APPENDIX G. ARI STANDARD OPERATING PROCEDURES

Analytical Resources, Incorporated
Analytical Chemists and Consultants

Standard Operating Procedure

Tissue Extraction – EPA Method 3550B
for
Pesticide & PCB Analysis
EPA Methods 8081B & 8082

SOP 303S
Revision 001

Revision Date: 8/4/05
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Prepared by:

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Approval:

Laboratory / Section Manager

Quality Assurance

Analytical Resources,Incorporated
Analytical Chemists and Consultants

Annual Review

SOP Number:	303S
Title:	Tissue Extraction for Pesticide & PCB Analyses
Revision:	001
Revision Date:	8/4/05
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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. Bebee	[signature]	8/4/05

Standard Operating Procedure
Tissue Extraction by Tissuemizer
Pesticide & PCB Analysis – Methods 8081B & 8082

1.0 Scope and Application

1.1 This document outlines the procedure for the extraction of tissue by tissuemizer for Pesticide (EPA Method 8081) and PCB (EPA Method 8082) analysis. 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 heating in kiln at 400° C for 4 hours.

3.12 Hexane, Pesticide Grade.

3.13 Acetone, high purity

3.14 Methylene Chloride, high purity

3.15 1:1 mixture of acetone/methylene chloride.

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

3.17 0.45 µm Whatman Puradisc filter (25 mm) PTFE.

3.18 Top-loading balance, accurate to 0.02 g.

3.19 Silica Gel, 0% and/or 4% (see ARI SOP 302S)

3.20 Green label tape.

3.21 Stainless steel spatula.

3.22 Hamilton gastight syringes: 100, 250, 500 & 1000 µL and 5.0 & 10 mL.

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

3.24 Turbo Vap LV.

3.25 Boiling chips - Teflon (Chemware).

3.26 4 mL glass vial with PTFE-lined screw cap.

3.27 2.5 mL amber vial with PTFE-lined screw cap.

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

3.29 Aluminum weighing dishes.

3.30 1% nitric acid solution

3.31 Surrogate, LCS and Matrix Spike solutions composed as follows:

	Component	Concentration
Surrogate:	Decachlorobiphenyl(DCBP)	2 µg/mL
	Tetrachloro-m-xylene(TCMX)	2 µg/mL
Pesticide/PCB Matrix Spike:		
	γ BHC	5 µg/mL
	Heptachlor	5 µg/mL
	Aldrin	5 µg/mL
	Dieldrin	10 µg/mL
	Endrin	10 µg/mL
	4,4'-DDT	10 µg/mL
PCB (only) Matrix Spike and LCS Spike:		
	Aroclor 1660	80 µg/mL
LCS Pesticide Spike		
	Hexachlorobutadiene	2 µg/mL
	Hexachlorobenzene	2 µg/mL
	α-BHC	2 µg/mL
	β-BHC	2 µg/mL
	γ-BHC	2 µg/mL

δ-BHC	2 µg/mL
Heptachlor	2 µg/mL
Aldrin	2 µg/mL
Heptachlor Epoxide	2 µg/mL
γ-Chlordane	2 µg/mL
α-Chlordane	2 µg/mL
Endosulfan I	2 µg/mL
4,4'-DDE	4 µg/mL
Dieldrin	4 µg/mL
Endrin	4 µg/mL
4,4'-DDD	4 µg/mL
Endosulfan II	4 µg/mL
4,4'-DDT	4 µg/mL
Endrin Aldehyde	4 µg/mL
Endosulfan Sulfate	4 µg/mL
Endrin Ketone	4 µg/mL
Methyoxychlor	20 µg/mL

4.0 Documentation

4.1 Pesticide/PCB bench sheet (ARI form 3017F).

5.0 In-house Modifications to Referenced Method

5.1 Samples are extracted by Tissuemizer, not sonication.

5.2 Section 6.23: extracts are decanted and filtered through a 100 mm funnel with a neutral glass wool plug, not through filter paper.

5.3 Section 6.24: for the third Tissuemizer step methylene chloride only is used.

5.4 Section 6.31: The extract is removed from the water bath at a volume of 2-3 mL, not 1 mL.

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 <u>inspect</u> 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-10 mL FEV is the normal In-house extraction level for Pesticides & PCBs. 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 (Pesticide, PCB or Pesticide/PCB) 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 200 µL Pesticide/PCB 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 **Pest/PCB** – Add 100 µL of full list pesticide spike to the LCS and LCSD to result in a final concentration of 0.02, 0.04 and 0.20 µL. To verify that the spiking is accurate, the spike addition will be witnessed and documented by a second laboratory technician.

6.19 **Pest/PCB** – Add 100 µL of Pesticide matrix spike solution to any MS samples to result in a final concentration of 0.05 µg/mL and 0.10 µg/mL. to verify that the matrix spiking is accurate, the spike addition will be witnessed and documented by a second laboratory technician.

6.20 **PCB only** – Add 63 µL of PCB matrix spike solution to the LCS and any MS samples, to result in a final concentration of 0.5 µg/mL. To verify that the matrix spiking is accurate, the spike addition will be witnessed and documented by a second laboratory technician.

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

6.22 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.

6.23 Decant the extraction solvent through a 100 mm funnel with a neutral glasswool plug in it. Pour the tissumized extract into the corresponding pre-labeled 500 mL Erlenmeyer flask with 100-150 g of sodium sulfate at the bottom.

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

6.25 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.

6.26 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.

6.27 Prepare one drying column for each sample by putting a neutral glass wool plug inside at the narrow end. 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.

6.28 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.

6.29 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.

6.30 Once the extract has concentrated to 2-3 mL, exchange the extract to Hexane by adding 30 mL of Hexane through the Snyder column. Tilt the KD body to remove any solvent from the bottom of the apparatus, to ensure a thorough exchange. Mark the label with an "X" to indicate the exchange process has been done.

6.31 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.

6.32 Remove the Snyder column from the K-D apparatus. Transfer the labels with sample ID to the Hexane 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 Hexane and add the rinsate to the Turbo tube.

6.33 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 **40° 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.

6.34 Adjust the extract volume to 10 mL, place it in a 20 mL scintillation vial and transfer the sample ID to the vial..

6.35 Pesticide only or Pesticide/PCB extracts: Silica Gel (4%) cleanup as described in ARI SOP 334S is required

6.36 PCB only extracts: Silica Gel (0%) is required as described in ARI SOP 334S.

6.37 Note: The final volume will be 10 mL following all extract cleanup

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 5-20 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. 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.

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_____

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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:__________

QA LIMs # ____________
EXT LIMs #____________
Bid # ________________

Pest/PCB – Wipe/Soil/Sediment
In House_____/PSDDA_____
(3550B) Microtip_____/Shakeout_______
(3550B) Macrotip_____
(3545A) ASE_____
KD_____
Turbovap_____

Client Name:________________________

ARI Job No(s): ____________________________

Extraction Requirements (ARI Lab ID)	Client Sample ID	Amount Extracted	GPC Aliquot #1 / #2	Sillica Gel (Pest) Aliquot	Silica Gel (PCB) Aliquot	Acid Clean Y / N	Sulfur Clean-up	Final Effective Volume	Volume To Lab	Time Extracted________ Comments:
MB:										
SB:										
Date/Analyst:										

Surrogate Amount: ____________
Concentration: ____________
Added By: ____________/Spk. Witness_____
ID: ____________

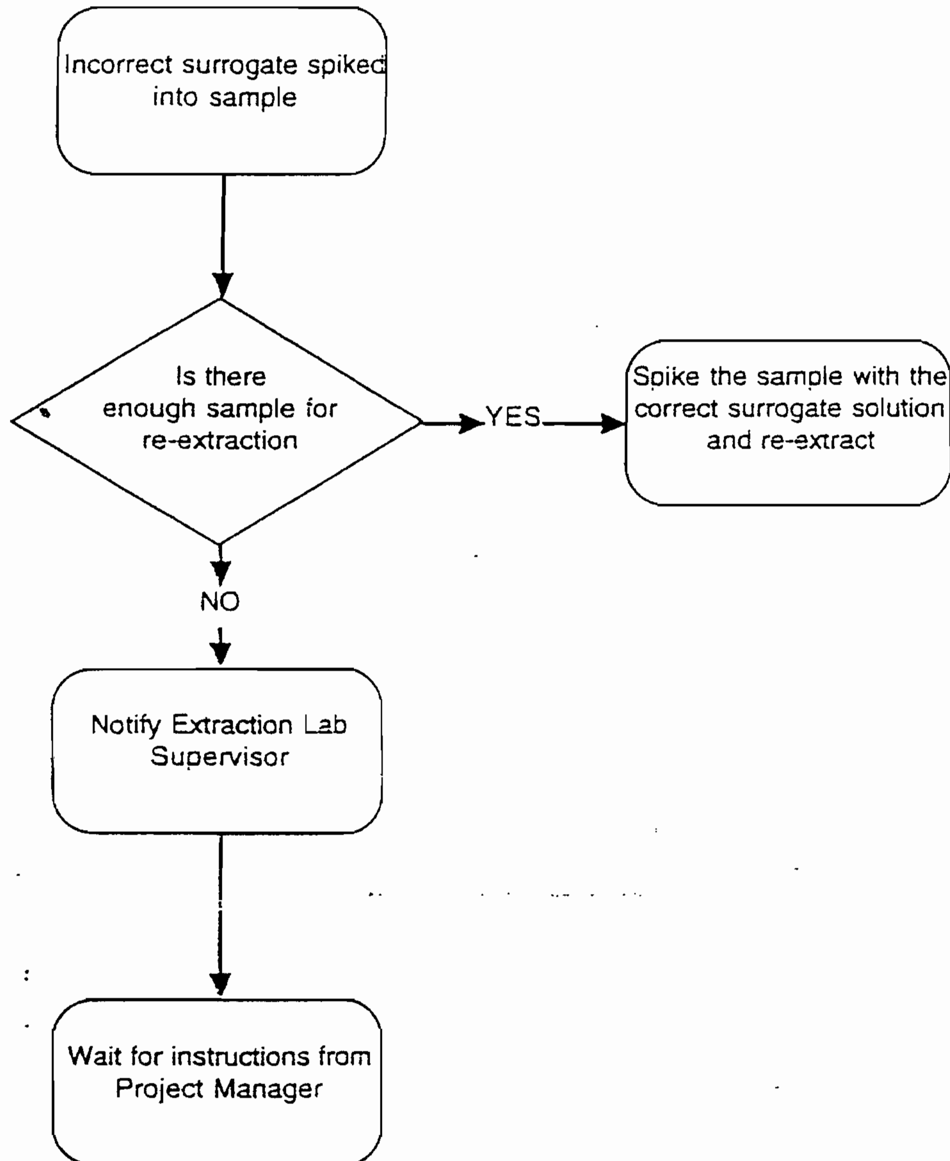
Spike Amount: ____________
Concentration: ____________
Added By: ____________/Spk. Witness_____
ID: ____________

1:1 DCM/Hex I.D.: ____________
(80:20 Hex/Acetone) Reagent ID: ____________
(Hexane) Solvent Lot ID: ____________
Wipe Lot ID: ____________
Conc. H2So4 ID: ____________
3% or 0% Silica Gel I.D. ______/______
Tetrabutyl Ammonium Sulfite Reagent I.D.: ____________
Na2So4 ID: ____________
Neutral glasswool ID: ______/______

SECTION 12.0

APPENDICES

Corrective Action for Incorrect Surrogate Addition



Corrective Action for Incorrect Spike Volume

Incorrect volume of spike added to sample

Is there enough sample to re-extract

YES

Spike correct volume and re-extract

NO

Record actual amount spiked on the bench sheet and Analyst Notes

Notify the Extraction Lab Supervisor

Proceed with the extraction

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

Excessive evaporation of sample on water bath or N-Evap

↓

Add proper solvent to sample as soon as error is discovered

↓

Is there sufficient sample to re-extract.

→ YES → Archive original extract and re-extract the sample

↓ NO

Notify the Extraction Lab Supervisor

↓

Proceed with extraction as directed by the the Extraction Lab Supervisor

Corrective Action for Extraction Spill

Extraction Spill

↓

Is there sufficient sample for re-extraction

YES → Archive original extract and re-extract the sample

NO ↓

Record the approximate amount of extract loss on the Bench Sheet

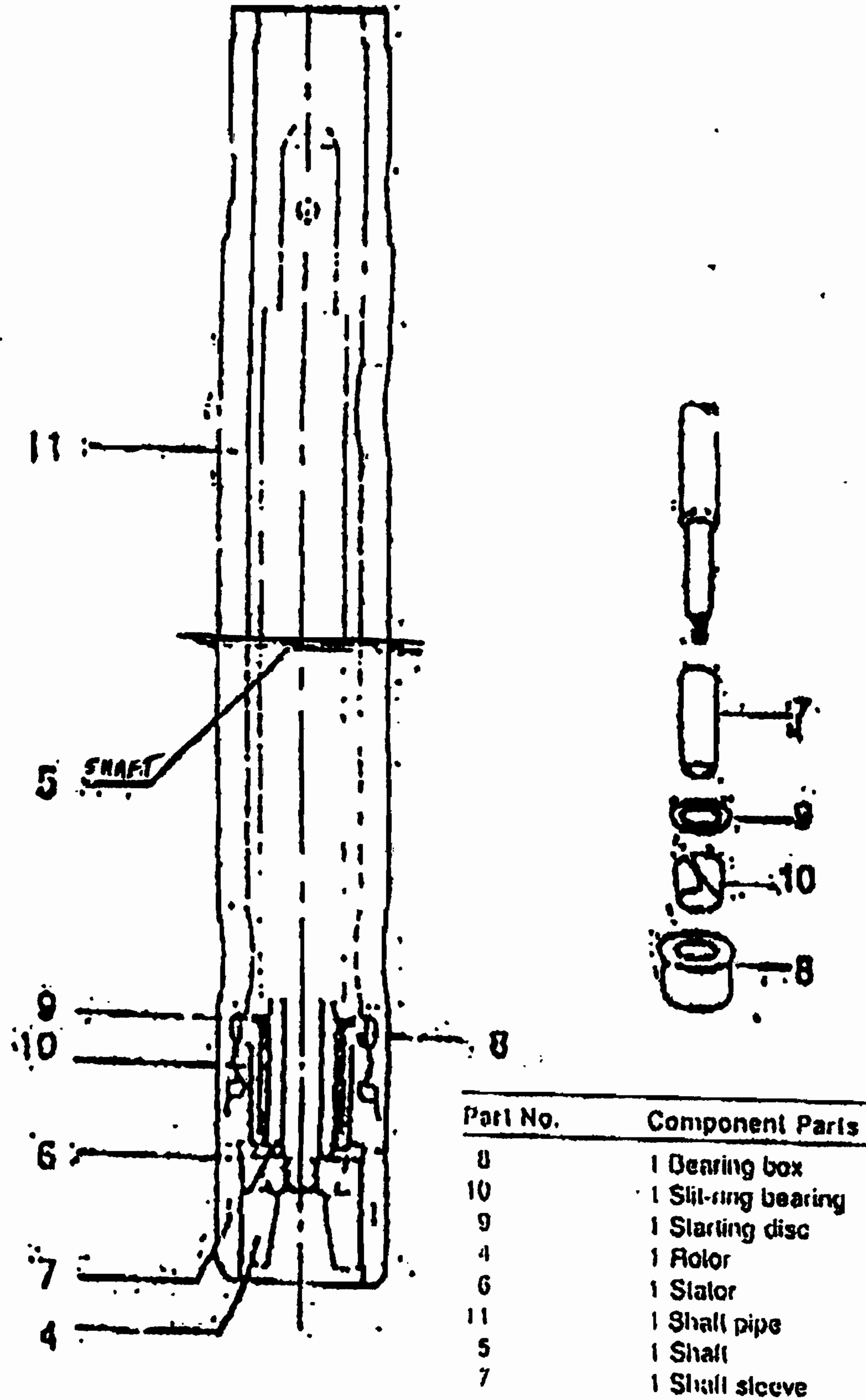
↓

Notify the Extraction Lab Supervisor

↓

Proceed with extraction as instructed by your supervisor

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