United States Environmental Protection Agency

Office of Research and Development

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

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

Battelle
Columbus, OH 43201
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Standard Operating Procedure **CTEPP-SOP-5.20**

Title: Extracting and Preparing Solid 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

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STANDARD OPERATING PROCEDURE (SOP) FOR EXTRACTING AND PREPARING SOLID 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 solid food samples for neutral persistent organic pollutants (POP).

2.0 Summary of Method

This method describes the procedures for extracting, and concentrating solid 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, PH, 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 33 mm S.S. ASE cells w/endcaps

6.1.3 Dionex D28, 1.983 cm ASE cell filters

6.1.4 Muffled Ottawa sand

6.1.5 Muffled spatulas

6.1.6 Extrelute (EM Science or equivalent)

6.1.7 Muffled 60 ml ASE collection vials

6.1.8 Muffled quartz fiber filters

6.1.9 Precision syringes

6.1.10 Muffled funnels

6.1.11 25 ml Kuderna-Danish tubes, 125 and 250 ml reservoir flasks, large and small Snyder condensers (muffled)

6.1.12 Muffled Hengar boiling chips

6.1.13 Acrodisc PTFE 25 mm, 0.45 micron syringe filters

6.1.14 Eppendorf pipettor 10-100 ul

6.1.15 Disposable 10 ml syringes

6.1.16 Muffled disposable culture tubes, 16 x 125 mm or 16 x 100mm

6.1.17 Muffled Pasteur pipettes

6.1.18 Muffled 160 ml GPC collection vials

6.1.19 Muffled and GC/MS autosampler vials

6.1.20 Florisil solid phase extraction (SPE) columns

6.1.21 Clean vials

6.1.22 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 Silylation vacuum oven. (Precision SN 600041701 or eqvivalent)

6.3.2 Dionex Accelerated Solvent Extractor (ASE)

6.3.3 Heated water bath

6.3.4 Vortex mixer

6.3.5 Balances

6.3.6 Heating lamp

6.3.7 Traceable timer/stopwatches

6.3.8 O.I. Analytical Autoprep 2000 GPC

6.3.9 Heating oven (Blue M or equivalent)

6.3.10 Freezers

6.3.11 Refrigerators

7.0 Procedure

7.1 Accelerated Solvent Extraction (ASE) for Homogenized Solid Food Samples.

7.1.1 Remove a set of solid 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 Weigh out 10 to 12 g of the solid food sample in the weighing pan and add 1 to 2 g of Extrelute to the same weighing pan. Record the weights on the laboratory record book (LRB). Mix the solid food sample and the Extrelute in the weighing pan using a muffled spatula.

7.1.3 Obtain 33mm ASE cells and end-caps. Screw on the bottom end-cap, insert filter and place a small amount of muffled Ottawa sand on the top of the filter.

7.1.4 Transfer approximately 2/3 of the food/Extrelute mixture to the ASE cell. Spike 50 µL of the surrogate recovery spiking solution onto the mixture using a syringe or a calibrated Eppendorf pipettor. Add the remaining mixture to the ASE cell and tamp gently. If the sample is a matrix spiked sample, 50 µL of matrix spiking solution will also be spiked onto the mixture. Fill the ASE cell to the top with muffled Ottawa sand and cap the cell. **Note that the cell should not be packed tightly because the ASE will not be processed properly.** The spiked level may be adjusted and the exact spiked amounts will be recorded in the LRB.

7.1.5 Place the ASE cells and the 60 mL muffled collection vials on the ASE unit.

Pressure: 2000 psi
Temperature: 100C
Solvent: DCM
Static: 5 min
Flush: 100%
Purge: 60 seconds
Cycles: 2

The extraction time for each sample is ~20 mins and the collection volume is ~50 mL.

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 7 days. Filter the sample extract to a KD tube equipped with a 125 mL reservoir through a muffled quartz fiber filter (or muffled glass wool) containing anhydrous sodium sulfate. Rinse down the sides of the ASE collection 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.

7.1.10 Remove a 25 µL aliquot of the extract and place in a tared or pre-weighed pan to determine the sample residue weight. Allow the sample to dry in a fume hood under a heat lamp for 2 to 3 mins. Weigh the pan containing the residue and calculate the residue weight for the entire sample. Record all weights in the LRB.

Residue Weight, g = (Weight of pan +residue, g) – Weight of pan, g
Total Sample Residue, g = (Residue Weight, g) *(7 mL /0.025 mL).

7.2 Gel Permeation Chromatography (GPC) for Solid 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 sample 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 ENIV-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 1mL 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 μg) 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.