the waterway where geoduck siphons have been observed,
- Area 2: northern portion of T-18, dredged in 2000 with elevated PCB concentrations (Figure 3)
- Area 3: the eastern shoreline south of Slip 36, video survey recorded a potential geoduck show in this area
- Area 4: the deep, undredged area midchannel between Terminal 30 and Terminal 18, and
- Area 5:the undredged area at the head of the waterway between Terminal 25 and the south end of Terminal 18

3.2 SAMPLING METHODS

3.2.1 Geoduck tissue collection

Up to 12 individual geoduck will be collected and analyzed from East Waterway, depending on how many areas are found to have geoducks. A minimum of 10 individual geoducks will be collected as required by EPA in order to ensure sufficient samples to calculate a 95th UCL for geoduck tissue concentrations. The area at the mouth of the waterway where they are known to occur will be visited first; evidence that geoduck siphons are still visible in that area will confirm the appropriateness of an early fall sampling period for the entire waterway. Three geoducks will be collected from Area 1. Then the divers will proceed to survey the remaining sampling areas. The divers will attempt to collect individuals located as far apart from one another as possible. If geoducks are not found in the subsequent survey efforts, the divers will return to Area 1 to collect an additional seven geoducks.

Geoducks will be collected by divers using a pressurized water nozzle that is inserted into the sediment adjacent to each clam. The water nozzle is typically about a meter long with a 5/8-inch diameter tip at the digging end and a shut-off valve on the other. This nozzle is made of rigid material attached to a flexible hose. Pressurized water is supplied by a pump or compressor on-board the sampling vessel. Geoduck will be located either by their siphon "shows" or by the figure-8 depression left after the clam retracts its siphon. The nozzle will be inserted next to the actual or likely siphon location and pressurized water (20 to 60 pounds per square inch) will be used to loosen the sediment in order to remove the geoduck. This hydraulic method is similar to that used by commercial geoduck harvesters. Individual geoduck will be placed in a mesh bag for transport to the surface where they will be wrapped in foil, labeled with the sample location, bagged and placed on ice.

Divers will attempt to obtain hand cores of sediment proximate to each geoduck sample. This sediment will be collected prior to the geoduck extraction and will be archived frozen. Following the analysis of tissue samples, EWG and EPA will determine which if any of these hand cores will be analyzed.

3.2.2 Geoduck diver survey

Divers will search for geoducks throughout the identified survey areas (Figure 2). They will traverse each area looking for visual evidence of the presence of geoduck (siphon shows) and probing the sediment to determine if there is any evidence of geoduck. If evidence of geoducks is found then the coordinates will be noted and the method described in Section 3.2.1 will be followed to sample area minimum of ten individual geoducks.

3.3 IDENTIFICATION SCHEME FOR ALL LOCATIONS AND SAMPLES

The first two characters of the location ID are "EW" to identify the East Waterway project area. The next character will be S for subtidal location. The specific area is indicated by a two-digit number that follows the subtidal notation.

The next characters indicate the sample medium to be collected at that location, GD for geoduck tissue and SS for surface sediment. When more than one sample of a specific medium is collected at a given location, a two-digit numeric suffix greater than -01 will be added (original samples are all labeled -01). An example of sample naming conventions for the geoduck tissue is: EW-S03-GD-01 (East Waterway, Subtidal Area 3, first geoduck).

3.4 SAMPLE HANDLING AND CUSTODY REQUIREMENTS

Individuals will be analyzed separately for the chemicals of interest (see Table3-5 in main QAPP for a complete list of analytical methods), following removal of the gut ball and siphon skin (leathery outer layer). A subset of geoducks will be analyzed for PCB congeners and dioxin/furans. As noted in the main QAPP for clam sample collection, the subset of samples will be selected following receipt of Aroclor PCB results. A plan for the compositing and analysis of the geoduck tissue will be submitted to EPA for approval prior to sample compositing and analysis. The siphon skin will be removed prior to homogenization. The siphon skin is readily removed from the siphon once a frozen geoduck is defrosted. This preparation is consistent with standard practices prior to human consumption and minimizes the loss of edible tissue associated with the removal of the siphon skin. The removal of the gut ball and siphon skin will occur at ARI. The gutball will be analyzed for inclusion in the risk estimates (12% of adult Suquamish Tribal members reported the consumption of whole body geoduck in the Suquamish seafood consumption survey). The compositing and analysis plan for the geoduck tissue will provide the masses for the individual gutballs. It is likely that compositing will be required to obtain sufficient mass for the analysis of the full analyte list.

Homogenization will be conducted in accordance with standard operating procedure (SOP) provided by Analytical Resources Inc (ARI) for the Clam Survey (Attachment 1). If any amendments to the SOP are necessary, EPA will be notified and will approve the change prior to homogenization. Geoduck shells will be retained for further analysis to determine the ages of the individual geoducks.

Following the homogenization the samples will be identified as either "EM" –for edible meat or "GB-comp" for gut ball composite.

3.5 ANALYTICAL METHODS AND QUALITY ASSURANCE/QUALITY CONTROL

Section 3.4 of the Final Clam Survey QAPP provides a detailed discussion of the sample handling requirements, chemical analytes, laboratory preparation and analytical methods, and data quality objectives (DQOs) for the chemical analyses of the tissue

samples. The analysis of geoduck tissue will follow all the provisions in Section 3.4 of the final Clam Survey QAPP. All samples will be analyzed for PCB Aroclors, organochlorine pesticides, SVOCs, total metals including mercury, inorganic arsenic, butyltins, total solids, lipids. Three individual geoduck tissue samples and two geoduck gutball composite samples will be analyzed for PCB congeners and dioxins/furans. If pesticides are detected in the initial analysis, a second subset of samples may be analyzed for confirmation by GC/MS/MS. Geoduck shells will be submitted to researchers at the University of Washington for visual analysis to determine the age of the individual geoducks.

4 References

Windward. 2008. East Waterway Operable Unit supplemental remedial investigation/feasibility study. Quality assurance project plan: clam studies. Draft. Windward Environmental LLC, Seattle, WA.

ATTACHMENT 1

Analytical Resources, Inc.
SOP 340S

Analytical Resources, Incorporated
Analytical Chemists and Consultants

Standard Operating Procedure

Tissue Extraction & Lipid Determination
Semivolatile Organics Analysis
EPA Method 8270D

SOP 340S
Revision 9

Revision Date: 7/28/05
Effective Date: 7/28/05

Prepared by:

Jim Hawk

Approval:

Laboratory / Section Manager

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Quality Assurance

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Annual Review

SOP Number:	340S
Title:	Extraction of Tissue – SVOA Analysis – EPA Method 8270D
Revision:	009
Revision Date:	7/28/05
Effective date:	7/28/05

An annual review has been performed on this SOP as required by ARI's Quality Assurance Plan. The following ARI staff have reviewed this SOP and confirm that no modifications are required at this time.

Reviewer Name	Reviewer Signature	Date
Brian N. Tebo	Brian N. Tebo	08/4/05

Standard Operating Procedure
Tissue Extraction by Tissuemizer
Base/Acid/Neutral (8270 BAN) / % Lipid Determination

1.0 Scope and Application

1.1 This document outlines the procedure for the extraction of tissue by tissuemizer for 8270 semivolitile analysis and percent lipid determination. This SOP is written to meet the requirements of SW-846 Method 3550B.

2.0 Definitions

2.1 Surrogates - known quantities of compounds added to all samples and blanks to evaluate extraction efficiency.

2.2 Matrix Spike (MS) - known quantities of selected analytes added to a sample to evaluate the effect of sample matrix on analyte recovery.

2.3 Laboratory Control Sample (LCS) - a blank fortified with known quantities of selected analytes to monitor extraction efficiency.

3.0 Equipment

3.1 Tissuemizer (Tekmar Mark II Type T25-S1 or equivalent).

3.2 Tissuemizer Accessories: Stainless steel shaft tube, shaft, bearing box, rotor, shaft sleeve, grinder, PTFE slit ring bearing, starting disk (washers). tool set.

3.3 Blender (Waring Commercial Blender Model 31BL92 or equivalent)

3.4 Chopper or grinder (Chefmate Model #CC12 or equivalent).

3.5 Cleaning station for tissuemizer, blenders or chopper parts to include three deep, gray bins, white plastic drying bins, paper towels, Contrex AP detergent, concentrated HCL, deionized water, aluminum foil, brushes and broad-range pH paper.

3.6 250 mL centrifuge bottles.

3.7 100 mm glass funnel.

3.8 500 mL Erlenmeyer flask.

3.9 Kuderna-Danish (K-D) concentrating apparatus including a 10 mL concentrator tube, 500 mL concentrator flask, and 3-ball Snyder column.

3.10 25 x 340 mm drying column.

3.11 Glass wool, prepared by rinsing with methylene chloride and heating in kiln at 400° C for 4 hours. Acidify glasswool by using 0.05% HCL/acetone solution. (0.5 mL/1.0 L).

3.12 Methylene chloride, high purity.

3.13 Acetone, high purity.

3.14 1:1 mixture of acetone/methylene chloride.

3.15 Anhydrous sodium sulfate prepared by heating in kiln at 400° C for 4 hours.

3.16 0.45 µm Whatman Puradisc filter (25 mm) PTFE.

3.17 Top-loading balance, accurate to 0.02 g.

3.18 Analytical balance, accurate to 0.0001 g.

3.19 Desiccators.

3.20 Vacuum apparatus.

3.21 Green and orange label tape.

3.22 Stainless steel spatula.

3.23 Hamilton gastight syringes: 250 µL, 500 µL, 1000 µL and 5.0 mL.

3.24 Gel Permeation Chromatograph (GPC) – (see ARI GPC SOP 306S).

3.25 Water bath set at 80 - 85° C and 90 - 95° C.

3.26 Turbo Vap LV.

3.27 Boiling chips - Teflon (Chemware).

3.28 4 mL amber vial with PTFE-lined screw cap.

3.29 2.5 mL amber vial with PTFE-lined screw cap.

3.29 Personal protective gear including gloves, goggles and laboratory coat.

3.31 Aluminum weighing dishes.

3.32 1% nitric acid solution

3.33 Surrogate and matrix spike solutions are composed as follows:

	Component	Concentration
Surrogate:	2-Chlorophenol-d4	150 µg/mL
	Phenol-d5	150 µg/mL
	2-Fluorophenol	150 µg/mL
	2,4,6-Tribromophenol	150 µg/mL
	p-Terphenyl-d14	100 µg/mL
	Nitrobenzene-d5	100 µg/mL
	2-Fluorobiphenyl	100 µg/mL
	1,2-Dichlorobenzene-d4	100 µg/mL
Spike:	Phenol	150 µg/mL
	2-Chlorophenol	150 µg/mL
	4-Chloro-3-Methylphenol	150 µg/mL
	4-Nitrophenol	150 µg/mL
	Pentachlorophenol	150 µg/mL
	Pyrene	100 µg/mL
	1,4-Dichlorobenzene	100 µg/mL
	1,2,4-Trichlorobenzene	100 µg/mL
	2,4-Dinitrotoluene	100 µg/mL
	Acenapthene	100 µg/mL
	n-Nitroso-Di-n-propylamine	100 µg/mL

4.0 Documentation

4.1 BAN bench sheet (ARI form 3009F).

4.2 % Lipid Determination bench sheet (ARI form 3071F).

5.0 In-house Modifications to Referenced Method

5.1 Samples are extracted by Tissuemizer, not sonication.

5.2 Section 6.22: extracts are decanted and filtered through a 100 mm funnel with an acidic glass wool plug, not through filter paper.

5.3 Section 6.23: for the third Tissuemizer step methylene chloride only is used.

5.4 Section 6.29 & 6.36: The extract is removed from the water bath at a volume of 2-3 mL, not 1 mL.

5.5 Section 6.33: samples are filtered with PTFE 0.45 µm Puradisc filter to remove gross particulate matter prior to GPC clean-up.

6.0 Tissue preparation:

6.1 It may be necessary to initiate some preparatory steps (dissection, manual chopping, removal of unwanted tissue, etc.) prior to extracting the tissue The portion of tissue to be extracted must be representative and amenable to the extraction process.. Dissection must be done by a qualified laboratory technician. See Laboratory Supervisor for specific instructions pertaining to tissue preparation requirements. NOTE: When preparing tissue samples that may be analyzed for trace metals, all metal instruments (spatulas, knives, razor blades, blender, etc) must be rinsed with copious amounts of 1% nitric acid between each sample to prevent metals contamination of the sample.

6.2 Clams and mussels should be shucked and homogenized in a blender or chopper. Other small tissue parts such as worms or organic material may also be blended provided there is enough free flowing tissue moisture. It may be necessary to cut or cube some tissues before blending chopping

6.3 Use a stainless steel spatula to pry open the shell of the clam or mussel and scrape the tissue into a jar with a PTFE-lined screw cap labeled with the sample ID. Clam or mussel tissue must be homogenized using a pre-cleaned blender. Blend the tissues thoroughly and place them back into the labeled sample jar. Clean the blender between samples by disassembling the blade, and washing as described in Sections 6.5 - 6.8.4

6.4 Fish may be filleted, ground whole or cut into small 3" cubes and homogenized in a blender or chopper. Place the ground tissue into a jar with a PTFE-lined screw cap

labeled with the sample ID. .All mechanical equipment must be claned between samples following the procedure in Sections 6.5 – 6.8.4.

6.5 Cleaning Preparation for Tissuemizer, blender or chopper parts:

Note: All Tissuemizer, blender and chopper parts that contact the sample must be disassembled completely and cleaned prior to initial use and after each sample to ensure that no cross-contamination will occur.

6.6 Prepare the Tissuemizer, blender or chopper parts and associated tools at a sink with hot running water. Disassemble the equipment completely then thoroughly rinse the parts with hot tap water and transfer them to the washing station. See Appendix 12.3 for Tissuemizer parts assembly diagram.

6.7 Prepare three water baths for washing the disassembled parts and tools as listed here in order of intended use:

6.7.1 Bin #1 - 1/2 cup Contrex AP detergent in approximately 12 L hot tap water. The tub will be 3/4 full. The resulting solution will be basic (pH ~12).

6.7.2 Bin #2 - 20 mL concentrated HCL in approximately 12 L hot tap water. This rinse water will be pH 2-acidic to remove soapy, basic residue left on parts.

6.7.3 Bin #3 - Deionized water. This rinse water should be pH 5-9 neutral to neutralize parts. Check the DI water frequently to ensure correct pH range, using a broad range pH paper. Change the DI water if it is acidic (<pH 4).

6.8 Parts Cleaning:

6.8.1 Operating at the washing station, remove all disassembled parts, including the tools used to disassemble the equipment and clean them sequentially in the three baths listed above. In Bath #1, scrub each piece thoroughly with brushes and inspect each part, making sure there is no residual tissue on the part or in any small crevices. Rinse all parts thoroughly with hot tap water, and then submerge all pieces in Bin #2, then in rinse bin #3.

6.8.2 Following the final rinse in Bin #3, place each part in a drying bin lined with paper towels to air dry.

6.8.3 Each part must then be rinsed with acetone three times and methylene chloride three times before assembling for use on next tissue sample.

6.8.4 Reassemble the parts. Perform one final rinse using methylene chloride. Extract the next sample or store for later use.

6.9 Extraction Procedures

6.10 Review the Special Analytical Requirements (SAR) form prior to extracting samples to determine if special procedures are required. Note: a 20 g-1.0 mL FEV is the normal In-house extraction level for semivolitiles. If insufficient volume is provided or other detection limits are required, see Laboratory Supervisor for details.

6.11 Fill out a Bench Sheet for the job.

6.12 Warm the samples to room temperature.

6.13 If percent total solids are required, see ARI SOP 359S for details.

6.14 Label each 250 mL centrifuge bottle with green label tape containing the following information: job number, sample ID letter, matrix ID, extraction type (BAN) and, for the method blank and the LCS, the date.

6.15 Using a top-loading balance, prepare two 250 mL centrifuge bottles with 20 g of anhydrous sodium sulfate for use as a method blank and an LCS.

6.16 Weigh 20 g of each homogenized tissue sample into the corresponding pre-labeled 250 mL centrifuge bottle. **<u>DO NOT</u> ADD ANHYDROUS SODIUM SULFATE AT THIS POINT.**

6.17 Add 250 µL BAN surrogate solution to each sample, the method blank and the LCS to result in a final concentration of 25 µg/mL for each base/neutral analyte and 37.5 µg/mL for each acid analyte. To verify that the surrogate spiking is accurate, surrogate additions will be witnessed and documented by another laboratory technician. If the sample is an LCS or matrix spike, proceed to step 6.18. After the surrogate and spike solutions have been added, immediately pour approximately 125 mL of 1:1 methylene chloride/acetone into each sample.

6.18 Add 250 µL BAN matrix spike solution to the LCS and any matrix spike samples to result in a final concentration of 25 µg/mL for each base/neutral analyte and 37.5 µg/mL for each acid analyte. To verify that the matrix spiking is accurate, spike additions will be witnessed and documented by another laboratory technician.

6.19 <u>To check the GPC for accuracy:</u> Add 250µL of GPC spike solution to the LCS and any matrix spike samples to result in a final concentration of 25 µg/mL for each analyte. To verify that the spiking is accurate, spike additions will be witnessed and documented by another laboratory technician.

6.20 Add 40-50 g of anhydrous sodium sulfate to the sample to be tissuemized, **immediately prior** to extracting with the tissuemizer.

6.21 Tissuemize the samples as follows: place the clean assembled shaft onto the tissuemizer and place the shaft into the sample bottle with the tip below the surface and into the tissue layer. Turn on the tissuemizer with the speed set to 11,500 rpm (between the green and red dial). Extract each sample with the tissuemizer for approximately 45 seconds mixing in an up, down and around motion. Turn off the tissuemizer.

6.22 Decant the extraction solvent through a 100 mm funnel with a neutral glasswool plug in it. **Acidify the glasswool by using 0.05% HCL/acetone solution. Shake out the glasswool to remove most of the acid solution. Rinse the glasswool 3 more times using methylene chloride.** Pour the tissumized extract into the corresponding pre-labeled 500 mL Erlenmeyer flask with 100-150 g of sodium sulfate at the bottom.

6.23 Repeat steps 6.20 and 6.22 two more times. For the third sonication, use pure methylene chloride only as the extraction solvent.

6.24 After the third extraction, transfer the sample to the funnel and rinse the 250 mL centrifuge bottle with methylene chloride. Pour this rinse through the funnel, then rinse the funnel and extracted sample with methylene chloride. All rinses are collected in the 500 mL flask. Transfer the label with sample ID to the flask. Empty the tissue into Ziploc bags then discard in buckets marked for halogenated waste.

6.25 Assemble a Kuderna-Danish (K-D) concentrator by attaching a methylene chloride rinsed 10 mL concentrator tube to a methylene chloride-rinsed 500 mL evaporation flask. Add 2-3 boiling chips and a blue clip.

6.26 Prepare one drying column for each sample by putting a neutral glass wool plug inside at the narrow end. **Rinse the glasswool with 30 mL 0.05% HCL/acetone solution. Shake out the glasswool to remove most of the acid solution. Rinse the glasswool 3 more times using methylene chloride.** Fill the column with anhydrous sodium sulfate to a bed height of approximately 10 cm. Rinse the prepared column once with 30 mL methylene chloride.

6.27 Dry the extract by passing it through the drying column and collecting it in the K-D concentrator. Rinse the Erlenmeyer flask three times with methylene chloride and also add this to the column. Once the entire extract has passed through the drying column, rinse the column with methylene chloride. Transfer the label with the sample ID to the K-D.

6.28 Remove the drying column and discard the sodium sulfate in buckets marked for halogenated waste. Attach a methylene chloride-rinsed 3-ball Snyder column to the drying column, and put the entire apparatus on a water bath set at approximately 90-

95° C. Wet the inside of the Snyder column with 1-2 mL of methylene chloride before boiling starts.

6.29 Once the extract has concentrated to approximately 2-3 mL, remove the apparatus from the water bath. Place the apparatus in a cooling rack and allow cooling for approximately 10 minutes.

6.30 Remove the Snyder column from the K-D apparatus. Transfer the labels with sample ID to the methylene chloride rinsed, pre-labeled Turbo tube. Pour the extracts from the K-D apparatus into the corresponding Turbo tube. Rinse the K-D apparatus with 5-10 mL methylene chloride and add the rinsate to the Turbo tube.

6.31 Remove the labels from the Turbo tube. Verify that the Turbo tube was previously labeled **at the top** with a wide Sharpie pen. Mount the Turbo tubes onto the Turbo Vap tray and place inside the Turbo Vap set at **30° C**. The water inside the Turbo Vap must be as high as the level of the extract. Select the row of nitrogen nozzles to be used by pressing the row panel on the Turbo Vap. Place an empty 60 mL VOA vial under the nozzles not being used in the selected row to prevent splashing. Set the timer on the Turbo Vap for 5 minutes, initially, but analyst discretion is advised. Close the lid of the Turbo Vap and slowly bring the pressure of the nitrogen up to maximum psi by turning the pressure dial clockwise. The extract should not be splashing. It may take 1-5 minute intervals after the initial five minute setting to complete the concentration. When the concentration is complete, remove the extracts from the Turbo Vap immediately.

6.32 Concentrate the extract to 1-2 mL to reduce the volume of acetone, and vial using a 5.0 mL syringe at 4.0 mL using methylene chloride into a 4.0 mL amber vial. Transfer the sample ID.

6.33 Proceed with GPC clean-up. See the ARI GPC SOP 306S for details and further instructions. The GPC aliquot must be filtered using a 25 mm PTFE 0.45 µm filter.

6.34 **Note: if extracting 20 g - 1.0 mL FEV for In-house limits:** A 1:2 aliquot, (2.0 mL) is taken for GPC and one-half the extract is archived. **% Lipid Determination may be taken from the <u>unfiltered</u> archived 2.0 mL. See % lipid determine in section 6.40**

6.35 **Note: if lower detection limits are required, the whole extract will need to be concentrated to 2.0mL for GPC and no archive will remain. % Lipid Determination can not be achieved using this extract. Another analysis or extract must then be generated.**

6.36 After the GPC clean-up, the extract is once again concentrated by using a K-D apparatus on a water bath set at approximately 80-85° C. Once the extract has

concentrated to 2-3 mL, remove the apparatus from the water bath and put the K-D in the cooling rack and allow to stand for at least 10 minutes.

6.37 Remove the Snyder column from the K-D apparatus. Transfer the labels with sample ID to the methylene chloride rinsed, pre-labeled Turbo tube. Pour the extracts from the K-D apparatus into the corresponding Turbo tube. Rinse the K-D apparatus with 5-10 mL methylene chloride and add the rinsate to the Turbo tube.

6.38 Remove the labels from the Turbo tube. Verify that the Turbo tube was previously labeled **at the top** with a wide Sharpie pen. Mount the Turbo tubes onto the Turbo Vap tray and place inside the Turbo Vap set at **30° C**. The water inside the Turbo Vap must be as high as the level of the extract. Select the row of nitrogen nozzles to be used by pressing the row panel on the Turbo Vap. Place an empty 60 mL VOA vial under the nozzles not being used in the selected row to prevent splashing. Set the timer on the Turbo Vap for 5 minutes, initially, but analyst discretion is advised. Close the lid of the Turbo Vap and slowly bring the pressure of the nitrogen up to maximum psi by turning the pressure dial clockwise. The extract should not be splashing. It may take 1-5 minute intervals after the initial five minute setting to complete the concentration. When the concentration is complete, remove the extracts from the Turbo Vap immediately.

6.39 Once the extract reaches 0.5 mL (or another specified final volume), transfer it using a 500 µL syringe into a 2.5 mL amber vial with a PTFE-lined screw cap using methylene chloride. Homogenize well. Then transfer the orange label tape to this vial. Note: Final Effective Volume = 1.0 mL.

6.40 Determination of % lipids using the 1:2 archived aliquot of the extract:
It is important this aliquot has not been filtered. Filtering will remove % lipids.

6.40.1 Label an aluminum dish with the job number and sample ID letter.

6.40.2 Pre-weigh the labeled aluminum dish on an analytical balance and record the weight on a % lipids bench sheet.

6.40.3 Use forceps from this point to handle aluminum dishes. Do not use your hands.

6.40.4 Place 200 µL of the 2.0 mL archived <u>unfiltered</u> extract into the pre-weighed/labeled aluminum dish. (1:20 final split).

6.40.5 Archive the remaining 1.8 mL of extract.

6.40.6 Place the aluminum dish with its contents under a hood for approximately 2 hours to allow the solvent to evaporate.

6.40.7 Transfer the aluminum dish to a desiccator.

6.40.8 Use a vacuum manifold to pressurize the dessicator then allow the lipids sit for 2-3 hours or overnight.

6.40.9 Re-weigh the aluminum dish on an analytical balance. Record the weight on the Bench Sheet.

7.0 Review

7.1 The Organic Extractions Supervisor or Lead Technician will review all bench work and bench sheets before distribution.

7.2 Review all project documentation (special analytical requirements, etc.) prior to extracting samples to determine if special procedures are required.

8.0 Quality Control

8.1 One Method Blank will be extracted for each batch of 20 or fewer samples.

8.2 One LCS (spike blank) will be extracted for each batch of 20 or fewer samples.

8.3 Upon the Client's request; one MS/MSD will be extracted for each batch of 20 or fewer samples, providing sufficient sample is available for the analyses.

8.4 To verify that surrogate and matrix spiking is accurate spiking will be witnessed and documented by another laboratory technician.

8.5 MDL's/DL's/RL's are on record and available at the QA office.

9.0 Corrective Actions

9.1 See Corrective Action Charts.

10.0 Miscellaneous Notes and Precautions

10.1 Use a permanent marker to label all glassware and vials with sample IDs.

10.2 Constant attention must be given to K-Ds when they are on the water bath. Check K-Ds frequently when solvent levels are below the surface of the water bath to ensure that extracts do not boil dry.

10.3 When extracts are concentrating on the Turbo Vap, a timer should be used to ensure that extracts are periodically checked and do not completely evaporate.

10.4 Always check the sample ID label when transferring, vialing or pouring extracts, or when changing glassware.

10.5 Modified extraction levels may be required as either the GC/MS Supervisor or Organic Extraction Supervisor deems necessary. Such decisions will be based upon the initial analysis of the BAN extract. The particular extraction procedure will be determined on a case-by-case basis, however, it will usually involve an extraction of

5-15 g of sample in 1:1 acetone/methylene chloride. Appropriate amounts of sodium sulfate, surrogate spike, and matrix spike should be added based on anticipated final effective volume. GPC clean-up, centrifuging or filtration may be required. The final effective volume of the extract should be between 0.5-10 mL.

10.6 All solvent lots, new or reclaimed, are to be checked for purity prior to use.

11.0 Method References

11.1 U.S. EPA, "Ultrasonic Extraction", (SW-846), Method 3550B, Revision 2, December, 1996.

11.2 A Rapid Method of Total Lipid Extraction and Purification. (Canadian Journal of Biochemistry and Physiology – August 1959) E.G. Bligh and W.J. Dyer.

12.0 Appendices

12.1 Corrective Action Charts.

12.2 Bench Sheets.

12.3 Tissuemizer assembly diagram.

QA LIMs # _____
EXT LIMs #_____
Bid # _____

(8270) BAN/Skydrol/Phenol – Soil/Sediment
In House_____/PSDDA_____Medium Level_____
(3550B) Microtip_____
(3550B) Macrotip_____
Med.Shakeout_____
KD_____
Turbovap_____

ANALYTICAL RESOURCES INCORPORATED

Client Name: _____

ARI Job No(s): _____

Extraction Requirements (ARI Lab ID)	Sample Client ID	Amount Extracted	Volume Of Solvent Added	GPC Aliquot A / Old	Final Effective Volume	Aliquot Volume For Analysis	Time Extracted_____ Comments
MB:							
SB:							
	Date/Analyst:						

Surrogate Amount:_____ Added by:_____/Spk. Witness_____
Concentration:_____ ID:_____

Spike Amount:_____ Added by:_____/Spk. Witness_____
Concentration: _____ ID:_____

Xtra Spike Amount:_____Added by:_____/Spk.Witness_____
Concentration:_____ ID:_____

(DCM)solvent ID:_____
(1:1DCM/Acetone) Reagent ID:_____
(95:5DCM/Hexane) Reagent ID:_____
Neutral glasswool ID:_____
0.05% HCL/acetone Reagent ID:_____
Na2So4 ID:_____

% Lipids – Tissue

ANALYTICAL RESOURCES INCORPORATED

Client Name: _____

ARI Job No(s): _____

Calculated on LIMs

ARI Sample ID	Client Sample ID	Original Extracted Volume	Volume Taken	Tare Weight	Tare + Sample Weight	Sample Weight	% Lipids
	Date/Analyst:						

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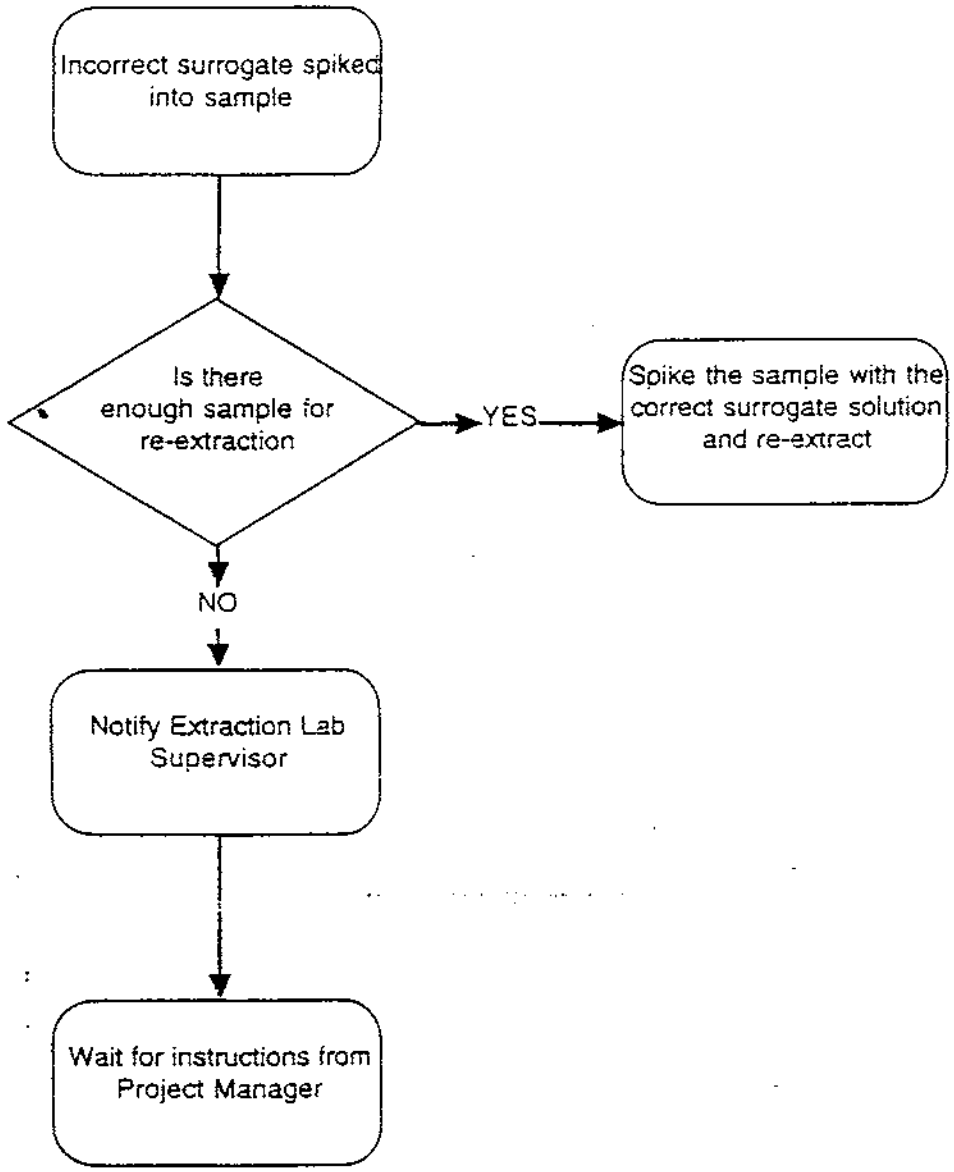
2/22/01

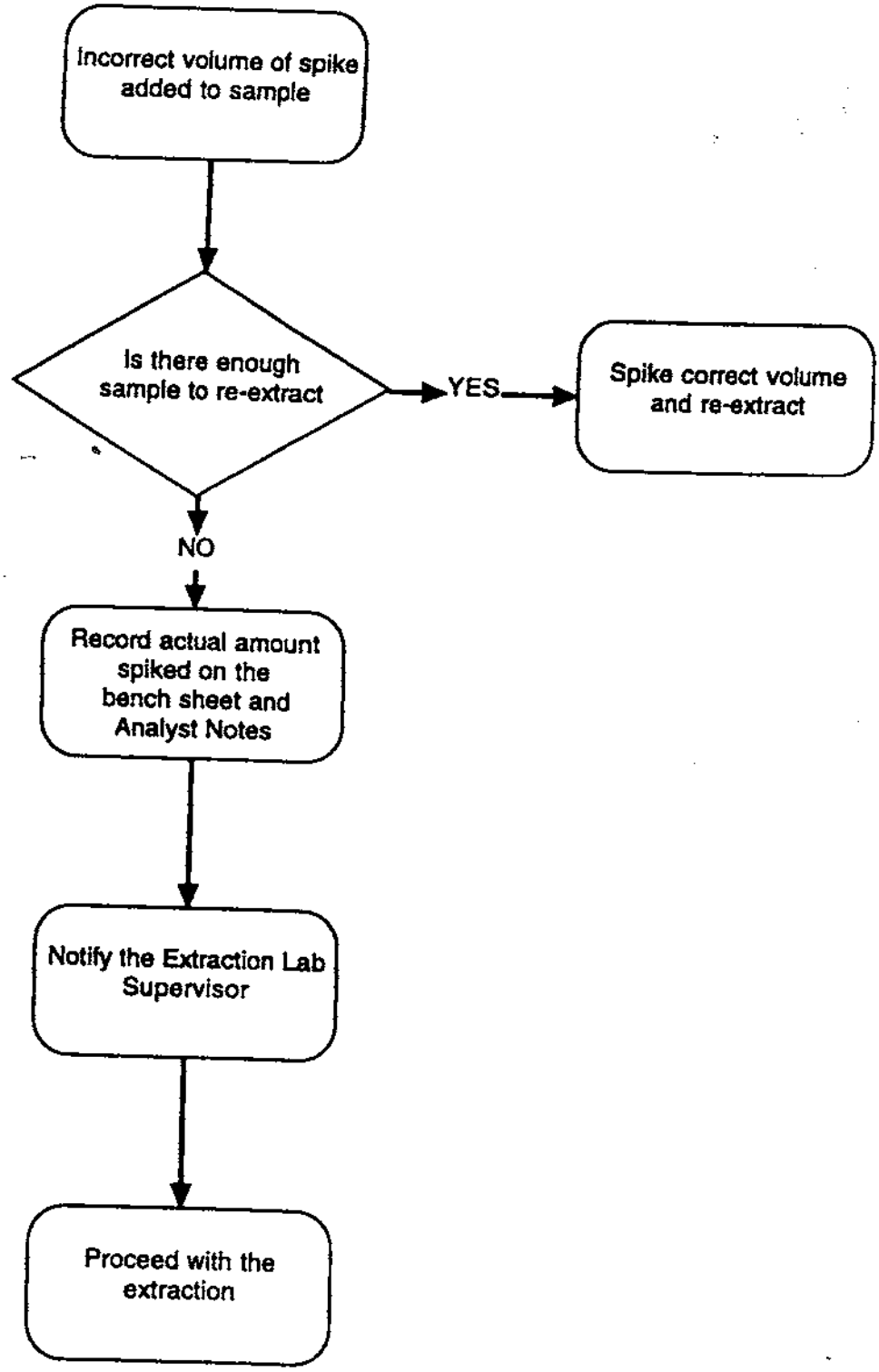
SECTION 12.0

APPENDICES

Corrective Action for Incorrect Surrogate Addition

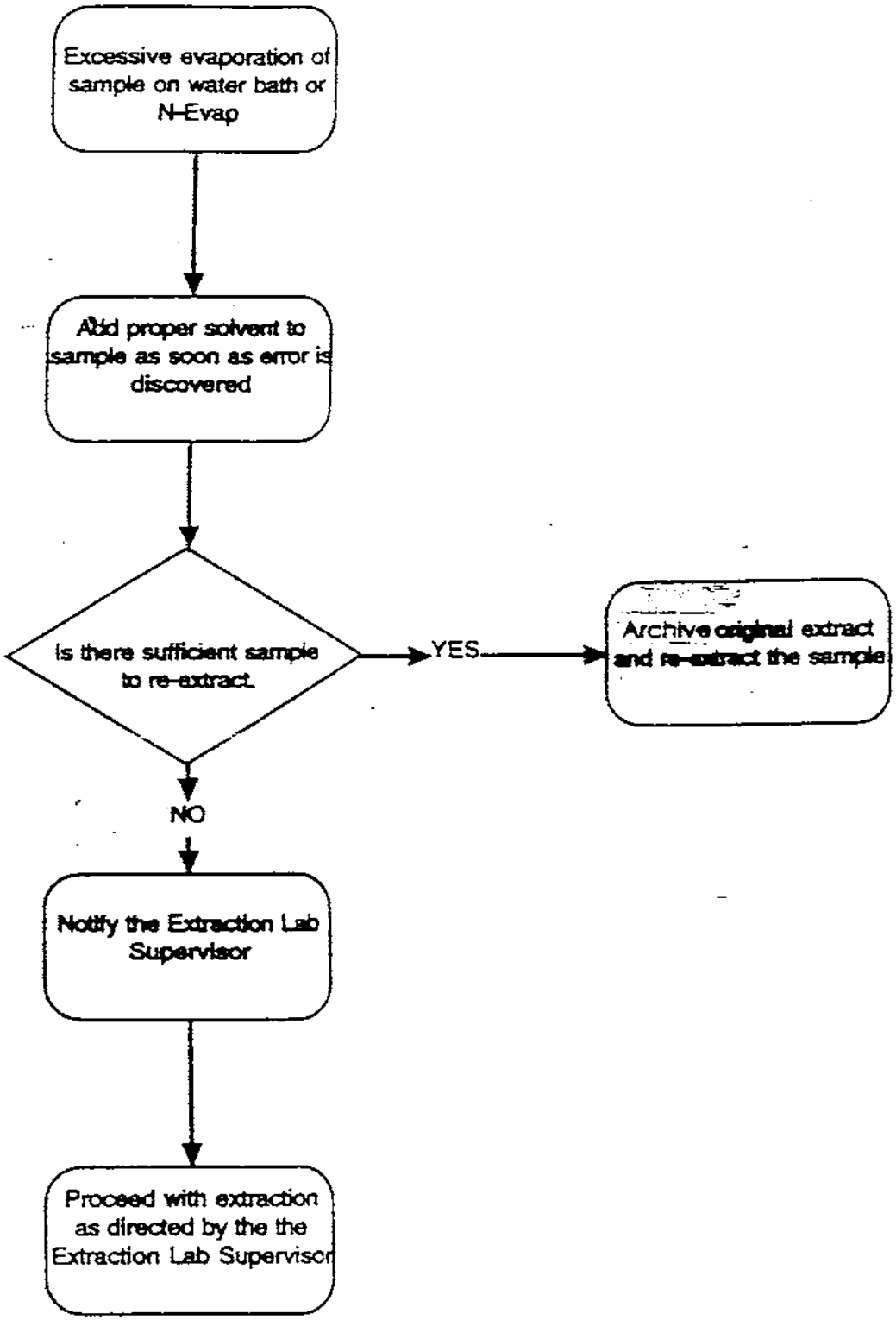


Corrective Action for Incorrect Spike Volume

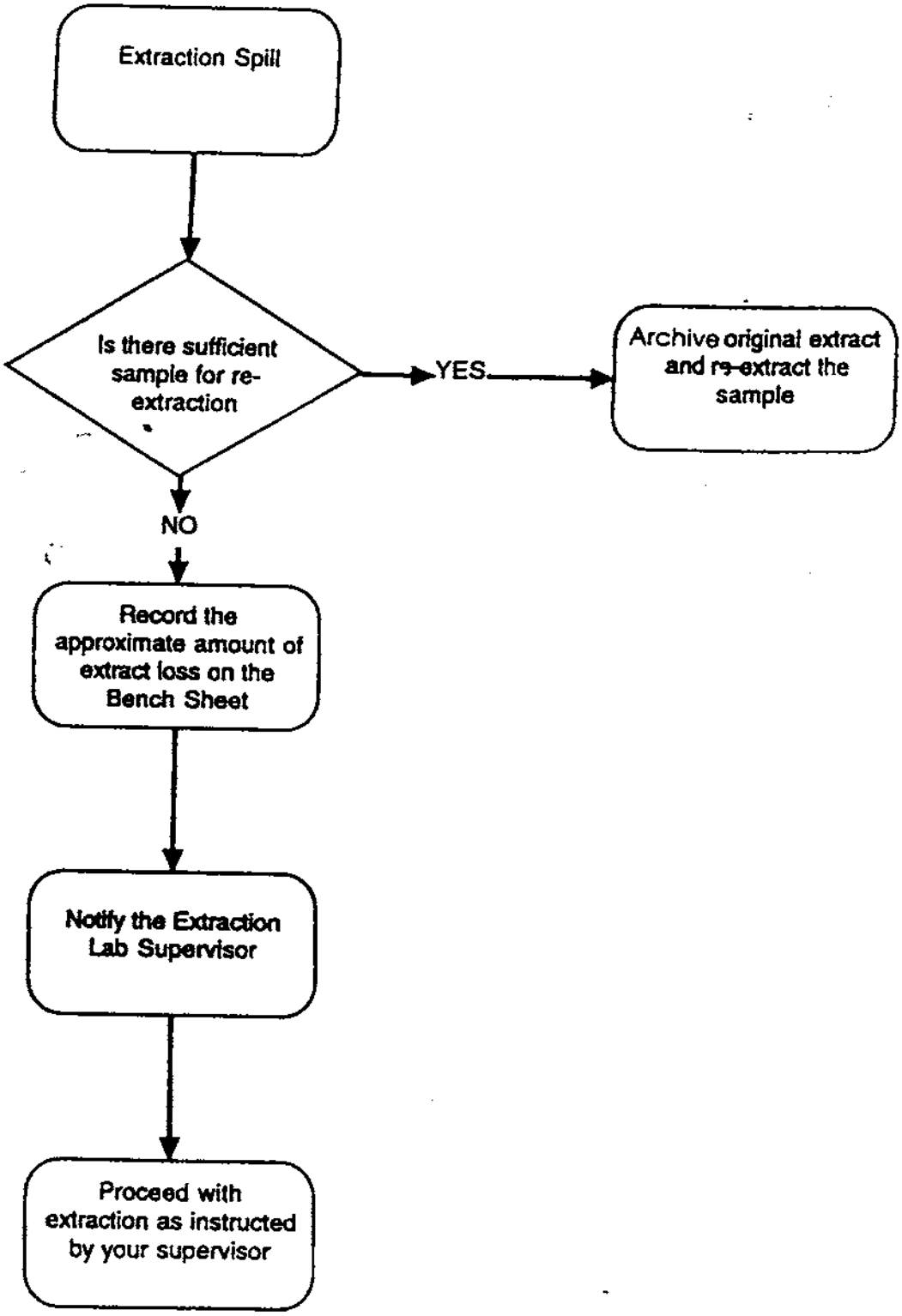


ANALYTICAL RESOURCES INCORPORATED

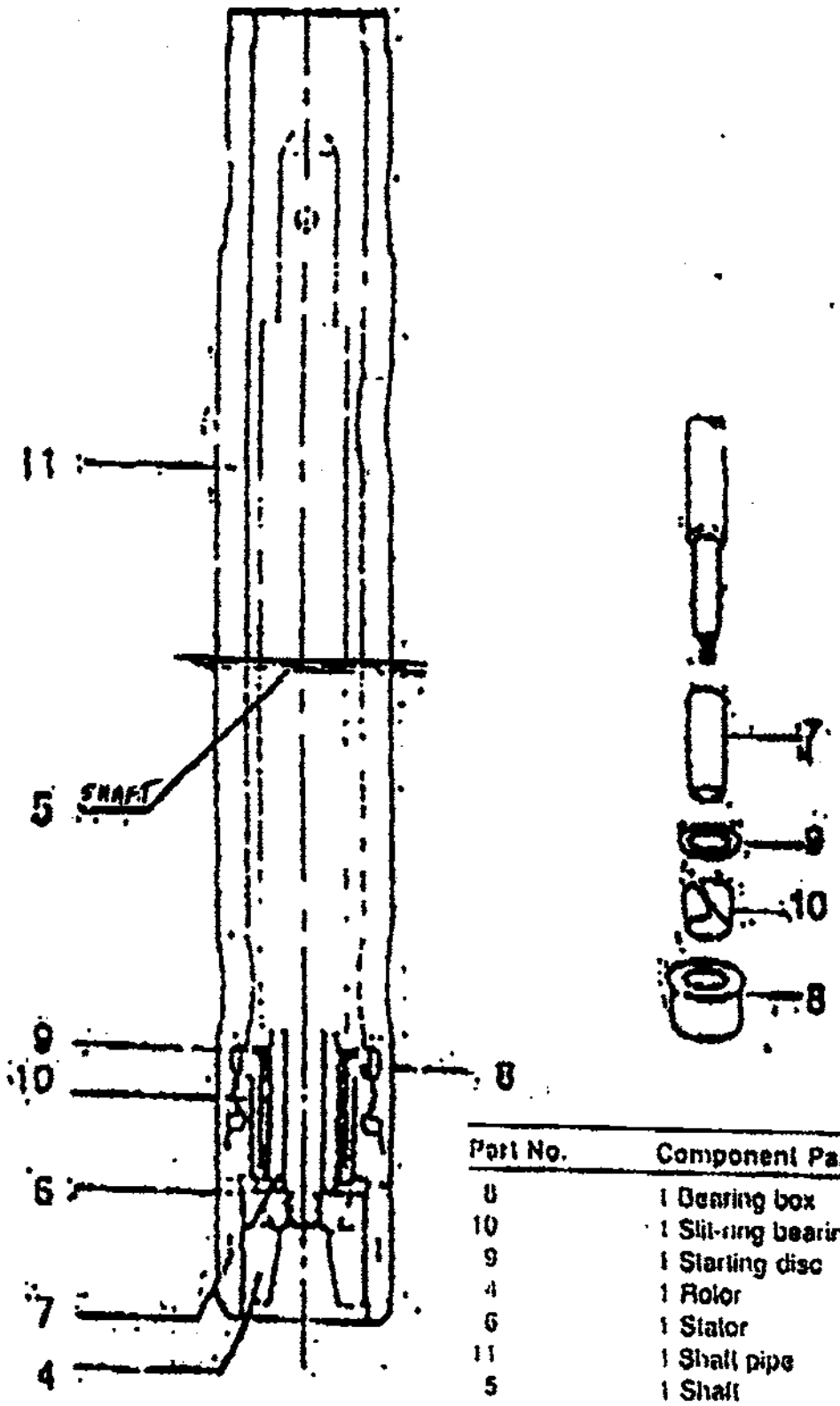
Corrective Action for Loss of Sample on Water Bath or N-Evap



Corrective Action for Extraction Spill



12.3 Appendix Tissuemizer assembly parts diagram:



Part No.	Component Parts
8	1 Bearing box
10	1 Slit-ring bearing
9	1 Starting disc
4	1 Rotor
6	1 Stator
11	1 Shaft pipe
5	1 Shaft
7	1 Shaft sleeve