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STANDARD OPERATING PROCEDURE

EXTRACTION OF ORGANOTINS IN SEDIMENT, WATER, AND TISSUE MATRICES

EXT-OSWT
Revision 6

November 25, 2009

Approved By: _____ Supervisor — 11/11/09 Date

_____ QA Manager — 11/11/09 Date

_____ Laboratory Manager — 11/11/09 Date

COLUMBIA ANALYTICAL SERVICES, INC.
1317 South 13th Avenue
Kelso, Washington 98626

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Annual review of this SOP has been performed and the SOP still reflects current practice.
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EXTRACTION OF ORGANOTINS IN SEDIMENT, WATER, AND TISSUE MATRICES

1. SCOPE AND APPLICATION

This procedure is used to extract selected butyltins from sediments, water and tissues. The procedure is a preparative step for determination of butyltins by gas chromatography (SOP SOC-BUTYL). The procedure can also be applied to porewater samples.

2. SUMMARY OF METHOD

Butyltin compounds are extracted with an organic solvent using the technique suitable for the sample matrix. Sediment and tissue samples are extracted by tumbling. Water samples are extracted by liquid-liquid extraction. Extracts are then derivitized to their hexyl form using hexylmagnesiumbromide. After derivitization, extracts from sediment and water samples are cleaned up with silica and alumina; tissue sample extracts are cleaned up with Florisil. Extracts are then taken to final volume and analyzed by GC/FPD.

3. DEFINITIONS

Refer to the determinative procedure SOP (SOC-BUTYL) for applicable definitions.

4. INTERFERENCES

4.1. Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines, causing misinterpretation of the chromatograms. All of these materials must be demonstrated to be free from interferences, under the conditions of the analysis, by running blanks.

4.2. In sediment samples, sulfur compounds cause the most direct interference with this procedure. Dimethyldioxirane may be used to help remove the sulfur that might otherwise interfere with the flame photometric detector.

5. SAFETY

5.1. The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined; however, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. A reference file of material safety data sheets is available to all personnel involved in these analyses. CAS also maintains a file of OSHA regulations regarding the safe handling of the chemicals specified in this method. Appropriate precautions must be taken when preparing, handling, and storing Grignard reagent.

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5.2. All samples should be treated as a potential hazard. Appropriate personal protective equipment must be worn when performing the procedure.

6. SAMPLE COLLECTION, CONTAINERS, PRESERVATION, AND STORAGE

Refer to the determinative procedure SOP (SOC-BUTYL).

7. APPARATUS AND MATERIALS

7.1. Materials used in sediment, tissue and water extractions.

7.1.1. pH paper, 0 - 14 range

7.1.2. 16x150mm and 25x150mm disposable glass culture tubes with Teflon lined screw caps

7.1.3. 0.5 ml, 1ml, 2 ml, 5 ml, and 10 ml serological pipettes

7.1.4. Pastuer pipets

7.1.5. 2 ml glass vials with Teflon lined crimp-top caps

7.1.6. Nitrogen evaporator

7.1.7. Centrifuge - capable of handling glassware in 7.1.2.

7.1.8. Vacuum pump and manifold

7.1.9. Kuderna-Danish (K-D) apparatus

7.1.9.1. Concentrator tube - 10 ml, graduated.

7.1.9.2. Evaporation flask - 250 ml. Attach to concentrator tube with springs, clamps or equivalent.

7.1.9.3. Three-ball Snyder column.

7.1.9.4. Springs - 1/2 inch.

7.1.10. Boiling chips - Pre-cleaned via Soxhlet extraction, approximately 10/40 mesh (silicon carbide or equivalent).

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7.1.11. Water bath - Heated, with concentric ring cover, capable of temperature control (± 5°C). The bath should be used in a hood.

7.2. Materials for extraction of sediments

7.2.1. 250 mL Teflon bottles with Teflon screw caps

7.2.2. Tumbler, capable of holding twelve 250 mL Teflon bottles

7.2.3. Modified vacuum filtration funnel

7.2.4. Whatman No. 41 filter paper

7.2.5. Polypropylene funnels

7.2.6. Scoopulas

7.2.7. 400 ml beakers

7.3. Materials for extraction of waters

7.3.1. Continuous liquid/liquid extraction body

7.3.2. 500 ml round bottom flask, with green Keck clip

7.3.3. 1000 ml graduated cylinder

7.3.4. Stir rod and pH paper

7.3.5. Allihn condensor

7.3.6. Pore water sample preparation

7.3.6.1. Polycarbonate centrifuge bottles, 1L.

7.3.6.2. Water-from-sand apparatus

7.3.6.3. Large spoon

7.3.6.4. Nitrogen Hood

7.3.6.5. Refrigerated Centrifuge capable of handling 1 liter bottles

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7.4. Materials for extraction of tissues

7.4.1. Vortex for VOA vials

7.4.2. Tumbler for VOA vials

7.4.3. 40 ml and 60 ml VOA vials with Teflon lined septa and screw caps

7.5. Materials for Grignard preparation

7.5.1. 500 ml round bottom three necked flask with glass stoppers

7.5.2. 30 cm condenser

7.5.3. 125 ml addition funnel

7.5.4. Two drying tubes filled with drierite and plugged with glass wool

7.5.5. Glass rod

7.5.6. Hot water bath

7.5.7. 100 ml graduated cylinders

7.5.8. 150 ml glass beaker

8. REAGENTS

8.1. Solvents: Dichloromethane (DCM), hexane, pentane, methanol, and anhydrous diethyl ether (J.T. Baker).

8.2. Tropolone in dichloromethane (DCM) - 0.1% (w/v) solution is prepared by adding 1 g tropolone per liter of DCM.

8.3. Tropolone in hexane - 0.1% (w/v) solution is prepared by adding 1g tropolone per liter of hexane.

8.4. Tropolone in water - 0.01% (w/v) solution is prepared by adding 0.4 g tropolone and 40 mL concentrated HCl to 4 L of reagent water.

8.5. Reagent water

8.6. D.I. Water

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8.7. Sodium sulfate powder.

8.8. Concentrated HCl.

8.9. Fisher PrepSepR 1 g silica cartridges, or equivalent.

8.10. Fisher PrepSepR 1 g alumina cartridges, or equivalent.

8.11. Magnesium turnings (from Fisher). It is recommended to investigate the use of semiconductor grade magnesium if blank contamination (tin) is exhibited.

8.12. Bromohexane (from Fluka).

8.13. Florisil cartridges (Supelco).

8.14. Surrogate and spiking solutions--see SOP SOC-BUTYL.

8.15. Hexylmagnesiumbromide - CH3(CH2)5MgBr (Grignard). Prepare as follows:

8.15.1. All glassware used in Grignard preparation is baked at least 4 hours at $\geq$ 100°C to remove water. Glassware is assembled while still warm.

8.15.2. Add 14 g of magnesium to the three necked flask during assembly. The condenser is placed in the middle neck of the flask with a drying tube at the top of the condenser. A glass stopper is put in one neck of the flask, with the 125 ml addition funnel in the last flask opening. The addition funnel contains 38 ml bromohexane in 95 ml anhydrous ether, and is topped with a drying tube.

8.15.3. Add 4 ml bromohexane and 10 ml ether to the flask. A glass rod is used to crush 2 or 3 Mg chips. A hot water bath may be used to help reaction start. After reaction has started, hot water bath is removed.

8.15.4. Once reaction is started, the bromohexane solution in the addition funnel is added at a rate of about 3 drops per second. Once nearly all of the bromohexane/ether solution has been added, an additional 95ml of anhydrous ether and 38ml of bromohexane are added to the addition funnel (in that order). Once all bromohexane solution has been added, the hot water bath is returned and reaction is refluxed for at least one hour. Grignard solution is now ready to use.

8.15.5. Laboratory records show Grignard lasts up to 2 months after preparation. Due to the reactive nature of Grignard reagent, it should be checked for reactiveness prior to continued use. After 2 months, test weekly prior to use. A derivatization

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blank, a 1 ppm standard, and a 10 ppm standard are typically prepared ahead of sample derivatization to assure the purity and reactiveness of each batch of Grignard.

8.16. Dimethyldioxirane (DMD) – see SOP SOC-DMD *DMD Synthesis*

9. PREVENTIVE MAINTENANCE

Routine cleaning of the extraction glassware is necessary. Refer to the SOP for *Organic Extractions Glassware Cleaning.*

10. RESPONSIBILITIES

10.1. It is the responsibility of the analyst to perform the analysis according to this SOP and to complete all documentation required for data review. Analysis and interpretation of the results are performed by personnel in the laboratory who have demonstrated the ability to generate acceptable results utilizing this SOP. This demonstration is in accordance with the training program of the laboratory. Final review and sign-off of the data is performed by the department supervisor/manager or designee.

10.2. It is the responsibility of the department supervisor/manager to document analyst training. Documenting method proficiency is also the responsibility of the department supervisor/manager.

11. PROCEDURE

11.1. Extraction of Organotins From Sediments

11.1.1. Mix sample thoroughly and weigh approximately 20g into a beaker. Record weight to nearest 0.01g. If possible, dry the sample overnight in a hood. Acidify with 2mL concentrated HCl. Mix samples with adequate sodium sulfate to dry sample. The method blank and laboratory control sample are made from an equal amount of sodium sulfate. Add sample to a 250 mL Teflon bottle and add appropriate surrogate and spike. Check pH to ensure pH <2. Add more acid if necessary. Add enough 0.1% tropolone/DCM solution to cover sample with at least 1/2" of solvent (approximately 150 ml). Cap tube, shake and vent to release pressure. Place bottles on tumbler, and tumble for at least 16 hours.

11.1.2. Pour sample into modified funnel with glass wool, and filter by vacuum into a 250mL KD flask. Evaporate the extract on a water bath (<75°C) to approximately 10 ml and transfer to a culture tube. Add 20 ml hexane

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11.1.3. Using the N-Evap, solvent exchange to hexane. Concentrate the extract to approximately 3 ml. Add 2 ml of reagent water and vortex. Centrifuge to separate precipitates. Using a Pastuer pipet and pentane, quantitatively transfer the extract (solvent) layer to a 2nd culture tube. Evaporate extract down to approximately 2 ml.

11.1.4. Add 2 ml of Grignard reagent and vortex every 5 minutes over a 45-minute period (or 45 minutes on a shaker). Add concentrated HCL slowly, vortexing as needed, until there is no reaction and the hexane layer is clear.

11.1.5. Transfer hexane layer to a new test tube. Take to 4.0mL final volume, remove 1mL for cleanup. Add 1mL DMD to this aliquot, vortex 3 minutes. Solvent exchange to hexane and proceed with cleanup.

11.1.6. Using the vacuum or positive pressure manifold, set up alumina/silica cartridges and condition with 5 ml hexane. Discard hexane. Place samples in cartridges and bring down to almost dry, and elute with 6 ml of pentane, allowing this to go dry. Take extracts to a 1.0 ml final volume in hexane, and place in a 2 ml vial.

11.2. Extraction of Porewater from sediments

11.2.1. All labware must be rinsed with 10% HCl followed by DI water prior to use. Labware must be dry prior to further rinsing. Air drying is best for polycarbonate labware; methanol may be used if time is limited. All polycarbonate labware must be rinsed with 0.1% w/v tropolone in hexane followed by hexane; all other labware must be rinsed with 0.1% w/v tropolone in Dichloromethane followed by Dichloromethane.

11.2.2. All transferring of samples must be carried out in an isolated environment under at least 2 atmospheres of positive N_2 pressure (15 psi). Sample centrifugation must be carried out at or below 10° C.

11.2.3. In the Nitrogen hood, transfer sediment with all standing water to two centrifuge bottles. Balance the bottles to within 1 gram before centrifugation.

11.2.4. Very sandy sediments often do not yield sufficient volumes of water. For the samples, it will be necessary to employ at least two water-from-sand recovery apparatuses; make a note of this as these samples will be exposed to the atmosphere.

11.2.5. Use 1000 mL of reagent water for the method blank and centrifuge along with the samples.

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11.2.6. Centrifuge the balanced bottles opposite each other for 30 minutes at 1000 - 3000G. (1100-3250 RPM for a 10 inch rotor radius).

11.2.7. Decant the water into clean polycarbonate centrifuge bottles and balance as above.

11.2.8. Centrifuge the samples a second time at 3000 G.

11.2.9. Decant the water from the centrifuge bottles into polycarbonate sample bottles. If the sediment is resuspended during the decantation, the samples must be centrifuged again before decanting.

11.2.10. Add 0.5-1 mL of concentrated HCl to each sample bottle to preserve them and store at or below 4° C.

11.2.11. Some projects may require samples to be filtered prior to preservation and storage. Check project specific requirements to see when filtration is necessary. Samples must be filtered using 0.4 μm polycarbonate filters in porcelain Buchner funnels. The samples are filtered into polycarbonate Erlenmeyer flasks.

11.2.12. Extract the recovered porewater following the procedure in section 11.3.

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11.3. Extraction of Organotins In Water

11.3.1. Refer to the SOP for Continuous Liquid-Liquid Extraction (SOP EXT-3520). Add 500 mL of DCM to the extractor. Measure and transfer 500 mL of sample to the extractor. Set up the method blank, laboratory control sample, and spike samples. Add surrogate and matrix spikes as appropriate.

11.3.2. Add 500 mL of 100ppm tropolone in 0.1% HCl in reagent water. Follow the instructions for continuous liquid-liquid extraction.

Note: For porewater samples, the available sample volume is typically limited to 500 ml or less. Determine the amount of sample available and adjust the volume extracted accordingly. Adjust the amount of reagents and surrogate and matrix spike amounts accordingly.

11.3.3. Make sure that the continuous liquid - liquid extractor is receiving adequate flow for cooling, and that the units are cycling properly. Check the chiller for proper temperature and operation. (Condensers should be cold to the touch before extraction begins).

11.3.4. Cycle the unit for 18 hours.

11.3.5. Transfer the extract (DCM) to a 250 mL KD flask. Evaporate the extract on a water bath (<75°C) to approximately 5 mL. Add approximately 10 ml hexane and continue to solvent exchange to hexane.

11.3.6. Transfer the extract to a culture tube and, using the N-Evap, blow down the extract to approximately 2 ml.

11.3.7. Add 2 ml Grignard, and vortex every 5 minutes over a 45 minute period. Add concentrated HCl slowly, vortexing as needed, until there is no reaction and hexane layer is clear.

11.3.8. Clean up using alumina and silica cartridge as described in section 11.1.6 and take to 1.0mL (nominal) final volume.

11.4. Extraction of Organotins In Tissue

11.4.1. Mix sample thoroughly and weigh approximately 10 g into a 40 ml VOA vial. Add surrogate and matrix spikes. Acidify with 10mL of 1.5M HCl. Add 10mL of 0.1% tropolone/DCM, vent to release pressure. Tumble for 1 hour. Centrifuge the extract and transfer tropolone/DCM layer to a culture tube. Repeat two more times with addition of tropolone/DCM each time.

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11.4.2. Evaporate the extract to 5 ml on the N-evap. Add 10 ml of hexane, evaporate to 2 ml, add 10 ml more hexane, evaporate to 2 ml again and take to 5 ml in hexane.

11.4.3. Continue with Grignard derivitization (11.1.4) and take to final volume as in section 11.1.6, using a Florisil cartridge instead of Alumina and silica gel cartridges.

12. QUALITY CONTROL

12.1. The QC samples required for the extraction batch is described in the *Butyltins* SOP (SOC-BUTYL) and the SOP for *Sample Batches* (ADM-BATCH). Any method blanks or laboratory control samples should be subjected to exactly the same procedures as those used in actual samples.

12.2. Follow the applicable quality control guidelines outlined in *Butyltins* SOP (SOC-BUTYL).

12.3. The laboratory must follow all quality control requirements outlined in Table F-1 of the *DoD Quality Systems Manual for Environmental Laboratories*, Version 4.1, 4/22/2009 for samples submitted for DoD ELAP projects. See the SOP *Department of Defense Projects Laboratory Practices and Project Management (ADM-DOD).*

13. DATA REDUCTION AND REPORTING

Use the appropriate benchsheet to record all sample preparation information. The benchsheet must be completed in full and reviewed by a 2nd analyst or supervisor. Sample preparation information recorded on the benchsheet is used to perform data reduction following GC analysis.

14. CORRECTIVE ACTION

14.1. Refer to the SOP for *Corrective Action* (ADM-CA) for procedures for corrective action. Personnel at all levels and positions in the laboratory are to be alert to identifying problems and nonconformities when errors, deficiencies, or out-of-control situations are detected.

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14.2. Handling out-of-control or unacceptable data

14.2.1. On-the-spot corrective actions that are routinely made by analysts and result in acceptable analyses should be documented as normal operating procedures, and no specific documentation need be made other than notations in laboratory maintenance logbooks, runlogs, for example.

14.2.2. Documentation of a nonconformity must be done using a Nonconformity and Corrective Action Report (NCAR) when: a) corrective action is not taken or not possible b) corrective action fails to correct an out-of-control problem on a laboratory QC or calibration analysis c) reanalysis corrects the nonconformity but is not a procedurally compliant analysis.

15. POLLUTION PREVENTION AND WASTE MANAGEMENT

15.1. It is the laboratory's practice to minimize the amount of solvents, acids, and reagents used to perform this method wherever feasibly possible. Standards are prepared in volumes consistent with methodology and only the amount needed for routine laboratory use is kept on site. The threat to the environment from solvents and/or reagents used in this method can be minimized when recycled or disposed of properly.

15.2. The laboratory will comply with all Federal, State, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions as specified in the CAS Environmental Health and Safety Manual.

16. METHOD PERFORMANCE

Available method performance data is given in the reference method and determinative method. In addition, this procedure was validated through single laboratory studies of accuracy and precision as specified in the determinative procedures.

17. TRAINING

17.1. Training outline

17.1.1. Review literature (see references section). Read and understand the SOP. Also review the applicable MSDS for all reagents and standards used. Following these reviews, observe the procedure as performed by an experienced analyst at least three times.

17.1.2. The next training step is to assist in the procedure under the guidance of an experienced analyst. During this period, the analyst is expected to transition from

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a role of assisting, to performing the procedure with minimal oversight from an experienced analyst.

17.1.3. Perform initial precision and recovery (IPR) study as described above for water samples. Summaries of the IPR are reviewed and signed by the supervisor. Copies may be forwarded to the employee's training file. For applicable tests, IPR studies should be performed in order to be equivalent to NELAC's Initial Demonstration of Capability.

17.2. Training is documented following the SOP *for Documentation of Technical Personnel Training*.

NOTE: When the analyst training is documented by the supervisor on internal training documentation forms, the supervisor is acknowledging that the analyst has read and understands this SOP and that adequate training has been given to the analyst to competently perform the analysis independently.

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18. REFERENCES

18.1. Unger, M.A.; MacIntyre, W.G. Greaves, J.; Huggett, R.J., *GC Determination of Butyltins in Natural Waters by Flame Photometric Detection of Hexyl Derivatives with Mass Spectrometric Confirmation, Chemosphere*, 1986, 15 (4): 461-470

18.2. Krone, C.A.; Brown, D.W.; Burrows, D.G.; Bogar, R.G.: Chan, S.: Varanasi, U., *A Method for Analysis of Butyltin Species and Measurement of Butyltins in Sediment and English Sole Livers from Puget Sound*, Environmental Conservation Division, Northwest and Alaska Fisheries Center, National Marine Fisheries Service, NOAA, November, 1988.

18.3. Stallard, M.O.; Cola, S.Y.; Dooley, C.A.; *Optimization of Butyltin Measurements for Seawater, Tissue, and Marine Sediment Samples*, Applied Organometallic Chemistry (1989)3, 105-114.

18.4. *DoD Quality Systems Manual for Environmental Laboratories*, Version 4.1, 4/22/2009

19. CHANGES SINCE THE LAST REVISION

19.1. Sec 8.5 removed ‘tap water’
19.2. Sec 8.15.1 Added 4 hours.
19.3. Sec 11.1.1 Changed drying sample from after addition of HCL and sulfate to before additions.
19.4. Sec 11.1.3 Changed D.I. water to reagent water.
19.5. Sec 12.1 Updated SOP references
19.6. Sec 12.3 is new.
19.7. Changed file name from SOC-OSWT to EXT-OSWT

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ATTACHMENT A

Preparation Forms
Benchsheets

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Additional Prep Information For Organotin Tissue Extractions

Service Request #_____**Work Group Number**_____

Pre-Prep Information:

_____

DCM Lot #: _____
HCl Lot #: _____
H_2O Lot #: _____
Tropolone Lot#: _____

Na_2SO_4 (G) Lot #: _____
Grignard Lot #: _____
Hexane Lot #: _____
Pentane Lot #: _____

Acidified? :_____

(1st) Tumbler (1hr.)
time/date/initial:
Start: _____
End: _____

(2nd) Tumbler (1hr.)
time/date/initial:
Start: _____
End: _____

(3rd) Tumbler (1hr.)
time/date/initial:
Start: _____
End: _____

Date Derivatization started (date/initial): _____
Date Derivatization ended (date/initial): _____

Florisil (3620)/Silica Gel (3630) (date/initial): _____

Comments/Observations:_____
_____
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_____

Bench Sheet Review Check List
❑ Hold Times Met (if no, Reason: _____)
❑ Prep date, dept, method, product code correct in stealth
❑ Spike Information correct
❑ Weights/Volumes and units correct on raw and final bench sheets
❑ Sample IDs have been checked—Bottle numbers appended if required
❑ Names present for: Started by, Completed by, relinquished by, and witnessed by.
❑ Training has been circled
❑ Extract Storage recorded
❑ Additional Prep Sheet completely filled out (NA or line out Blanks)
❑ All clean-ups have been noted on additional prep sheet
❑ Signed service request with Form V, if applicable, has been attached

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ORGANOTINS IN TISSUE

1. Mix sample thoroughly and weigh approximately 10 grams into a 40 ml VOA vial. Record weight to the nearest 0.01 gram.

2. Acidify with 10 ml of 1.5M HCl (12.5 mL acid diluted to 100 ml deionized water).

3. Add exactly 10.0 ml methylene chloride/0.1% tropolone (DCM/tropolone)

4. Cap VOA vial and shake. Place VOA vials on tumbler and tumble for 1 hour.

5. Centrifuge extract. Transfer entire DCM layer into a 60 ml disposable test tube.

6. Add another 10.0 ml of DCM/0.1% tropolone and shake. Tumble for 1 hour.

7. Centrifuge extract. Transfer entire DCM layer into the same disposable test tube. Repeat steps 6-7.

8. Evaporate on the N-Evap and solvent exchange to Hexane.

9. Concentrate extract to approximately 1mL

10. Slowly add 2 ml of Grignard to each sample, cap and vortex for 45 minutes.

12. Add conc. HCl one drop at a time to the samples and standards until there is no visible reaction. Be very careful not to add acid too quickly. Then add a few drops at a time, vortexing occasionally. Keep adding acid and vortexing until hexane layer is clear. Evaporate hexane layer to ~1 ml on the N-evap.

13. Clean ups: Using the vacuum or positive pressure manifold, set up florisil columns. Add 1-2cm of 100-200 mesh **deactivated** silica gel to the top of the florisil. Deactivate silica gel by adding 3.3% reagent water to silica gel that has dried overnight at 130°C. Mix contents thoroughly and let sit ovenight to equilibrate. Store in a sealed glass jar.

14. Condition silica/florisil columns with 5 mL of hexane. Do not let the columns go dry. Discard hexane. Quantitatively transfer extracts from test tube into the rinsed columns and bring down to almost dry. Elute samples at rate no faster than 3-4 drops per second with 6 mL of pentane, allowing the columns to go dry at the end.

15. Concentrate extracts on N-Evap and bring to a 1mL final volume in hexane. Put into a blue vial and mark level.

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Additional Prep Information For Organotin Water Extractions (3520)

Service Request #_____**Work Group Number**_____

Pre-Prep Information:

_____

DCM Lot #: _____
HCl Lot #: _____
H_2O Lot #: _____
Pentane Lot #: _____

Na_2SO_4 (G) Lot #: _____
Grignard Lot #: _____
Tropolone Lot #: _____
Hexane Lot #: _____

Acidified? : _____

Continuous (18 hr.) start time/date/initial: _____
Continuous (18 hr.) end time/date/initial: _____

Date Derivatization started (date/initial): _____
Date Derivatization ended (date/initial): _____

Alumina (3610)/Silica Gel (3630) (date/initial): _____

Comments/Observations:_____
_____
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Bench Sheet Review Check List
❑ Hold Times Met (if no, Reason: _____)
❑ Prep date, dept, method, product code correct in stealth
❑ Spike Information correct
❑ Weights/Volumes and units correct on raw and final bench sheets
❑ Sample IDs have been checked—Bottle numbers appended if required
❑ Names present for: Started by, Completed by, relinquished by, and witnessed by.
❑ Training has been circled
❑ Extract Storage recorded
❑ Additional Prep Sheet completely filled out (NA or line out Blanks)
❑ All clean-ups have been noted on additional prep sheet
❑ Signed service request with Form V, if applicable, has been attached

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PROCEDURE FOR EXTRACTION OF ORGANOTINS IN WATER- EPA 3520

1. Rinse all labware employed in sample preparation, with 1% HCl, DI water, acetone, 0.1% tropolone in DCM and DCM.

2. Pour 500 mL DCM into continuous extractor body.

3. Pour at least 500 mL of the following solution into the continuous body: 1% HCl and 100 ppm tropolone in reagent water (0.4 g tropolone and 40 mL concentrated HCl diluted to 4 L in reagent water).

4. Pour NO MORE THAN 500 mL of sample into continuous body. Use reagent water for method blank and lab control sample(s). It may be necessary to add more reagent water into the continuous body to push enough DCM into the flask.

5. Check the pH of the samples with wide-range pH paper, and adjust to a pH of <2 with concentrated HCl if necessary.

6. Add surrogate and matrix spikes to samples.

7. Fit condensors in continuous bodies and extract for 18 to 24 hours. Record start and stop times on bench sheet.

8. Remove the flasks from the continuous bodies, being very careful not to introduce water into the extracts; carefully pipet off any water that does make it into the flask.

9. Pour extracts into KDs/collectors or into 280 mL Holden Collectors. Drop a clean boiling chip into the extract, fit snyder columns and evaporate extracts on an S-Evap at a temperature of no greater than 75°C.

10. Solvent exchange extracts to Hexane.

11. Quantitatively transfer extracts to 16x150 mm disposable culture tubes. Evaporate extracts, **unheated**, under N_2 to about 2 mL in order to ensure no more DCM is present.

12. Derivatization: Slowly add 2 mL of Grignard to each sample, cap and vortex. Vortex for at least 45 minutes.

13. Add concentrated HCl one drop at a time to the derivatized extracts. Be very careful not to add acid too quickly. Then add a few drops at a time, vortexing occasionally. Keep adding acid and vortexing until hexane layer is clear; usually less than 3 mL of concentrated HCl is necessary. The presence of precipitates at this point is normal and does not affect analysis; however if analyst wishes, addition of about 0.5 mL of DI water followed by vortexing is useful for dissolving massive precipitates.

14. Clean ups: Using the vacuum or positive pressure manifold, set up alumina columns. Add 1-2cm of 100-200 mesh **deactivated** silica gel to the top of the alumina. Deactivate silica gel by adding 3.3% reagent water to silica gel that has dried overnight at 130°C. Mix contents thoroughly and let sit ovenight to equilibrate. Store in a sealed glass jar.

16. Condition silica/alumina columns with 5 mL of hexane. Do not let the columns go dry. Discard hexane. Quantitatively transfer extracts from test tube into the rinsed columns and bring down to almost dry. Elute samples at rate no faster than 3-4 drops per second with 6 mL of pentane, allowing the columns to go dry at the end.

16. Concentrate extracts on the N-Evap and bring to a 1mL final volume in hexane. Put into a blue vial and mark level.

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Additional Prep Information for Organotin Soil Extractions

Service Request #:_____ Workgroup #:_____

Prep Information

Date/Time/Initials Weighed:_____ Balance ID:_____ Calibration Verified ☐

Storage Location (if not extracted same day):_____

0.1% Tropolone in DCM Lot #:_____ Na_2SO_4 (P) Lot #:_____
Acid Lot #:_____

Tumbler (16hr.) Start (Time/Date/Initial):_____
Tumbler (16hr.) Stop (Time/Date/Initial):_____

Cleanup Information

S-Evap (Time/Date/Initial):_____ Temp/Thermometer ID:_____
Hexane Lot #:_____

Water Wash (Time/Date/Initial):_____ Reagent Water Lot #:_____

Derivatization Started (Time/Date/Initial):_____ Grignard Lot #:_____
Derivatization Stopped (Time/Date/Initial):_____

DMD Cleanup (Time/Date/Initial):_____ DMD Lot #:_____

Alumina (3610)/Silica Gel (3630) (Time/Date/Initial):_____ Pentane Lot #:_____
Alumina Lot #:_____ Silica Gel Lot #:_____

Vial:_____ Vial Storage:_____

Archived Extract Location:_____

Comments/Observations:_____

_____

Bench sheet Review Check List
☐ Hold Times Met (if no, Reason: _____)
☐ Prep date, dept, method, product code correct in stealth
☐ Spike Information correct
☐ Weights/Volumes and units correct on raw and final bench sheets
☐ Sample IDs have been checked—Bottle numbers appended if required
☐ Names present for: Started by, Completed by, relinquished by, and witnessed by.
☐ Training has been circled
☐ Extract Storage recorded
☐ Additional Prep Sheet completely filled out (NA or line out Blanks)
☐ All clean-ups have been noted on additional prep sheet
☐ Signed service request with Form V, if applicable, has been attached

PROCEDURE FOR THE PREPARATION OF ORGANOTINS IN SOIL AND SEDIMENT

1. Rinse all labware once with 0.1% tropolone in DCM followed by twice with DCM.
2. Mix sample thoroughly and weigh 20 grams into a 250mL beaker. Use unmuffled sulfate for the laboratory control samples and the method blank.
3. If possible, allow the samples to dry overnight in the hood.
4. Acidify with 2 mL concentrated HCl.
5. Mix soil with enough unmuffled sodium sulfate to dry the sample. Sample is sufficiently dry once it has attained a loose, freely-flowing texture.
6. Transfer samples to 250mL Teflon tumblers.
7. Add surrogate and spiking solutions as necessary.
8. Add enough 0.1% tropolone in DCM to cover the sample by ½ inch. Cap tumbler tightly and tumble for at least 16 hours.
9. Pour the entire sample in to a modified funnel and filter into a 250mL KD. Rinse the bottle and cap three times with DCM, transferring rinses to funnel. Rinse funnel and sample three times with DCM under vacuum until there is no color visible in the glass wool.
10. Concentrate the extract on the S-evap and solvent exchange into Hexane.
11. Transfer extract to a disposable test tube and concentrate down to around 1mL.
12. Add between 2 and 5mL of reagent water and vortex for 1 minute.
13. Centrifuge for 5 minutes and transfer the top hexane layer to a new test tube.
14. Concentrate the extract on the N-Evap down to around 1mL.
15. Add 2mL of Grignard to each extract and cap tightly. Vortex for 45 minutes.
16. To deactivate the unreacted Grignard reagent, add concentrated HCl one drop at a time until the excess Grignard is deactivated. At this point, add approximately 1 mL HCL followed by 1mL of reagent water and vortex the mixture vigorously in order to dissolve all the solids (salts) that were generated in the course of the derivatization. Repeat the HCl and water addition until there are no more solids present.
17. Transfer the top hexane layer to a new test tube.
18. Evaporate the extract to approximately 1mL.
19. Bring extract to 4mL intermediate volume in hexane and remove a 1mL aliquot to a new test tube. Archive the remaining 3mL.
20. Add 1 mL dimethyldioxirane (DMD) in acetone to the 1mL aliquot, cap the tube and vortex for at least 3 minutes.
21. Add about 2mL of hexane and concentrate on the N-evap until extract is at approximately 1mL.
22. Set up an alumina column (1 g alumina in a 6 mL PE reservoir topped with a PE frit) in a vacuum or positive pressure manifold. Add 2-3 cm of 100-200 mesh deactivated silica gel to the top of the frit. Condition cleanup column with 5mL of Hexane. Add a small amount of unmuffled granular sodium sulfate to the top of the silica bed.
23. Set up manifold to collect eluents and quantitatively transfer the extract with pentane to the cleanup column. Collect the extract in a small, disposable test tube, allowing it to elute through the column under force of gravity. If necessary, apply vacuum (or positive pressure) to speed elution, but no faster than approximately 3 drops per second.
24. Once all of the extract has drained from the reservoir, add 3 mL of pentane and allow it to elute completely with the extract. Again add 3 mL pentane and allow it to elute completely.
25. Once all of the rinse has drained from the reservoir vacuum (or blow) the column to dryness.
26. Concentrate the extract (unheated) and bring to a 1 mL final volume using Hexane. Place in autosampler vial and cap.

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COLUMBIA ANALYTICAL SERVICES, INC.

Service Request No.: _____ Date Extracted: _____

Analyst: _____ *HOLD TIME FOR ORGANOTIN EXTRACTION:* _____

Interstitial / Pore Water Extraction

Lab ID	Client ID	Sample Description	1st/2nd spin # tubes	Start Time 1st Spin	End Time 1st Spin	Start Time 2nd Spin	End Time 2nd Spin	Approx. Volume Extracted

Comments: _____

_____

_____

Spike ID: _____

Extracts Filtered? (Circle one) : YES / NO *EXTRACT STORAGE:* _____

Witnessed By:	Date:
Reviewed By:	Date:

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PROCEDURE FOR EXTRACTION OF INTERSTITIAL / PORE WATER FROM SEDIMENT

1. LABWARE RINSING: **Polycarbonate glassware must not be allowed to come into contact with acetone or methylene chloride!** All labware used for extraction must be rinsed with dilute HCl followed by DI water. All labware must be dried prior to further rinsing (air drying works best for polycarbonate labware; methanol may also be used if time is limited. Acetone may be used for all non-polycarbonate labware). All polycarbonate labware must then be rinsed with 0.1% w/v tropolone in hexane, followed by hexane; all other labware must be rinsed with 0.1% w/v tropolone in hexane or methylene chloride, followed by hexane or methylene chloride. Inspect all centrifuge bottles to be employed; if any appear to be in danger of breakage, they must be discarded.

2. All transferring of samples must be carried out in an isolated environment under at least 2 atmospheres positive N_2 pressure. Sample centrifugation must be carried out at or below 10° Celsius.

3. Note and record the nature of the samples.

4. Transfer sediment with all standing water to centrifuge bottles.

5. If the client requested a method blank, use 1000 mL reagent water and polycarbonate labware previously employed in an interstitial water from sediment extraction. If the client requested matrix spikes, use 1000 mL reagent water spiked with analytes at the same concentration as in the routine organotins in water extraction; do not add surrogates.

6. Balance bottles to be spun opposite each other to within 1 gram and centrifuge for 30 minutes at 1000 - 3000 G (1100 - 3250 RPM for a 10 inch rotor radius).

7. Very sandy sediments often do not yield sufficient volumes of water. For these samples, it will be necessary to employ at least two water-from-sand recovery apparatuses; make a note of this on the benchsheet as these samples will be exposed to the atmosphere during their interstitial water extraction.

8. Decant water into (a) clean polycarbonate centrifuge bottle(s) and balance as above. It is expected that some sediment will be transferred in this step. If necessary, balance the bottles with centrifuge bottles filled with water.

9. Centrifuge a second time at 3000 G.

10. If the client requested that the samples be filtered, use 0.4 µm polycarbonate filters, polycarbonate Erlenmeyer flasks and porcelain Büchner funnels.

11. Very carefully decant water from the centrifuge bottles (if the water was not filtered) or Erlenmeyer flasks (if the water was was filtered) into polycarbonate sample bottles. If sediment is resuspended during decantation, the samples must be recentrifuged before any more water is decanted.

12. Add 0.5-1 mL concentrated HCl to each sample bottle to preserve (i.e., prevent growth of algae or other life) and store at or below 4° Celsius.

13. Record any difficulties, oddities or deviations from procedure.

Approved by / Date
15-8-00
Extraction Manager
5-9-00
Inorganic Department Manager
Quality Control