National Human Exposure Assessment Survey (NHEXAS)

Maryland Study

Quality Systems and Implementation Plan for Human Exposure Assessment

Emory University
Atlanta, GA 30322

Cooperative Agreement CR 822038

Standard Operating Procedure **NHX/SOP-L14**

Title: Determination of Pesticides and PAHs by GC/MS

Source: Harvard University/Johns Hopkins University

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

1. Title of Standard Operating Procedure

   Harvard University/Johns Hopkins University Standard Operating Procedures:
   L14 Determination of Pesticides and PAHs by GC/MS, Rev. 1.0

2. Overview and Purpose

   This procedure defines the method for determination of pesticides and polynuclear aromatic hydrocarbons (PAHs) in prepared extracts by GC/MS using selected ion monitoring (SIM).

   This SOP is a modification of SwRI SOP 01-17-02 "Determination of Pesticides, Acid Herbicides, Phenols, and PAHs by GC/MS."

3. Discussion

   This procedure is applicable to soil, house dust, water, air and a variety of media employed for pesticide exposure assessment. Specific SOPs for extraction, derivatization, and cleanup (see References) are to be followed for sample preparation prior to GC/MS analysis.

   This method is limited to target analytes in which GC/MS performance has been validated. Specific target analytes will be specified by the client, study protocol or quality assurance narrative for each project.

4. Personnel Responsibilities

   It is the responsibility of the project manager and the laboratory manager to assure that all steps described in this procedure are performed.

   It is the responsibility of the study personnel to comply with all the criteria described in this procedure.

5. Required Equipment and Reagents

   5.1 Equipment

   VG Fisons MD800 GC/MS equipped with LAB-BASE data acquisition and analysis software
   30 m x 0.32 mm i.d., or 30 m x 0.25 mm i.d., DB5 column, or equivalent
   MD800 Instruction Manual
   carrier gas (helium, chromatography grade)
   balance (precision 0.001 g)

   5.2 Reagents

   n-hexane, Fisher Optima grade or equivalent
   p-Terphenyl-d_{14} 1-2 mg/mL stock solution in n-hexane
   Internal standards: 1,2-Dichlorobenzene-d4, Naphthalene-d8, Acenaphthene-d10, Chrysene-d12, and Perylene-d12: commercially available solution at a concentration of 2 mg/mL

6. Procedure

6.1 Preparation of Standards

All pesticide and PAH standards must be certified when available. Certificates of analysis are maintained on file in the pesticides laboratory.

Stock standard solutions for each analyte will be prepared at concentration level 1-2 mg/mL (precision of weighing should be ± 0.0001 g). The amount weighed, the final solvent volume, the lot number of the standard, and lot numbers of all solvents will be recorded in the laboratory notebook.

Each standard will be labeled with the common name of the analyte, solvent, concentration, date of preparation, control number, expiration date and storage instructions. The control number will be assigned as "xx-yy-zz" where xx is the notebook page number, yy is the sequential number of the standard prepared on page xx, and zz is the notebook identification. The expiration date of the stock standards will be one year from the date of preparation or the expiration date of the neat standard or stock solution, whichever is sooner.

p-Terphenyl-d_{14} is used as the surrogate for neutral pesticide analysis. A 1-2 mg/mL stock solution of p-Terphenyl is prepared in n-hexane.

Specific spiking levels of the surrogate vary with the sampling medium and the extract final volume and are specified in appropriate extraction SOPs.

1,2-Dichlorobenzene-d4, Naphthalene-d8, Acenaphthene-d10, Chrysene-d12 and Perylene-d12 are used as internal standards. A commercially available solution containing the internal standards at a concentration of 2 mg/mL is used as stock.

A stock internal standard solution is prepared by diluting the individual internal standards in n-hexane so that each internal standard is present at a concentration of approximately 10 ng/µL.

Stock standard solutions are prepared from individual standards using n-hexane as a solvent so that each pesticide is present at a concentration of approximately 10 ng/µL. For the stock solution, the lot number of each standard used, the volume of each standard used, the final concentration and final volume will be recorded in the laboratory notebook. Each standard will be labeled and assigned a lot number as described above. The expiration date of stock group standards will be six months or the expiration date of the neat standard, whichever is sooner.

Five different levels of working standards are prepared, corresponding to a range from 0.005 to 0.10 ng/µL for the most sensitively detected pesticides and PAHs, by serial dilutions of the stock standard using n-hexane. Calibration ranges for specific target analytes will be as specified by the client or as established in the protocol or quality assurance narrative. Stock internal standard solution is added to each standard so that the concentration of each internal standard in 0.1 ng/µL.

6.2 INSTRUMENT PREPARATION

6.2.1 Gas Chromatograph Preparation

a. If the correct column is not already installed, the following procedure is followed:

b. One end of the chromatography column is inserted into the mass spectrometer source so that it extends approximately 2 mm inside the source. The other end of the column is inserted into the injector a distance of 64 mm from the 1/16" nut. If necessary, consult the MD800 Instruction Manual for proper column installation.

c. The injector liner is removed and cleaned and the injector septum is replaced when a new column is installed or peak tailing is observed in the chromatograms.

d. GC operating parameters are set. Typical values are as follows:

Carrier Gas (Helium, Chromatography Grade)	1.5-2 mL/min
Column Head pressure	7-9 psi
Injector Temperature	240° C
Transfer line Temperature	275° C

e. The GC oven program is set as follows on the parameter page in the LAB-BASE software accessed by the commands ACQU-INLET-EDIT.

Initial Temperature	60° C
Initial Time	5.0 min
Rate	15° C/min
Final Temperature	200° C
Final Time	3.0 min
Rate A	15° C/min
Final Temperature A	295° C
Final Time A	10. min
Injection Volume	3 µL
Purge Delay	1 min.

The GC operating parameters will be automatically downloaded to the GC upon initiation of the sequence.

f. The above GC oven parameters are provided as a guide only. The analyst may modify GC oven parameters to obtain sufficient separation of target analytes.

g. The GC oven parameters used are entered into the instrument logbook.

6.2.2 Mass Spectrometer Preparation

a. The mass spectrometer is tuned by entering the LAB-BASE by the selections ACQU-TUNE-MANUAL.

b. The following mass spectrometer parameters are set:
- Source Temperature 200° C
- Electron Energy 70 eV
- Emission Current 100- 200 µA

c. The FC43 leak is opened and the mass spectrometer is tuned so the 69, 219 and 502 m/z peaks are resolved from 70, 220 and 503 m/z peaks. Consult the instrument operating manual if necessary for procedures for proper tuning.

d. The mass spectrometer is then tuned so that the resulting FC43 mass spectrum containing the following peaks is approximately the abundances indicated: 69 m/z (100%), 131 m/z (50 %), 219 m/z (30 %), 264 m/z (10 %), 414 m/z (10 %), 502 m/z (5 %).

6.2.3 If the elution order of the target analytes, surrogate and target analytes for the particular GC column and instrument and temperature program are not known, it is determined using the following procedure:

a. The full-scan acquisition parameter page is accessed by initiating the commands ACQU-MS-OPTION-SCN-EDIT

b. The mass spectrometer is set to scan from 35 to 500 m/z at 1 s per scan.

c. 2-3 µL of a solution containing all of the target analytes, surrogate and internal standards at a level of approximately 1 ng/µL is injected.

d. Upon completion of the acquisition, the mass spectrum of each chromatographic peak is compared to the reference mass spectra of the target analytes for positive identification.

6.2.4 The GC/MS selected-ion-monitoring acquisition is then set up using the following procedure:

1. Based upon the survey chromatogram acquired in section 6.2.3, arrange the target analytes into groups.

   a. The number of analytes in the group depends upon the required sensitivity. The greater the required sensitivity, the smaller the number of monitored ions that can be placed in the group.

   b. Typically, good sensitivity can be obtained when four to six target analytes, surrogates, or internal standards are in a group.
   c. Re-examine the groups and the survey scan to insure that the groups do not

overlap and that no target analytes elute during the switching from one group to another.

d. Recommended monitored quantitation and secondary ions for each internal standard:

Compound	Monitored Ions	Quantitation Mass
GC/MS Internal Standards:		
1,2-Dichlorobenzene-d4	152 & 150	152
Naphthalene-d8	136 & 137	136
Acenaphthene-d10	164 & 162	164
Phenanthrene-d10	188 & 94	188
Chrysene-d12	240 & 120	240
Perylene-d12	264 & 132	264
Surrogate:		
p-Terphenyl-d14	244 & 122	244

e. Primary and secondary monitored ions for target analytes are as specified by the client, study protocol or project quality assurance narrative. If monitored ions are not specified, ions are selected based upon their relative abundance. The highest mass ion possible should be selected to minimize interferences.

2. The total scan time for each group of target analytes is then determined.

a. The dwell time for each mass is selected so that the sum of all monitored ions in the group, and the interchannel delay (the time between each monitored ion) is approximately 1.0 s.

b. The SIM acquisition program is then entered by the commands ACQU-MS-OPTION-SIR-EDIT.
The masses to be monitored, group start time, group end time and time each ion is to be monitored are entered in the matrix.

6.3 Sample Preparation

6.3.1 Prior to extract analysis, the gas chromatograph is calibrated using the five working standards. If the instrument has already been calibrated from a previous sequence, only a continuing calibration verification is required.

If the continuing calibration does not meet criteria given in section 6.5.2, then the instrument must be re-calibrated using five working standards.

6.3.2 A series of clean autosampler vials are arranged and labeled with the sample ID and dilution factor according to the injection sequence listed below.

- Solvent Blank
- Standard-1 (lowest conc.)
- Standard-2
- Standard-3
- Standard-4
- Standard-5 (highest conc.)
- Solvent blank
- Standard 3 (CCV1)
- Extraction Blank(s)
- Sample Matrix Spike (when available)
- Sample Matrix Spike duplicate (when available)
- Sample extracts (15 maximum)
- Solvent Blank
- CCV2

6.3.3 For extremely dirty samples such as house dust, solvent blanks will be required between undiluted samples in the injection sequence.

6.3.4 A mid-level continuing calibration standard is analyzed at the beginning and ending of each analytical sequence and after analysis of every 15 extracts so that no more than 15 extracts are analyzed without a solvent blank and continuing calibration verification.

6.3.5 A volume of approximately 200 µL of each solution is pipetted into its respective vial. Internal standard solution is added to the sample vial so that the concentration of each internal standard in the extract is 0.1 - 0.2 ng/µL. The vial is immediately closed with a crimp cap. The meniscus level on each vial is marked. The sample vials are then inserted in an autosampler tray in the proper sequence.

6.3.6 The sequence is then entered into the instrument logbook. Each sequence must contain the data filename, the sample I.D., and the dilution factor for each injection. In addition, the date the sequence was initiated, the project number and chromatographic conditions will be entered for each sequence. Each standard used will be identified by its lot number as assigned in section 6.1.

Filenames are assigned as "ABBCCDD" where A is the instrument used, BB is the number of the month, CC is the day of the month and DD is the sequential number of the sample in the sequence.

6.3.7 The sample sequence is then initiated on the LAB-BASE software using the following procedure:

1. Enter the sequence editor by the commands ACQU-SEQUENCE-EDIT.

2. Enter the filename, GC temperature program name, user, instrument ID, sample ID, and any dilution factor.

3. Initiate the sequence by the commands ACQU-SEQUENCE-GO.

4. The autosampler is started by pressing its INJ button.

6.4 Instrument Calibration

6.4.1 As soon as possible upon completion of the five calibration standards, the data will be evaluated to verify proper calibration of the instrument.

6.4.2 All quantitation mass ion profiles are inspected by the analyst to insure that the baseline is properly assigned. All profiles that are not properly integrated will be manually re-integrated.

6.4.3 Calibration is to be performed using the reconstructed ion chromatograms of the selected masses. Relative response factors (RRF) for each target analyte will be determined by:

$$RRF = (A_t \times C_{is})/(A_{is} \times C_t)$$

where

A_t = the integrated area of the quantitation mass selected ion chromatogram peak for the target analyte

A_{is} = the integrated area for the quantitation mass of the selected ion chromatogram for the quantitation mass of the internal standard

C_{is} = the concentration of the internal standard

C_t = the concentration of the target analyte in the standard.

6.4.4 Over the calibration range, the mean relative response factor (mean RRF) and the percent relative standard deviation (%RSD) are calculated for each target analyte.

6.4.5 Once the appropriate concentration value has been entered into the "CONC" field for each entry in the QUAN data base, the calibration file and the calculation of the RRF, mean RRF and the %RSD using the LAB-BASE software are performed using the following procedure:

1. Use the QUAN facility from the LAB-BASE Menu by executing the command sequence: OTHER, FILE, CALI.

2. Delete any pre-existent files (from previous calibration) by the command sequence CALI, DELETE, ALL.
3. Enter the appropriate five point initial calibration files by the command sequence:

CALI, ADD and the file name at the prompt; entering the filenames for all five calibration standards.

4. Once all the files for the five point standards are entered, calculate the RRFs, mean RRFs, and %RSDs for each target analyte and surrogate by the command sequence: CALI, CALCULATE, CALIBRATION.

5. An Initial Calibration File (ICAL) is generated by the above sequence. The calibration curve data for each target analyte is generated by executing the command sequence: CALI, GRAPH, CURVE and the list entry number for that target analyte.

6.4.6 Calibration is successful when the %RSD for each target analyte is less than or equal to 30% or as specified by the client, the study protocol or the study quality assurance narrative.

6.4.7 If a target analyte has a calibration outside the accepted range, the sequence will be aborted. An attempt will be made by the analyst to ascertain the source of the calibration drift. If the problem is corrected, the calibration process repeated. If the problem cannot be determined, the project manager, analytical manager or laboratory supervisor is contacted for further instructions.

All maintenance will be recorded in the instrument maintenance logbook.

6.5 Data Processing

6.5.1 As soon as possible upon completion of an analytical sequence, the RRFs for all target analytes in the continuing calibration standards will be calculated according to the following procedure:

All selected ion chromatograms will be generated.

Continuing calibration RRFs are calculated using the macros (EPALs) resident in the LAB-BASE software. This is executed by the commands @EPAL-RUN-MENU or F2. The continuing calibration option is selected followed by entering the raw data filename of the continuing calibration standard and the calibration raw data filename against which the continuing calibration is being evaluated.

6.5.2 Analytical results will only be considered valid when sample extract chromatograms are bracketed by continuing calibration standards with %D for each target analyte of less than 30% or as specified by the client, study protocol or study quality assurance narrative.

6.5.3 If a target analyte has a continuing calibration outside the accepted range, an attempt will be made by the analyst to ascertain the source of the calibration drift. If the problem is corrected, the calibration and analytical sequence will be repeated. If the problem cannot be determined, the laboratory supervisor or the analytical manager will be contacted for further instructions.

6.5.4 Selected ion chromatograms will then be generated for all analyzed extracts using the procedure described above.

6.5.5 The chromatograms will then be reviewed by the analyst for detected target analytes using the following procedure:

1. Each sample data will be analyzed for target analytes by criteria including retention times, mass spectra, and unique ion profiles.

2. A search is made for each target analyte within a pre-defined time window, typically 20 seconds in width.

3. If a peak is observed in the selected ion chromatogram for the quantitation ion of a target analyte, the ion selected chromatogram of the secondary ion is inspected to determine whether a peak exists.

4. If both the quantitation ion mass and the secondary selected mass are present in the chromatograms, in approximately the same proportions as in the NIST reference spectrum, and both peaks are within ± 2 seconds of the retention time of the target analyte peak in the nearest calibration standard, the target analyte is considered tentatively detected and is submitted for quantitation. Otherwise it is reported as not detected.

5. The retention time criterion may sometimes be waived when, in the judgement of the analyst, a retention time shift occurred as a result of column overload by an extremely contaminated sample. These instances will be recorded in the laboratory notebook and reported to the project manager or the analytical manager.

6.5.6 The LAB-BASE software then performs quantitation by comparing the integrated intensity of the chromatograms for quantitation masses of identified compounds to the quantitation mass of the internal standard using the equation:

$$\text{Amount} = (A_i \times C_{is})/(\text{RRF} \times A_{is})$$

where

A_i = the area of the quantitation mass chromatographic intensity.

The procedure for quantitation using the LAB-BASE software is as follows:

1. The sample raw datafile is retrieved by the command sequence: OTHER, FILE, QUAN and the appropriate filename.

2. This procedure uses the Initial Calibration File (ICAL) generated in section 6.4.

3. The QUAN report is then printed using the commands @EPAL-RUN B-HCPY2 or F4.

6.6 Records

6.6.1 A hardcopy and a floppy disk copy of the QUAN report will then be delivered to the analytical manager or his representative for review and tabulation.

6.6.2 Sample extracts that contain suspected target analyte peaks outside the calibration range, or that, in the judgment of the analytical manager, cannot be properly quantitated owing to a large quantity of contaminants, will be reanalyzed following dilution.

6.6.3 All dilutions will be performed using calibrated pipettes. The sample ID, initial volume, final volume, dilution factor and lot number the solvent used will be recorded in the laboratory notebook.

6.6.4 A full analytical report will be sent to the Principal Investigator.

7. Quality Assurance Procedures

In addition to 10% blanks and 10% duplicates, p-terphenyl-d14 is added to all samples to monitor both GC retention time and MS output.

8. References

Harvard University/Johns Hopkins University Standard Operating Procedures:
L05 Sieving and Division of Dust and Soil Samples
L10 Extraction of Neutral Pesticides and PAHs from Air Sampling Media
L11 Extraction of Neutral Pesticides and PAHs from House Dust and Soil
L12 Extraction of Neutral Pesticides from Isopropanol Handwipes

SwRI SOP 01-17-02 Determination of Pesticides, Acid Herbicides, Phenols, and PAHs by GC/MS