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 Urine Samples for Analysis of Hydroxy Polycyclic Aromatic Hydrocarbons, Pentachlorophenol and 2,4-D

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

Standard Operating Procedure **CTEPP-SOP-5.21**

Title: Extracting and Preparing Urine Samples for Analysis of Hydroxy Polycyclic Aromatic Hydrocarbons, Pentachlorophenol and 2,4-D

Source: Battelle

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

STANDARD OPERATING PROCEDURE (SOP) FOR EXTRACTING AND PREPARING URINE SAMPLES FOR ANALYSIS OF HYDROXY POLYCYCLIC AROMATIC HYDROCARBONS, PENTACHLOROPHENOL AND 2,4-D

Prepared by: _____ Date: _____

Reviewed by: _____ Date: _____

Approved by: _____ Date: _____

Approved by: _____ Date: _____

Approved by: _____ Date: _____

1.0 Scope and Applicability

This standard operating procedure (SOP) describes the method for extracting and preparing urine samples for analysis of hydroxy polycyclic aromatic hydrocarbons (PAH), pentachlorophenol (PCP), and 2,4-D.

2.0 Summary of Method

The method for extracting and preparing urine samples for analysis of hydroxy-PAH, PCP, and 2,4-D pollutants is summarized in this SOP. It covers the extraction, concentration, and methylation of samples that are to be analyzed by gas chromatography/mass spectrometry (GC/MS).

3.0 Definition

3.1 Surrogate Recovery Standard (SRS): The compounds that are used for QA/QC purposes to assess the extraction and recovery efficiency obtained for individual samples. Known amounts of these compounds are spiked into the urine sample prior to extraction. The SRSs are quantified at the time of analysis and their recoveries indicate the probable extraction and recovery efficiency for native analytes that are structurally similar. The SRSs are chosen to be as similar as possible to the native analytes of interest, but they must not interfere in the analysis.

3.2 Internal Standard (IS): The compounds that are added to sample extracts just prior to GC/MS analysis. The ratio of the detector signal of the native analyte to the detector signal of the corresponding IS is compared to ratios obtained for calibration curve solutions where the IS level remains fixed and the native analyte levels vary. The IS is used to correct for minor run-to-run differences in GC injection, chromatographic behavior, and MS ionization efficiency.

3.3 Matrix Spike Standard (MSS): The compounds that are used for QA/QC purposes tp assess the recovery efficiency obtained for the individual samples. Known amounts of the target analytes are spiked into the urine sample prior to extraction. The matrix spikes (MSs) are quantified at the time of analysis and their recoveries indicate the probable extraction and recovery efficiency for the target analytes. The MSs are only generated for duplicate aliquots of selected urine samples.

4.0 Cautions

Standard laboratory protective clothing, gloves, and eye covering is required.

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, and Task Order Leader (TOL) will oversee the sample extraction operation and ensure that SOPs are followed by all project staff.

6.0 Apparatus, Materials, and Reagents

6.1 Apparatus and Materials

6.1.1 Silylated clean glass wool

6.1.2 Clean quartz fiber filters

6.1.3 Eppendorf reference adjustable pipettes in ranges of 10 to 100 and 100 to 1000 micorliters

6.1.4 Silylated separatory funnels (muffled)

6.1.5 Analytical or micro-liter syringes

6.1.6 Silylated glass funnels (muffled)

6.1.7 Large Kim-wipes (15" x 15")

6.1.8 Latex gloves

6.1.9 Silylated 1 dram glass vials with Teflon-lined screw caps; muffled

6.1.10 Silylated 1.8 mL glass GC vials with Teflon-lined screw caps; muffled

6.1.11 Silylated Kuderna-Danish concentrators (large 24/40 3-ball Snyder condenser, 125 mL reservoir flask and 25 mL tube); (Kontes 570000)

6.1.12 Silylated small 19/22 3-ball Snyder condensers

6.1.13 Pasteur glass pipettes (muffled and stored in clean glass jar)

6.1.14 Vortex mixer (American Scientific Products)

6.1.15 Graduated cylinders

6.1.16 Heated water bath

6.1.17 Oven (Blue-M or comparable)

6.2 Reagents

6.2.1 Dichloromethane (DCM); distilled-in-glass

6.2.2 n-Hexane (distilled-in-glass)

6.2.3 Boiling chips (Hengar crystals)

6.2.4 Surrogate Recovery Standard Spiking Solution

6.2.5 Internal Standard Spiking Solution

6.2.6 Matrix Spiking Solution

6.2.7 Ethyl ether (distilled-in-glass)

6.2.8 Diazald

6.2.9 Carbitol

6.2.10 37% aqueous potassium hydroxide (KOH)

6.2.11 Methanol

6.2.12 Sodium sulfate anhydride

6.2.13 SPE cartridges (Florisil)

6.2.14 Hydrochloric acid (HCl)

6.2.15 20% aqueous sodium chloride (NaCl)

6.2.16 Chlorobutane

6.2.17 Distilled, deionized water (DI water)

7.0 Procedure

7.1 Extraction and Concentration.

7.1.1 Samples are removed from the freezer and thawed to room temperature.

7.1.2 Transfer an aliquot (10 mL) of a urine sample to a 60 mL centrifuge tube.

7.1.2 Add 20 µL of surrogate recovery standard solution, 500 µL of concentrated hydrochloric acid, and 1 mL of chlorobutane to the sample. Add 20 µL of matrix spike standard solution to the matrix spike sample. *Note that the spike amount may vary and the actual amount used are recorded in a laboratory record book (LRB).*

7.1.3 Tightly cap the centrifuge tube and place in an oven at approximately 80°C. The exact temperature and starting time are noted in the LRB.

7.1.4 Remove the sample from the oven after one hour heating time has elapsed. The exact temperature and the ending time are noted. Cool the sample to room temperature.

7.1.5 Transfer the resulting mixture to a separatory funnel and rinse the centrifuge tube with 10 mL of 20% sodium chloride solution and then with 10 mL of DCM. Add both rinses to the separatory funnel.

7.1.6 Shake the funnel vigorously for about 30 seconds. Remove the DCM layer and filter through a quartz fiber filter or glass wool containing sodium sulfate anhydride into a KD tube.

7.1.7 Add 10 mL of DCM to the funnel and repeat step 7.1.6.

7.1.8 Repeat step 7.1.7 and concentrate the sample extract to 1 mL using KD evaporation. Add 10 µL of internal standard solution to the KD tube; vortex for about 3 seconds; and transfer the sample to a GC vial.

7.1.9 Add an aliquot (50 µL) of methanol to the concentrated extract and methylate the extract with etheral diazonethane generated in situ from Diazald, carbitol, and 37% aqueous KOH.

7.1.10 After methylation, depending upon the sample matrix, cleanup steps (7.1.11 to 7.1.15) may be required.

7.1.11 Add 2 mL of hexane to the solution and use KD concentration to reduce volume to 1 mL. Vertex for about 3 seconds to mix.

7.1.12 Place Florisil SPE cartridges on the SPE manifold and condition each cartridge in sequence with 6 mL of 50% ethyl ether in hexane, followed by 100% of hexane. Close the valve stem on the manifold to prevent the cartridge from going dry between solvents.

7.1.13 Using a clean Pasteur pipette, transfer the methylated sample extract to an SPE cartridge.

7.1.14 Elute the cartridge into a clean vial with 18 mL of 50% ethyl ether in hexane.

7.1.15 KD concentrate the extract to 0.6 to 0.8 mL and rinse down the sides of the tube with hexane to bring the volume to 1 mL.

7.1.16 Transfer the extract, using a muffled disposable glass Pasteur pipette, to a clean prelabeled 1.8 mL GC vial for GC/MS analysis. Label the sample vial with its respective sample ID.

7.2 Store the extract in a $\leq$-10˚C freezer until GC/MS analysis.

8.0 Records

8.1 Records of the preparation of field samples, blanks, and matrix spikes will be retained in a LRB that is kept in the extraction laboratory. This LRB will record all sample preparation activities. These samples will be recorded in the LRB by field sample ID and the laboratory generated QA/QC sample will be assigned with a laboratory sample number (a unique number that combines the 5 digit LRB number-2 digit page number-2 digit line number). The date of extraction, the lot number of solvents used for extraction, and the spike level of the surrogate recovery standards and internal standards will be recorded in the LRB.

8.2 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 A field blank and laboratory method blank consists of a urine sample vial that will be extracted together with the field samples. The field blank analyses are performed to verify that minimal contamination occurs through sample handling during shipping and field operations. The laboratory method blank analyses are performed to verify that minimal contamination occurs through sample preparation. The laboratory fortified blank analyses are performed to verify the recoveries of analytes preparation procedures.

9.2 Field crews will be reminded to wear clean clothing and shoes, to remove all pesticide products from their residences that may contain the target analytes, and to refrain from using these materials during the field study. Field crews will also be reminded to obtain clean clothing after visiting a home where they know or suspect that these pesticides have been applied within the previous week. Cigarette smoking is not permitted during the field sampling. Field crews should store the samples in a clean environment away from any known combustion sources.

9.3 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% or greater than 150%, the data will be flagged. For recoveries greater than 130%, the concentration of the surrogate spiking solution will be checked against a calibration curve to determine whether inadvertent solvent loss has resulted in higher spike levels. If this has occurred, the surrogate spiking solution will be re-prepared and re-analyzed.

9.4 One laboratory method blank that is analyzed as a sample concurrently with a field sample set will be analyzed for typically every 50 samples processed. If significant target analyte levels (>0.01 μg) are found in the field blanks or laboratory blanks, the source of contamination must be identified and more laboratory blanks, together with additional field blanks, trip 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.