National Human Exposure Assessment Survey (NHEXAS)

Arizona Study

Quality Systems and Implementation Plan for Human Exposure Assessment

The University of Arizona
Tucson, Arizona 85721

Cooperative Agreement CR 821560

Standard Operating Procedure **SOP-BCO-L-11.0**

Title: Extraction of Air Samples for GC/MS Analysis of Pesticides

Source: The University of Arizona

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

Extraction of Air Samples for GC/MS Analysis of Pesticides

1.0 Purpose and Applicability

This standard operating procedure (SOP) describes the procedures for extracting and preparing an air sample consisting of a PUF plug and Teflon-coated glass fiber filter (Pallflex T60A20) for analysis of pesticides. This procedure covers sample preparation for samples that will be analyzed using GC/MS.

2.0 Definitions

2.1 Surrogate Recovery Standard (Surrogate or SRS): The compound that is used for QA/QC purposes to assess the extraction and recovery efficiency obtained for individual samples. A known amount of this compound is spiked into the PUF plug prior to extraction. The "surrogate" is quantified at the time of analysis and its recovery indicates the probable extraction and recovery efficiency for native analytes that are structurally similar. The surrogate recovery standard is chosen to be as similar as possible to the native analytes of interest, but it must not interfere in the analysis.

2.2 Internal Standard (IS): The compound that is added to sample extracts just prior to GC/MS analysis. The ratio of the detection signal of the native analyte to the detection signal of the 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.

3.0 References

3.1 Roinestad, K.S.; Louis, J.B.; Rosen, J.D., "Determination of Pesticides in Indoor Air and Dust," J. AOAC Intl., **76**, 1121-1126 (1993).

3.2 Bogus, E.R., Watschke, T.L, Mumma, R.A., "Utilization of Solid-Phase Extraction and Reversed-Phase and Ion-Pair Chromatography in the Analysis of SevenAgrochemicals in Water," J Agric. Food Chem., **38**, 142-144 (1990).

3.3 Bagnati, R., Benfenati, E., Davoli, E., Fanelli, R., "Screening of 21 Pesticides in Water by Single Extraction with C18 Silica Bonded Phase Columns and HRGC-MS," Chemosphere, **17**, 59-65, (1988).

3.4 Loconto, P.R., Gaind, A.K., "Isolation and Recovery of Organophosphorous Pesticides from Water by Solid-Phase Extraction with Dual Wide-Bore Capillary Gas Chromatography," J. Chromatogr. Sci., **27**, 569-573 (1989).

3.5 Sherma, J., Bretschneider, W., "Determination of Organo-Phosphorous Insecticides in Water by C18 Solid Phase Extraction and Quantitative TLC," J. Liquid Chromatogr., **13**, 1983-1989 (1990).

4.0 Discussion

4.1 This procedure involves spiking the PUF plug with a surrogate recovery standard, Soxhlet extraction of the filter and PUF plug together with 150 mL of acetone for 14 h, and Kuderna-Danish evaporation to 1 mL, solid phase extraction cleanup, addition of an internal standard and then analysis using GC/MS for detection and quantification of the pesticides. SOP BCO-L-15.0 covers the GC/MS analysis and quantification of the extract.

4.2 The procedure outlined here provides for the addition of a structurally similar surrogate recovery standard (fenchlorphos). This surrogate is not used in residential applications and is rarely used in agricultural applications (suggesting that it is not likely to be encountered in samples as a native analyte). This structurally similar surrogate recovery standard provides essential QA/QC data on extraction efficiency and recovery for each sample. The use of a structurally similar IS for GC/MS quantification corrects for minor run-to-run variation in injection, chromatography, and ionization.

5.0 Responsibilities

5.1 The sample extractions will be performed by staff of the Atmospheric Sciences and Applied Technology Department at Battelle who are engaged in the NHEXAS program.

5.2 These staff will be responsible for obtaining samples from the sample coordinator, entering relevant information in the extraction/preparation log books, sending final extracts for analyses, and filing analyte concentration values with the database coordinator.

6.0 Materials and Reagents

6.1 Materials

6.1.1 Small Soxhlet extraction apparatus consisting of condenser, extractor (31 mm id x 135 mm length), and flask (250 mL round bottom); (Kontes 585000-0021).

6.1.2 Heating mantle for 250 mL round bottom flask.

6.1.3 Variac controller.

6.1.4 Silanized glass wool.

6.1.5 Analytical syringes.

6.1.6 Wide-neck glass funnels

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

6.1.8 Latex gloves.

6.1.9 Tweezers and tongs

6.1.10 1 dram glass vials with Teflon-lined screw caps; muffled and vacuum silylated

6.1.11 1.8 mL glass GC vials with Teflon-lined screw caps; muffled and vacuum silylated

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

6.1.13 Small 19/22 3-ball Snyder condensers.

6.1.14 Disposable glass pipettes (muffled and stored in clean glass jar).

6.1.15 Vortex mixer (American Scientific Products)

6.1.16 C18 SPE cartridges (Baker, 500 mg, 6mL)

6.1.17 60 mL SPE reservoirs (Supelco)

6.1.18 12 port SPE manifold (Supelco)

6.1.19 Graduated cylinders

6.2 Reagents

6.2.1 Acetone (high purity).

6.2.2 Boiling chips (Hengar crystals).

6.2.3 Surrogate Recovery Standard Spiking Solution (see SOP BCO-L-21.0).

6.2.4 Internal Standard Spiking Solution (see SOP BCO-L-21.0).

6.2.5 Methyl-t-butyl-ether (mtbe; high purity)

6.2.6 Distilled, deionized water (DI water)

6.2.7 Methanol (high purity)

6.2.8 4% Acetone in DI water (4 mL of acetone diluted to 100 mL with DI water)

7.0 Procedure

7.1 Extraction and Concentration

7.1.1 Retrieve the appropriate sample from the freezer and place the sealed Zip-lock bag on the laboratory bench for 10 min to come to room temperature.

7.1.2 Put on clean gloves.

7.1.3 Place ~ 2 g of glass wool on bottom of the Soxhlet extractor body.

7.1.4 Spike the middle of the PUF plug with 50 µL of the surrogate recovery standard spiking solution, using a 50 µL syringe.

7.1.5 Using acetone-rinsed tongs and tweezers, place the T60A20 filter and PUF plug in the body of the Soxhlet extractor and wedge the PUF plug against the side of the extractor body so that it does not float.

7.1.6 Assemble the Soxhlet extractor, add 100 mL of high purity acetone to the flask with 3 boiling chips. (see SOP BCO-L-2.0 for details)

7.1.7 Adjust the temperature of the flask with the Variac so that the acetone boils smoothly and drips at a constant rate from the condenser into the Soxhlet body. Solvent should fill the extractor body and dump into the flask in approximately 15-20 min. Continue extraction overnight (14 h).

7.1.8 Turn off the Variac, and remove the heating mantle. Allow the Soxhlet apparatus to cool for 15 min, then remove the condenser.

7.1.9 Tilt the Soxhlet body to dump the remaining solvent from the extractor body into the round-bottom flask.

7.1.10 Using a wide-neck funnel, pour the extract from the round-bottom flask into a Kuderna-Danish concentrator (KD) which has a 125 mL reservoir flask. Rinse the round-bottom flask twice with 2 mL acetone and add to the KD flask.

7.1.11 Add 3-5 boiling chips to the KD tube/flask. Add the large Snyder condenser to the flask.

7.1.12 Concentrate the extract in a heated (65°C) water bath to 0.6-0.8 mL. Remove the KD assembly from the water bath and let it stand in the hood to cool.

7.1.13 Rinse down the insides of the flask and tube with acetone to bring the volume to 1 mL. Remove the flask from the tube.

7.1.14 Vortex the tube 2-3 sec to mix.

7.1.15 Place SPE cartridges on the SPE manifold, and condition each cartridge in sequence with 6 mL of methanol, DI water, and 4% acetone in DI water. Close the valve stem on the manifold when the top frit of the cartridge is just wet so that the cartridge does not go dry between solvents. Add a reservoir to each cartridge.

7.1.16 Using a clean Pasteur pipette, transfer and dilute each 1 mL sample extract to 25 mL with DI water in a 25 mL volumetric flask. Invert several times to mix.

7.1.17 Using a Pasteur pipette, transfer a sample extract to an SPE reservoir.

7.1.18 Load the extract onto the cartridge under reduced pressure (20 in Hg on manifold gauge)

7.1.19 When all the extract has run through the cartridge, remove the reservoir and dry the cartridge for 20 min by drawing air through the cartridge.

7.1.20 Elute the cartridge into a muffled/silanized 1 dram vial with 2 mL of mtbe followed by 1 mL of mtbe.

7.1.21 Use N_2 evaporation to concentrate the extract to 1 mL.

7.1.22 Spike the extract with 50 µL of the Internal Standard spiking solution (trichloronate at 5 µg/mL), and vortex 3 s to mix.

7.1.23 Transfer the extract, using a muffled disposable glass Pasteur pipette, to a clean prelabeled 1.8 mL GC vial for GC/MS analysis. Mark the volume on the side of the vial and label the sample with the laboratory notebook number and field sample ID.

7.1.24 Store extract in -20 °C freezer until GC/MS analysis.

7.2 Calculations

None.

7.3 Quality Control

7.3.1 As discussed in SOP BCO-L-2.0, the pre-shipment verification for residue levels of pesticides in filter and PUF plug batches are performed individually on one PUF plug and one filter, rather than on a combined set of a filter and a PUF plug. This is done to pinpoint the specific media that may be contaminated, before use.

7.3.2 A field blank or field spike sample consists of a filter and PUF plug that will be extracted together. The field blank analyses are performed to verify that minimal contamination occurs through sample handling during shipping and field operations. The field spike analyses are performed to verify retention of analytes through shipping and handling procedures.

7.3.3 If field blank levels exceed 0.05 μg/sample, the data from the corresponding 20 homes will be flagged and inspected for possible blank correction. Additional sampling media (filter and PUF) will be sent to the field within 3 days for additional field blank measurements. The field team responsible for the flagged data will be requested to process these field blanks under field conditions as quickly as possible; their analyses at Battelle will be carried out as soon as samples are received.

7.3.4 Field crews will be reminded to wear clean laboratory coats and shoes, to remove all pesticide products from their home/residence that contain these analytes and to refrain from using these materials while a member of the field crew. Field crews will also be reminded to obtain a clean laboratory coat after visiting a home where they know or suspect that these pesticides have been applied within the previous week.

7.3.5 Field spike recovery values of 70-130% of the true value will be acceptable. Recoveries of less than 70% will require a review of field and analytical protocol to verify that procedures are being correctly implemented, especially those having to do with storage at Blue Ice/ freezer temperatures after field collection. For recoveries greater than 130%, the preparation date of the field spiking solution will be checked, and recoveries in a second field spike sample (any matrix) prepared by the same field crew will be checked. If the expiration date on the spike is imminent, and/or another field spike has a high recovery, a new field spiking solution will be prepared and shipped immediately.

7.3.6 Surrogate recovery values of 70-130% in blanks, field spikes, and actual samples will be deemed acceptable, and no correction to the data will be made. For recoveries less than 70%, the data will be flagged, and the analyte concentrations will be corrected (divided) by the percent recovery of the surrogate. For recoveries greater than 130%, the concentration of the surrogate spiking solution will be checked against a calibration curve to determine whether inadvertant solvent loss has resulted in higher spike levels. If this has occurred, the surrogate spiking solution is re-prepared.

7.3.7 Laboratory blanks of PUF plugs and filters (those that are analyzed as a sample concurrently with a field sample set) will not be performed unless significant pesticide levels (>0.050 μg) are found in the field blanks. In that case, the source of contamination must be identified, and laboratory blanks, together with additional field blanks, trip blanks, and storage blanks, will be analyzed.

8.0 Records

8.1 Records of the field blank levels and field spike recovery values will be retained in a NHEXAS laboratory notebook that is kept in the pesticide extraction laboratory. This notebook will serve as a continuing file for reference on expected performance of the methods and likely contaminant levels that will arise as a result of field handling. These samples will be identified in the laboratory notebook by field sample ID and the assigned laboratory analysis number (a unique number that combines the 5 digit laboratory book number-2 digit page number-2 digit line number), the date of extraction, the lot number of acetone used for extraction, the batch number of the PUF plug and filter, and surrogate recovery value. This notebook will be transferred to the Battelle co-PI's office at the conclusion of the program.

8.2 The record of the extraction of samples will be maintained in a NHEXAS laboratory notebook that is kept in the pesticide extraction laboratory. This notebook will contain the field sample ID, the assigned laboratory analysis number (see above), the date of extraction, and the lot number of acetone used for extraction.