National Human Exposure Assessment Survey (NHEXAS)

Arizona Study

Quality Systems and Implementation Plan for Human Exposure Assessment

The University of Arizona
Tucson, Arizona 85721

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Standard Operating Procedure **SOP-BCO-L-14.0**

Title: Extraction of Soil/House Dust 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

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Extraction of Soil/House Dust Samples for GC/MS Analysis of Pesticides

1.0 Purpose and Applicability

This standard operating procedure (SOP) describes the procedures for extracting and preparing a dust or soil sample for GC/MS (or GC/ECD) analysis of pesticides.

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 collected sample (dust, soil, dermal wipe, PUF, etc.) 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, detector efficiency, and final extract volume.

3.0 References

3.1 Roinestad, K.S., Louis, J.B., and 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, and Mumma, R.A. "Utilization of Solid-Phase Extraction and Reversed-Phase and Ion-Pair Chromatography in the Analysis of Seven Agrochemicals in Water," J. Agric. Food Chem, **38**, 142-144 (1990).

3.3 Bagnati, R., Benfenati, E., Davoli, E., and 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. and 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. and 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 of the dust/soil with a surrogate recovery standard, sonication extraction in 5 mL of acetone, followed by solid phase extraction (SPE) cleanup with a C18 cartridge, and 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). The compound 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 analysts of Battelle's pesticide extraction laboratory who are completely familiar with the methods and procedures listed here. The analyst will be responsible for obtaining samples from the Sample Coordinator and ensuring the chain-of-custody form is properly documented, entering relevant information in the extraction/preparation log books, and sending final extracts for analyses.

5.2 After receipt of the analysis results, the Project Laboratory Director in the pesticide extraction laboratory will review the data. Once verified, the analyst will be responsible for filing analyte concentration values with the Data Coordinator.

5.3 The analyst will be responsible for following this SOP, for reporting deviations and changes to the supervisory scientist, for making sure that the materials and

reagents used are of sufficient purity (as indicated by manufacturer's labels), and for ensuring that the holding times for solutions used have not expired.

6.0 Materials and Equipment

6.1 Materials

6.1.1 15 mL conical centrifuge tubes.

6.1.2 10 mL graduated volumetric pipettes.

6.1.3 Balance with 4-place accuracy (x.xxxx g).

6.1.4 Balance with 2-place accuracy (x.xx g).

6.1.5 Large Kim-Wipes (15 in. x 15 in.).

6.1.6 Latex gloves.

6.1.7 Tweezers and spatulas.

6.1.8 Ultrasonic water bath (Bransonic 52, or equivalent).

6.1.9 1.8 mL glass vials with Teflon-lined screw-caps, muffled and vacuum silylated.

6.1.10 Kuderna-Danish concentrators (small 19/22 3-ball Snyder condenser and 25 mL tube).

6.1.11 Glass loaf pan (8" length x 4" width x 4" depth) filled ¾-full with glass beads (4 mm diameter) and distilled water.

6.1.12 C18 SPE cartridges (Baker, 500 mg, 6mL).

6.1.13 60 mL SPE reservoirs (Supelco).

6.1.14 12 port SPE manifold (Supelco).

6.1.15 2 dram glass vials with Teflon-lined screw caps; muffled and vacuum silylated.

6.1.16 Laboratory-bench centrifuge.

6.2 Reagents

6.2.1 Boiling chips (Hengar crystals).

6.2.2 High purity acetone.

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 Methanol (high purity).

6.2.7 Distilled, deionized water (DI water)

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

7.0 Procedures

7.1 Extraction of House Dust Samples

7.1.1 Retrieve the appropriate sample from the Sample Custodian, and sign and date the chain-of-custody form.

7.1.2 Put on latex gloves.

7.1.3 Weigh out approximately 1 g of the dust into a prelabeled 15 mL centrifuge tube using a 2-place balance and record the weight in the NHEXAS pesticide extraction laboratory notebook. If 1 g of dust is not available, then weigh out the amount that is available.

7.1.4 Spike 50 µL of the Surrogate Recovery Standard Spiking solution (fenchlorphos at 5 µg/mL) onto the dust and allow the solvent to disperse before addition of the solvent (~ 15 min).

7.1.5 Add 5 mL of acetone to the dust, put on the cap and swirl to wet. Place up to 20 samples along the loaf pan, with the tip resting in the water/beads and the side wall near the cap resting on the edge of the pan.

7.1.6 Sonicate the samples in an ultrasonic bath in which the water is present to a depth of approximately 8 cm of water. Sonicate for 15 min.

7.1.7 Centrifuge the samples for 15 min to settle the dust.

7.1.8 Pipette 4 mL of each extract into a Kuderna-Danish (KD) evaporator tube (80% of total), add 3-5 boiling chips, and the condenser column.

7.1.9 Concentrate in a heated (65 °C) water bath to 0.6-0.8 mL.

7.1.10 Remove the KD tube from the water bath and stand it upright in the hood to cool for ~ 5 min.

7.1.11 Remove the condenser and rinse down the sides of the tube with acetone to bring the volume to 1 mL. Vortex for 3 s to mix.

7.1.12 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 to prevent the cartridge from going dry between solvents. Add a reservoir to each cartridge.

7.1.13 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 the flask several times to mix.

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

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

7.1.16 When all of the extract has run through the cartridge, remove the reservoir and dry the cartridge for 20 min.

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

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

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

7.1.20 Transfer the extract, using a muffled disposable glass Pasteur pipette, to a clean prelabeled 1.8 mL vial for GC/MS analysis. Mark the volume on

the side of the vial and label the sample with the laboratory notebook number and the field sample ID.

7.1.21 Store the extract in a -20 °C freezer until required for GC/MS analysis.

7.2 Extraction of Soil Samples

7.2.1 Carry out the extraction of soil in a manner identical to that described above (Steps 7.1.1 through 7.1.21) with the exception that the extract does not need to be centrifuged. Instead, the tube containing the extract is placed upright in a rack for ~15 min to allow the soil particles to settle.

7.2.2 The following procedure is used to select the smallest soil particles for extraction, because the soil sample cannot be dried and sieved without loss of pesticides.

7.2.2.1 Hold the Zip-lock bag containing the soil on a 45°-angle and gently shake the bag up and down for about 20 seconds. Look to see that the larger soil particles rise to the top and the smaller particles sink to the bottom.

7.2.2.2 While holding the bag in one hand, use the thumb and forefinger of the other hand to grasp the tip of the bag and clamp off ~2 cc of soil below the fingers. Squeeze the fingers together to separate the soil in the bag.

7.2.2.3 Tilt the bag an additional 45° until the soil above the fingers rolls to the side. Open the bag and scoop some soil from that 2 cc amount of soil left in the corner of the bag.

7.3 Calculations

None.

7.4 Quality Control

7.4.1 For every 20 homes where dust and soil are sampled, one home will be designated as the site for field duplicates. Because it is impossible to have two identical sites, especially for house dust collection, the field duplicates will consist of split dust and split soil extracts. The sample extract will be split and the two replicate aliquots will be analyzed; the precision of the GC/MS analyses should be <10% deviation from the mean.

7.4.2 For every twenty homes, one additional QA/QC sample will be analyzed, consisting of either a laboratory blank or a reference dust. The laboratory blank will be analyzed to determine laboratory contamination, if any, and the reference dust sample will be analyzed as a measure of accuracy. The house dust to be used has been collected in large quantities from a single home and contains detectable levels of chlorpyrifos and diazinon.

7.4.3 These analyses will provide 5% QA as field duplicates and 10% overall QA.

7.3.4 Surrogate recovery values of 70-130% in 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 inadvertent solvent loss has resulted in higher spike levels. If this has occurred, the surrogate spiking solution is re-prepared.

8.0 Records

8.1 The record of the extraction of samples will be maintained in a NHEXAS laboratory notebook that is retained in the pesticide extraction laboratory. This notebook will contain the field sample ID, the assigned laboratory analysis number (a unique number that combines the 5 digit lab book number-2 digit page number- 2 digit line number), the date of extraction, and the lot number of acetone used for extraction. Check-off columns will be included for addition of the surrogate and IS, and removal of an aliquot for ELISA. After completion of the analysis, the sample analysis form will be filed with the Data Coordinator. This form will record not only the analyte values (μg/g) but also the recovery of the surrogate recovery standard.

8.2 Records of the laboratory blank levels and reference dust analyte levels 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 analyses. These samples will be identified in the laboratory notebook by the same laboratory analysis coding used for field samples, including the date of extraction, the lot number of acetone used for extraction, and the surrogate recovery value. This notebook will be transferred to the Battelle co-PIs office at the conclusion of the program.