

The Children's Total Exposure to Persistent Pesticides and Other Persistent Organic Pollutants (CTEPP) Study

Extracting and Preparing Liquid Food Samples for Analysis of Persistent Organic Pollutants

Battelle
Columbus, OH 43201
Contract No. 68-D-99-011

Standard Operating Procedure

CTEPP-SOP-5.19

Title: Extracting and Preparing Liquid Food Samples for Analysis of Persistent Organic Pollutants

Source: Battelle

U.S. Environmental Protection Agency
Office of Research and Development
Human Exposure & Atmospheric Sciences Division
Exposure Measurements & Analysis Branch

Notice: *The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development (ORD), partially funded and collaborated in the research described here. This protocol is part of the Quality Systems Implementation Plan (QSIP) that was reviewed by the EPA and approved for use in this demonstration/scoping study. Mention of trade names or commercial products does not constitute endorsement or recommendation by EPA for use.*

STANDARD OPERATING PROCEDURE (SOP)
FOR EXTRACTING AND PREPARING LIQUID FOOD SAMPLES
FOR ANALYSIS OF PERSISTENT ORGANIC POLLUTANTS

Prepared by: _____ Date: _____

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1.0 Scope and Applicability

This standard operating procedure (SOP) describes the method for extracting liquid food samples for persistent organic pollutants (POP).

2.0 Summary of Method

This method describes the procedures for extracting, and concentrating liquid food samples for analysis of target POP including semi-volatile organic compounds (SVOC) and non-volatile organic compounds (NVOC) such as organochlorine (OC), organophosphate (OP) pesticides, polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), substituted phenols (PH), and phthalates.

3.0 Definitions

- 3.1 Persistent Organic Pollutants: SVOC and NVOC which include the compound classes: OC and OP pesticides, PAH, PCB, substituted phenols, and phthalates.

4.0 Cautions

- 4.1 Appropriate laboratory safety equipment such as lab coats, safety glasses, and protective gloves should be worn when performing these procedures.

5.0 Responsibilities

- 5.1 The project staff who performs the sample extractions will be responsible for obtaining samples from the sample coordinator, entering relevant information in the extraction/preparation laboratory record books, and sending final extracts for analyses.
- 5.2 The CTEPP Laboratory Team Leader (LTL), the QA Officer or designee, and Task Order Leader (TOL) will oversee the sample extraction operation and ensure that SOPs are followed by all project staff.

6.0 Materials and Reagents

6.1 Materials

- 6.1.1 Disposable nitrile or latex gloves
- 6.1.2 Round bottom flasks
- 6.1.3 Reflexing condenser
- 6.1.4 Muffled spatulas

- 6.1.5 Muffled quartz fiber filters
- 6.1.6 Precision syringes
- 6.1.7 Muffled funnels
- 6.1.8 25 ml Kuderna-Danish tubes, 125 or 250 ml reservoir flasks, large and small Snyder condensers (muffled)
- 6.1.9 Muffled Hengar boiling chips
- 6.1.10 Separatory funnels
- 6.1.11 Eppendorf pipettor 10-100 ul
- 6.1.12 Graduated cylinder
- 6.1.13 Muffled Pasteur pipettes
- 6.1.14 Muffled 160 ml GPC collection vials
- 6.1.15 Muffled GC/MS autosampler vials
- 6.1.16 Florisil solid phase extraction (SPE) columns
- 6.1.17 Clean vials
- 6.1.18 Muffled glass wool
- 6.2 Reagents
 - 6.2.1 Dichloromethane (DCM); distilled-in-glass
 - 6.2.2 Granular sodium sulfate (analytical grade)
 - 6.2.3 Hexane (distilled-in-glass)
 - 6.2.4 Methanol (distilled-in-glass)
 - 6.2.5 Ethyl ether (EE) distilled-in-glass
 - 6.2.6 Surrogate Recovery Standard Spiking Solution
 - 6.2.7 Internal Standard Spiking Solution

6.2.8 Matrix Spike Standard Solution

6.2.9 GPC calibration solution

6.2.10 Compressed helium

6.3 Equipment

6.3.1 Heated water bath

6.3.2 Vortex mixer

6.3.3 Balances

6.3.4 Traceable timer/stopwatches

6.3.5 O.I. Analytical Autoprep 2000 GPC

6.3.6 Heating oven (Blue M or equivalent)

6.3.7 Freezers

6.3.8 Refrigerators

7.0 Procedure

7.1 Extraction for Liquid Food Samples.

7.1.1 Remove a set of liquid food samples from the freezer and place in the refrigerator to thaw for 1 to 3 days. Remove the food samples to the food preparation laboratory. Record the date/time of removal from the freezer in the laboratory record book (LRB).

7.1.2 Measure 30 mL of the liquid food sample using a clean graduated cylinder and transfer the food sample to a round bottom flask. Spike 50 μ L of the surrogate recovery spiking solution onto the mixture using a syringe or a calibrated Eppendorf pipettor; add 60 mL of dichloromethane (DCM) and few boiling chips to the flask. For matrix spike sample, spike 50 μ L of the matrix spiking solution onto the mixture before refluxing. Note that the spike level may vary from samples. The spike amount will be recorded in the LRB.

7.1.3 Reflux the food mixture for about one and half hours. Record the refluxing time in the laboratory record book.

7.1.4 Filter the food mixture to a clean separatory funnel and collect the DCM extract in a clean vial. Extract the aqueous layer with 2 x 20 mL of DCM and collect the DCM extract in the same vial.

- 7.1.6 Add up to ~three grams of sodium sulfate to the sample extracts and store the sample extracts in a freezer up to 5 days. Filter the sample extract to a KD tube equipped with a reservoir through a muffled quartz fiber filter (or muffled glass wool) containing anhydrous sodium sulfate. Rinse down the sides of the vial with a trace amount of DCM and collect the rinse in the same KD tube.
- 7.1.7 Concentrate the filtered sample extract to ~ 8 mL using KD evaporation. Rinse down the sides of the reservoir and further KD concentrate to ~5 mL. Rinse the Snyder column with trace amount of DCM and collect the rinse in the KD tube.
- 7.1.8 Wet the 0.45 micron acrodisc disposable PTFE filter with ~0.5 mL of DCM and filter the concentrated DCM extract into a muffled disposable culture tube. Rinse the KD tube with ~0.5 mL of DCM; vortex and filter the rinse to the same culture tube. Repeat the rinsing of the KD tube with ~0.5 mL of DCM. Add a final rinse ~0.5 mL of DCM to the filter and collect the rinse in the same vial.
- 7.1.9 Add 7 mL of DCM to a clean culture tube and mark the tube at the height of the solvent. Then mark all culture tubes that contain the samples to the same height as the clean solvent tube. Add more DCM to the filtered sample extract to the mark.

7.2 Gel Permeation Chromatography (GPC) for Liquid Food Sample Extracts.

- 7.2.1 Calibrate the GPC with the GPC calibration solution using the following conditions and determine the dump and collect times:

Flow Rate: 5 mL/minute
Mobile Phase: DCM.

- 7.2.2 Inject the samples and collect the necessary fraction in GPC collection vials. Typical dump and collection times are as follows:

Dump Time: 0 minutes to valley between corn oil and phthalate in the standard

Collect Time: from the end of the dump time to valley between phthalate and methoxychlor (F1)

Dump Time: 0.5 minutes from the end of F1 collection time

Collect Time: from the end of the 2nd dump time to ~60 minutes (F2)

Wash Time: ~15 minutes

- 7.2.3 Store the samples extracts in the freezer overnight, if necessary. Store the F1 fractions in a freezer for further cleanup and process only the F2 fraction. Concentrate the F2 using KD evaporation using a 250 mL reservoir. The sample extract is concentrated to ~0.5 mL. Spike the concentrated sample extract with 10 µL of Internal Standard spiking solution. Mix well and transfer the extract to a GC vial. Store the sample in a freezer until GC/MS analysis. Note that Florisil Solid Phase Extraction (SPE) column clean up

procedures described in Section 7.3 may be needed and will be recorded in the LRB. Further cleanup the F1 fraction using ENVI-Carb column is described in Section 7.4.

7.3 Florisil SPE Cleanup.

- 7.3.1 Condition 1 gram Florisil SPE columns with 6 mL 15% ethyl ether (EE) in hexane, then 12 mL hexane; discard conditioning solvents. Solvent exchange the sample extract into hexane by adding ~2 mL of hexane and KD concentrate to ~1mL.
- 7.3.2 Apply the sample to the conditioned column and collect the eluant. Rinse the sample tube with ~1 mL of hexane; add the rinses to the column. Allow the liquid to drain to just above the column packing and collect the eluant into a clean vial. Elute the column with 3*6 (18) mL aliquots of 15% EE in hexane, collecting the eluant in the same tube used above. Elute the column with 2*6 (12) mL aliquots of 100% DCM, collecting the eluant in the same tube used above.
- 7.3.3 Concentrate the collected eluant to ~0.5 mL using KD evaporation. Transfer the sample to the GC vial. Store the samples in a freezer until analyzed by GC/MS.

7.4 ENVI-Carb Column Cleanup.

- 7.4.1 Condition the ENVI-Carb columns with 12 mL of acetonitrile (ACN), then 12 mL of 10% methanol in ACN, 12 mL of 20% DCM in ACN and 12 mL of ACN. Solvent exchange the sample extract into ACN by adding ~2 mL of ACN and concentrate to ~1mL.
- 7.4.2 Solvent exchange the F1 fraction from DCM to 1 mL of ACN and apply the ACN sample extract to the conditioned column and collect the eluant. Rinse the sample tube with ~1 mL of ACN; add the rinses to the column. Allow the liquid to drain to just above the column packing and collect the eluant into a clean vial. Elute the column with 48 mL of ACN, collecting the eluant in the same tube used above.
- 7.4.3 Concentrate the collected eluant to ~0.5 mL using KD or TurboVap evaporation. If the sample is to be analyzed by GC/MS, add 10 µL of the internal standard solution; vortex for about 5 seconds; and transfer the sample to a GC vial. Store the samples in a freezer until analyzed by GC/MS.

8.0 Records

- 8.1 The field samples will be identified in the LRB by field sample ID. The QC samples generated in the laboratory will be assigned a laboratory analysis number (a unique number that combines the 5 digit laboratory book number-2 digit page number-2 digit line number).
- 8.2 The date of extraction, the lot numbers of solvents, surrogate recovery values, matrix spike standard values, and internal standard values will be recorded in the LRB. The

extraction activities of samples will be also be recorded in the LRB that is kept in the extraction laboratory. This LRB will contain the field sample ID, the assigned laboratory analysis number (see above), the date of extraction, and the lot number of solvent used for extraction. The LRB will be retained in the laboratory where these operations are performed until the conclusion of the study and will be archived in a secure room for three years after completion of the study.

9.0 Quality Control and Quality Assurance

- 9.1 Four types of QC samples will be processed together with the field samples. The QC samples are laboratory method blank, field blank, duplicate sample aliquot, and matrix spiked sample aliquot. The laboratory method blank is to verify that minimal contamination occurs through sample preparation in the laboratory. The field blank is to verify that minimal contamination occurs through field handling, shipping, and preparation processes. The duplicate and matrix spiked samples are used for assessing the overall method precision and the accuracy, respectively.
- 9.2 Surrogate recovery values of 50-150% in blanks and actual samples will be deemed acceptable, and no correction to the data will be made. For recoveries less than 50% and or greater than 150%, the data will be flagged.
- 9.3 If significant target analyte levels ($>0.1 \mu\text{g}$) are found in the field blanks and or laboratory blanks, the source of contamination must be identified and more laboratory blanks and storage blanks will be analyzed.

10.0 Reference

- 10.1 J. C. Chuang, C. Lyu, Y-L Chou, P. J. Callahan, M. Nishioka, K. Andrews, M. A. Pollard, L. Brackney, C. Hines, D. B. Davis, and R. Menton, "Evaluation and Application of Methods for Estimating Children's Exposure to Persistent Organic Pollutants in Multiple Media." EPA/600/R-98/164a, EPA/600/R-98/164b, and EPA/600/R-98/164c (Volume I, II, and III), 1999.