Appendix 1

Standard Operating Procedure

for the USGS Reston, Virginia Environmental Organic Geochemistry Laboratory

Procedures for Preparation of Clean Reagents and Labware: Trace Organics

Note: A version of this Standard Operating Procedure was accepted by the National Oceanic and Atmospheric Administration (NOAA) in August 1993 as part of the Analytical Chemistry Quality Assurance Plan for the Southern California Natural Resource Damage Assessment.

Contents

Procedures for Preparation of Clean Reagents and Labware: Trace Organics

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1. **Application:** Following are procedures for the preparation of clean reagents and labware to be used in the processing of sediment samples for trace organic analysis. Because this is not a method of analysis, subsections normally found here relating to ***Tested Concentration range***, ***Sensitivity***, ***Detection limit***, ***Interferences*** and ***Processing rate*** have been omitted. For more information on these subjects, see Appendix 4.

2. **Chemistry:** No chemical reactions are involved in these procedures.

3. **Apparatus:**

a. ***Instrumentation.*** The only instrumentation required for these procedures are the GC/ECD (gas chromatography/electron capture detector) and GC/MS (gas chromatography/mass spectrometer), which are used to evaluate the purity of solvents (hexane, dichloromethane, and methanol) and pre-extracted water. Information on these instruments and their operating conditions can be found in Appendices 7 and 5, respectively.

b. ***Hardware/glassware***. Following is a list of laboratory equipment and supplies used in this procedure.

Miscellaneous pieces of equipment used in these procedures include the following:

-Bransonic Model 3200 ultrasonic cleaner
-Thermolyne Model F30428C muffle furnace
-Baxter Scientific Model DX-41 drying oven
-Büchi Model R200 rotary evaporator with V-800 vacuum controller
-Custom made stainless steel four-position nitrogen gas blowdown system

Glassware and implements used in these procedures include the following:

-microsyringes: 10, 25, 50, 100, 250, 500 and 1000 μL
-separatory funnels: 250, 500 mL, 2000 mL
-graduated cylinders: 10, 25, 50, 100, 250 and 1000 mL
-chromatography columns (1.1 cm x 25 cm; Kontes)
-all glass B-D syringes: 5 mL
-8-oz. jars
-vacuum dessicator
-blunt tip 18-gauge, 6" Popper stainless steel pipetting needles
-reagent bottles: 250, 500 mL
-glass funnels
-stainless steel spatula
-aluminum foil
-250-mL flat bottom flasks
-50-mL pear-shaped flasks

-cork rings
-½-dram borosilicate vials with Teflon™-lined caps (National Scientific, #B7800-1)
-Target™ amber DP vials (National Scientific, #C4000-2W)
-Target™ 250-µL conical glass inserts (National Scientific, #C4010-629L)

c. ***Chemicals***. Chemicals used in these procedures include the following:

-glass-distilled dichloromethane (Burdick & Jackson, High Purity grade)
-glass-distilled hexane (Burdick & Jackson, High Purity grade)
-glass-distilled methanol (Burdick & Jackson, High Purity grade)
-hydrochloric acid (VWR, Reagent grade)
-anhydrous sodium sulfate (Mallinckrodt AR)
-fine copper granules (Mallinckrodt AR)
-deionized water (Milli-Q)
-silica gel (EM Science, #7752-3)
-alumina (Fisher, #A941, 80-200 mesh)
-nitrogen gas (Valley National Gases, grade 5.0)

4. Standards:

a. ***Calibration standards.*** See Appendices 4,5, and 7.

b. ***Surrogates (recovery).*** See Appendices 4,5, and 7.

c. ***Internal (quantitation) standards.*** See Appendices 4,5, and 7.

5. Procedures:

a. ***Preparation of clean adsorbents***. Details of these procedures are given in Appendix 2.

b. ***Preparation of clean (reagent) water***. The external surfaces of two empty amber Burdick & Jackson (B&J) solvent bottles are cleaned by completely removing the label with water (and scraping) followed by solvent. The caps are cleaned by adding a small amount of dichloromethane to the bottle, replacing the caps, and shaking vigorously. The solvent is discarded, and the caps are placed seal-end down on solvent-rinsed aluminum foil. The bottles are then placed in the muffle furnace and heated at 450 °C overnight. After cooling, the bottles are sealed with the clean Teflon™-lined cap.

Water is collected in a B&J bottle (cleaned as described above) from the Milli-Q water system in the "common use" lab at the USGS/WRD (Reston, VA). Five 400-mL aliquots of this water are transferred to clean 500-mL separatory funnels. To each separatory funnel is added 40 mL of dichloromethane. Each funnel is shaken vigorously for 1 minute, venting often, and the phases are allowed to separate. The dichloromethane layer is drained and discarded. This procedure (extraction) is repeated two more times after which the water is drained into a clean B&J bottle. Extractions continue until the bottle is filled, at which time the bottle is labeled according to date of extraction, batch, and bottle number.

c. ***Preparation of activated copper***. Fine granular copper is obtained commercially and can be used directly from the bottle. It must, however, first be "activated." This means removing the surface layer of copper oxide so that a fresh layer of elemental copper is exposed for reaction with elemental sulfur in sediment extracts.

Using a small funnel, copper granules are added to a chromatography column (1.1 cm x 25 cm) until a layer approximately 25 cm deep has been formed. The copper is washed (into a waste beaker) with three rinses of 20 mL 6N HCl (pre-extracted; *cf*. below) using a graduated cylinder. Before the last rinse has been applied, a reagent bottle with glass-distilled methanol and a clean B-D syringe should be prepared. As the last of the hydrochloric acid reaches the surface of the copper, 20 mL of methanol is added. The inside walls of the column are thoroughly rinsed with the methanol so that no traces of acid solution are left on them. This is followed by two additional washes with 20 mL of methanol. Each wash is added to the column before the meniscus reaches the top of the copper. Finally, the same procedure is performed with dichloromethane (DCM) using three successive washes, 20 mL each time. When the DCM has stopped dripping, the copper granules are immediately added to a 250-mL amber reagent bottle half-filled with dichloromethane previously dried over anhydrous sodium sulfate (~125 mL DCM). If necessary, additional dry dichloromethane is added so that it covers the copper. The bottle is labeled with the analyst's initials and dated.

d. ***Preparation of clean anhydrous Na_2SO_4***. Sodium sulfate is combusted at 450 $^{\circ}$C in a Pyrex™ beaker for $\geq$ 4 hours. While still warm, the sodium sulfate is transferred (by pouring) into a pre-kilned glass jar and sealed with a precleaned cap with a Teflon™ liner. The jar is stored in a vacuum dessicator dedicated for that purpose.

e. ***Preparation of clean 6N HCl***. Fresh Milli-Q water is placed in a precleaned B&J solvent bottle dedicated for this purpose. Using a 1000-mL graduated cylinder, 500 mL of Milli-Q water is measured and transferred to a clean 2-liter separatory funnel equipped with a Teflon™ stopcock (using a glass funnel). Five hundred mL of concentrated HCl is then measured in the same 1000-mL graduated cylinder and gently transferred to the separatory funnel. The funnel is removed and a glass stopper is placed in the separatory funnel. The water and acid are mixed by gently rolling the separatory funnel and inverting it, venting as needed. Then 100 mL of glass-distilled DCM is measured in a 250-mL graduated cylinder and added to the separatory funnel. The mixture is agitated vigorously for 1 minute, with frequent venting, after which the phases are allowed to separate. The lower DCM layer is then drained into a beaker up to the DCM-water interface. The extraction is repeated twice more with successive additions of 100 mL DCM and draining of the DCM layer into the beaker. On the last extraction, the separatory funnel is drained just beyond the DCM-water interface to ensure that no excess DCM is present in the 6N HCl remaining in the separatory funnel. The solvent-extracted 6N HCl is now drained into a precleaned B&J bottle that is sealed with a Teflon™-lined cap. The label on the bottle is marked with the analyst's name and the date of preparation. This bottle is kept in an acids cabinet

beneath the hood until it is needed. The DCM is poured into a waste solvent bottle maintained in the hood.

f. ***Cleaning of labware***. All (Pyrex™) glassware (not including syringes) is kilned @ 450 °C overnight prior to use. Afterwards, it is stored with aluminum foil covering any openings and placed into an appropriate drawer or cabinet. Just prior to use, all glassware is rinsed (using a Teflon™ wash bottle or B-D syringe) with previously tested dichloromethane so that all internal and external surfaces that could conceivably come into contact with the sample are wetted and thereby cleaned. Relatively clean Teflon™ and metal objects must be sonicated for 30 minutes in 1:1 dichloromethane/methanol. This does not include the microsyringes, which require special handling (see below). A beaker or other glass vessel is used for this purpose. The vessels are covered with aluminum foil to reduce the volatilization of solvent during sonication. The solvent is decanted, and the objects are placed into a 135 °C drying oven in the same beaker used for sonication or another glass vessel for > 1 hour. No plastic or wood or other organic material is ever placed into the oven. Generally, it is sufficient to air dry Teflon™ implements. If the implements are very dirty, they are cleaned first with detergent (Alcojet™) and water, rinsed thoroughly with water and methanol, and then sonicated as described above.

B-D syringes generally don't require soap and water washing, but must be cleaned by sonication. All items cleaned in this manner are handled (when transferred) with clean metal forceps and stored in aluminum foil to help keep them clean. Small implements such as Pasteur pipettes, vials, ampoules, needles, and syringes are stored in glass containers that are either covered with heavy duty aluminum foil or preferably glass closures.

The microsyringes (both fixed and removable-needle types) are generally cleaned by rinsing thoroughly with DCM. This involves disassembly and squirting DCM on the needle, the plunger, and into the barrel with reinsertion of the plunger and expulsion of the solvent. This should be done at least 3 times, more if the syringe was dirty or the material handled with the syringe was present at high concentrations (*e.g*. TEO-total extractable organics). If the syringe is unusually dirty, it should be disassembled and placed needle up in a container. Dichloromethane is added to the container until it just covers the syringe parts, and the parts are sonicated for 20 minutes. It is important that the solvent does not go above the fixed needle cement. The solvent is then decanted and the parts can be transferred to a clean piece of aluminum foil for air drying.

Cellulose Soxhlet thimbles (33 mm x 118 mm) are placed in previously cleaned, tall 32 oz. glass jars. Enough of a 1:1 mixture of dichloromethane and methanol is added to each jar so that it will cover the thimble. The mixture is sonicated for 30 minutes, after which the solvent is drained. The procedure is repeated 2 times, after which the thimbles are allowed to dry at room temperature with a piece of perforated aluminum foil over the jar. When the thimbles are dry, a clean Teflon™-lined cap is placed on the jar.

g. ***Evaluation of solvent purity***. In the case of hexane or dichloromethane, a 250-mL flat bottom flask, previously kilned, is rinsed with the solvent to be tested by adding about 10-20 mL and swirling so that all inner surfaces are washed. The wash is discarded. One hundred mL of the solvent to be tested is added to the 250-mL flat bottom flask which is then spiked with 50 µL of PCB-RS-05-1/1000 (chlorinated hydrocarbon surrogate solution; *cf*. Appendix 4) and 50 µL of LAB-RS-01-1/10 (long-chain alkylbenzene surrogate solution; *cf*. Appendix 4). A similarly pre-combusted and solvent-rinsed glass stopper is placed into the flask. The rotary evaporator is cleaned prior to use by rinsing the bump trap with dichloromethane (using a Teflon™ squeeze bottle) and replacing the receiving flask. A final rinse of the bump trap's ground glass joint (male end) is performed with the solvent to be tested using a clean B-D syringe. The flat bottom flask is attached to the rotary evaporator, and the solvent is concentrated at specified conditions (hexane: temp = 40 °C, pressure = 250 torr [± 5%]; dichloromethane: temp = 30 °C, pressure = 475 torr [± 5%]) until only about 1 mL remains. The vacuum is broken, and the flask is removed. The flask is sealed with the ground glass stopper (which should have been placed on a piece of solvent-rinsed aluminum foil in the interim). The remaining solvent in the flask is swirled to bring about contact with all of the inner surfaces. The stopper should not be contacted. The solvent is then transferred using a clean 5-mL B-D syringe to a clean ½-dram vial. A separate, clean, glass 5-mL B-D syringe is used to wash the flask with three successive 0.5-1.0 mL rinses of the solvent being tested (this rinse solvent should be in a clean reagent bottle) with transfers in between. The solvent in the vial is gently evaporated to dryness under a stream of dry nitrogen gas and immediately taken up in 100 µL of PCB-IS-02-1/200 (chlorinated hydrocarbon internal standard solution; *cf*. Appendix 7). The vial containing the sample is gently rotated to ensure dissolution of any residues left on the inner walls. It is labeled with a reference number (indicating the analyst's notebook number and page), the analyst's initials, and the date. It can then be placed in the freezer. This solution can be analyzed by manual injection on the GC/ECD. Alternatively, the solvent in the ½-dram vial (before addition of the internal standard solution) can be blown to a low volume (about 100 µL) and transferred to an autosampler (A/S) microvial using a microsyringe. The transfer is completed with three successive rinses of the ½-dram vial using 200 µL of dichloromethane with evaporation of the solvent in the A/S microvial under nitrogen gas in between. Once transfer to the A/S microvial is complete, solvent in the A/S microvial is gently evaporated to dryness under a stream of dry nitrogen gas and immediately taken up in 100 µL of PCB-IS-02-1/200 (chlorinated hydrocarbon internal standard solution; *cf*. Appendix 7). After the chlorinated hydrocarbon (*i.e.* DDTs, PCBs) analyses are complete, the sample is evaporated to dryness and immediately taken up in 100 µL of an equal mixture of LAB-IS-03-1/10 (long-chain alkylbenzene internal standard solution; *cf*. Appendix 5) and hexane and is analyzed on the GC/MS. Instructions for the analysis, data archiving, and criteria for acceptability are given in Appendices 5 and 7.

The same procedure is used for evaluating <u>methanol</u> with the exception that the 100-mL test sample is evaporated to dryness while on the rotovap (temp = 40 °C,

pressure = 220 torr [± 5%]) and immediately transferred to the vial with dichloromethane prior to final evaporation (under nitrogen) and addition of the internal standard solution. Instrumental analysis of the concentrated solvents is performed with a single-point PCB calibration standard (PCB-CS-02-1/1; DDT-CS-01-1/1; *cf*., Appendix 7) using the GC/ECD and a single-point long-chain alkylbenzene calibration standard (LAB-CS-05-1/5; *cf*., Appendix 5) using the GC/MS.

h. ***Evaluation of water purity***. A 125-mL separatory funnel, previously kilned, is rinsed with dichloromethane by adding about 10-20 mL and swirling so that all internal surfaces are washed. The wash is discarded. One hundred mL of the water to be tested is added to the 125-mL separatory funnel that is then spiked with 50 µL of PCB-RS-05-1/1000 and 50 µL of LAB-RS-01-1/10 (surrogate solutions; *cf*. Appendix 4). A similarly pre-combusted and solvent-rinsed glass stopper is placed into the funnel. Ten mL of dichloromethane is added to the funnel (using a B-D syringe or clean graduated cylinder). The funnel is shaken vigorously for 1 minute, venting often, and the phases are allowed to separate. The dichloromethane phase is then drained into a clean 50-mL pear-shaped flask that is immediately sealed with a ground glass stopper. The extraction procedure is repeated twice more with the extracts being combined in the 50-mL flask. After three extractions are completed, the water can be discarded.

The rotary evaporator is cleaned by rinsing the bump trap with dichloromethane (using a Teflon™ squeeze bottle) and replacing the receiving flask. A final rinse of the bump trap's ground glass joint (male end) is performed with dichloromethane using a clean B-D syringe. The 50-mL flask is attached to the rotary evaporator, and the solvent is concentrated until only about 1.0 mL remains (temp = 30 °C, pressure = 475 torr [± 5%]). The vacuum is broken, and the flask is removed. The flask is sealed with the stopper (which should have been placed on a piece of clean aluminum foil in the interim). Solvent remaining in the flask is swirled to facilitate contact with all of the internal surfaces with which the original solvent aliquot had come in contact. The solvent is then transferred using a clean 5-mL B-D syringe to a clean ½-dram vial. A separate clean glass 5-mL B-D syringe is used to wash the flask with three successive 0.5-1.0 mL rinses of the solvent being tested (this could be in a clean flask or reagent bottle) with transfers in between. The solvent in the vial is gently evaporated to dryness under a stream of dry nitrogen gas and immediately taken up in 100 µL of PCB-IS-02-1/200 (chlorinated hydrocarbon internal standard solution; *cf*. Appendix 7). The vial containing the sample is gently rotated to ensure dissolution of any residues left on the inner walls. The vial is labeled with a reference number (indicating the analyst's notebook number and page), the analyst's initials, and the date. It can then be placed in the freezer. This solution is analyzed either by manual injection (GC/ECD) or using the A/S microvials (see description above for solvent testing) using a single-point calibration (PCB-CS-02-1/1; DDT-CS-01-1/1; *cf*. Appendix 7). After this analysis is complete, the sample is gently evaporated to dryness and immediately taken up in 100 µL of an equal mixture of LAB-IS-03-1/10 (long-chain alkylbenzene internal standard solution; *cf*. Appendix 5) and hexane and

analyzed using the GC/MS. Instructions for the analysis, data archiving, and criteria for acceptability are given in Appendices 5 and 7.

6. **Calculations:** All calculations for the DDT and PCB determinations for solvents and pre-extracted water are carried out automatically by the PerkinElmer data system using the TotalChrom Workstation v. 6.2.1 software. The internal standard method of quantitation is used whereby all analyte concentrations (including those of the surrogates) are determined by comparing responses of the analytes in the sample with those of the analytes and internal standards in the calibration standard along with the response of the internal standard in the sample. In this case, a single-point calibration using a linear calibration curve with forcing through the origin is used. Because the concentrations of the components in the calibration standard and the internal standard in the sample are known, the equation(s) developed from the single-point calibration curves can be applied to the sample to obtain concentrations not corrected for recovery (equation 1). Recovery is computed as the percent of the amount of each added surrogate that is measured in the sample (equation 2). PCB congeners 30, 121, and 198 are used as surrogates to monitor recovery for the PCBs and DDTs.

Computation of alkylbenzene concentrations in the solvents and pre-extracted water is carried out by the Agilent ChemStation data system. Previous multipoint calibration exercises with the solutions described here using the GC/MS have been linear over the entire concentration range (2-275 ng/μL). Consequently, a linear model with forcing through the origin is used. Under these circumstances, equation 1 is employed for computation of alkylbenzene concentrations.

The equation used for computing concentrations of the analytes using a linear model with forcing through the origin is given below.

$$[C_i]_{solv} = \left[\frac{A_i \times C_{IS}}{A_{IS}}\right]_{solv} \times RRF_{CS}^{-1} \times \frac{V_{final}}{V_{solv}} \qquad (1)$$

where: $[C_i]_{solv}$ = concentration of analyte *i* in the solvent (pg/mL),
A_i = area of analyte peak *i* in the final solution,
C_{IS} = concentration of the internal standard in the final solution (pg/μL),
A_{IS} = area of the internal standard peak in the final solution,
RRF_{CS}^{-1} = relative response factor of the calibration standard run,
V_{final} = total volume of the final solution (μL),
V_{volv} = volume of solvent tested (mL).

The equation for estimating recovery is given below.

$$R_i = \left[\frac{[C_{RSi}]_{solv} \times V_{final}}{M_{RSi}}\right] \times 100 \qquad (2)$$

where: R_i = recovery of surrogate *i* in percent,

$[C_{RSi}]_{solv}$ = measured concentration of surrogate *i* in the final solution (pg/µL),

M_{RSi} = mass of surrogate *i* added to the sample (pg).

7. QA/QC Considerations: Information on the QA/QC considerations applicable to the procedures described here can be found in Appendices 4, 5, and 7.

8. Health, Safety, and Waste-Disposal Information:

a. ***Personal protection.*** Safety glasses and protective gloves are recommended whenever reagents or samples are handled. For other precautions and safety procedures, consult the Material Safety Data Sheets (MSDS) for each chemical used. They are on file in the laboratory; *http://www.ilpi.com/msds/#Manufacturers* provides links to MSDSs of most chemical companies.

b. ***Electrical hazards.*** Electrical systems must conform to the National Electric Code, the National Fire Protection Association Code (NFPA 70-1971), and the American National Standards Institute (ANSI) Code (C1-1971). Consult the U.S. Geological Survey's Safety and Environmental Health Handbook (U.S. Geological Survey, 2002). Shock hazards exist inside the instruments. Only an authorized service representative or an individual with training in electronic repair should remove panels or circuit boards where voltages are greater than 20 V. The instruments require a third-wire protective grounding conductor. Three-to-two wire adapters are unsafe for these instruments.

c. ***Chemical hazards.*** Hydrochloric acid is a strong acid used to remove the oxide layer of the copper granules (activation). Gloves should be worn when handling strong acids. If contact occurs, the affected area should be rinsed thoroughly with water. Hexane, dichloromethane, and methanol are solvents used in cleaning glassware and the preparation of clean adsorbents and reagents. They also are evaluated for purity. Gloves should be worn when handling organic solvents and, whenever possible, manipulations should be conducted in a fume hood. Waste solvents accumulated during rotary evaporation or other cleaning operations should be stored in a capped glass bottle (satellite accumulation point) and arrangements made for its disposal through the USGS Materials Management Office.

d. ***Gas cylinder handling.*** Compressed gas cylinders must be handled and stored according to the Safety and Environmental Health Handbook (U.S. Geological survey, 2002). Each cylinder must be 1) carefully inspected when received, 2) securely fastened at all times with an approved chain assembly or belt, 3) capped at all times when not in use, 4) capped when transported, 5) transported only by a properly designed vehicle (hand truck), and 6) stored separately with other full, empty, flammable, or oxidizing tanks of gas, as appropriate.

e. ***Sharps.*** Microsyringes with fixed or removable needles should be handled with care to avoid accidental skin punctures.

9. References: Following are some additional sources of information about the procedures that have been described here.

Eganhouse, R.P., R.W. Gossett and G.P. Hershelman. 1990. Congener-specific Characterization and Source Identification of PCB input to Los Angeles harbor. *Report to the Los Angeles Regional Water Quality Control Board*, September 20, 1990, 34pp.

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Eganhouse, R.P., E.C. Ruth and I.R. Kaplan. 1983b. Determination of long-chain alkylbenzenes in environmental samples by argentation thin-layer chromatography/high-resolution gas chromatography and gas chromatography/mass spectrometry. *Analytical Chemistry*, 55:2120-2126.

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Hendricks, T.J. and R.P. Eganhouse. 1992. Modification and Verification of Sediment Deposition Models. *Final Report to the California Water Resources Control Board*, September, 1992, 330pp.

U.S. Geological Survey. 2002. USGS Handbook 445-3-H, Safety and Environmental Health Handbook, 435p.