# The Arizona Border Study

***An Extension of the***
***Arizona National Human Exposure Assessment Survey (NHEXAS)Study***
***Sponsored by the Environmental Health Workgroup of the Border XXI Program***

# Quality Systems and Implementation Plan for Human Exposure Assessment

The University of Arizona
Tucson, Arizona 85721

Cooperative Agreement CR 824719

**Standard Operating Procedure** **SOP-BCO-L-15.1**

**Title:** Analysis of Pesticide Samples by GC/MS

**Source:** The University of Arizona

U.S. Environmental Protection Agency
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# Analysis of Pesticide Samples by GC/MS

## 1.0 Purpose and Applicability

1.1 This standard operating procedure (SOP) describes the methods used for detection and quantification by GC/MS of pesticides in a variety of matrices, including air, house dust, soil, and handwipes. Other SOPs (BCO-L-11 through BCO-L-14) detail the extraction of these samples. This analysis involves automated GC/MS analysis using a high-resolution capillary column, electron impact ionization and selected ion monitoring with a mass selective detector (HP 5973).

## 2.0 Definitions

2.1 Extract: the 1.0-mL sample volume that contains native analytes, surrogate recovery standard, and internal standard. Each sample (dust, soil, wipe, air) is reduced to this type of extract for analysis.

## 3.0 References

3.1 J.P. Hsu, H.G. Wheeler, Jr., D.E. Camann, H.J. Schattenberg III, R.G. Lewis, and A.E. Bond, "Analytical Methods for Detection of Nonoccupational Exposure to Pesticides," J. Chromatogr. Sci., **26**, 181-189 (1988).

3.2 Hewlett Packard 6890 GC Operator's Manual, HP Part No. G1530-90310.

3.3 Hewlett Packard 5973 Mass Selective Detector Hardware/Operator's Manual.

3.4 On-Line Hewlett Packard ChemStation Software Manual B.02.05.

## 4.0 Discussion

4.1 For analysis, a small aliquot of the extract (2 μL) is injected splitless into a heated GC injector (250 °C). Analytes and solvent vapors are swept onto the GC column by the helium carrier gas. The GC column temperature is then increased (temperature-programmed) and the components eluting from the column are identified and quantified by mass spectrometry (MS). Component identification is normally accomplished on the basis of the GC retention time and mass spectral fragmentation characteristics.

4.2 Both the range and limit of detection depend strongly on the properties of the individual compounds of interest. Absolute limits of detection may vary from tens of picograms (pg) to a few nanograms (ng), depending on the multiplier voltage used. Precision also depends greatly on the chemical; for GC introduction, repeatability is typically ± 20% at a 1 ng level.

## 5.0 Responsibilities

5.1 The GC/MS analyst will have been trained in the use and maintenance of the GC/MS instrument, and this will be verified either through the use of training records or through years of service at Battelle with known participation in programs where GC/MS operating skill was required.

5.2 It will be the responsibility of the Mass Spectrometry Laboratory Director to certify that GC/MS operators are fully trained.

5.3 It will be the responsibility of the operator to verify the correct operation of the instrument, through mass calibration and standard calibration curve analyses, prior to analysis of samples. It will be his/her responsibility to promptly report any deviations in instrument performance to the Laboratory Director, to seek guidance in the correction of the problems that go beyond routine repairs and maintenance (e.g., replacing blown filament, cleaning source), and to reanalyze samples which were acquired under poor instrument operating conditions.

5.4 The Laboratory Director will review data before submission, and will review especially high end and low end sample analyses for correct interpretation of the data (e.g., was signal saturated, was signal-to-noise ratio sufficient for quantification. etc.).

## 6.0 Materials and Reagents

### 6.1 Materials

6.1.1 60 m x 0.25 mm id fused silica column coated with nonpolar 5% phenyl methylsilicone stationary phase (DB-5).

6.1.2 Automated Hewlett-Packard gas chromatograph/mass selective detector (6890/5973 GC/MSD) equipped with an autosampler. The instrument can be operated in either the full-scan (SCAN) or selected ion monitoring (SIM) mode. In the SCAN mode, the detector scans all masses repeatedly during the GC run between a lower and an upper mass limit. This mode is best suited for analyzing unknown compounds because it provides a

complete mass spectrum for each GC peak. The mass spectrum may then be used to identify the compound using a computer-based compilation of standard spectra along with a suitable library search algorithm. In the SIM mode, the mass spectrometer monitors only preselected ions, rather than scanning all masses continuously between two mass limits. This results in increased sensitivity. With the GC/MSD system and the SIM mode, detection limits of ~0.01 µg/mL in the extract are attainable.

6.1.3 Liquid microliter syringes, 10 µL, for injection of standards and sample extracts into GC/MS system.

### 6.2 Reagents

6.2.1 Helium carrier gas (purity >99.995%).

6.2.2 Perfluorotributylamine (FC-43) for MS standardization.

6.2.3 Calibration solutions as detailed in SOP BCO-L-21.1.

6.2.4 Methyl-t-butyl ether (mtbe); high purity.

6.2.5 Sony 120 MB 2120 Mini Data Cartridges; formatted QIC-80.

## 7.0 Procedure

### 7.1 Initial Preparations

#### 7.1.1 GC/MS Instrument Set-Up

7.1.1.1. The helium sweep flow (across the GC injector septum) and carrier gas flow are set at approximately 3-5 mL/min and 1-2 mL/min, respectively.

7.1.1.2 Once the column and other system components are assembled and the various flows established, the column temperature is initially increased to about 300 °C for at least 4 hours to condition the column.

7.1.1.3 The MS and data system are set according to the manufacturer's instructions. Electron ionization (70 eV) and an electron multiplier gain of about $10^5$ should be used. Once the entire

GC/MS system has been set up, the system is calibrated as described in Section 7.1.3.

7.1.1.4 The injector module of the HP 7673A autosampler (used with either GC/ITMS or GC/MSD system) is positioned onto the injection port of the GC. It houses the syringe holder and a "turret"-type sample tray with space for 5 vials (one to three samples, one wash and one waste bottle), and associated electronics to perform the injection sequence. A tray module, which can position any one of 100 vials (sample extracts) into the injector, is located adjacent to the injector module. The turret tray rotates the 5 vials into position directly below the syringe. Settings for the sample volume (1 - 5 μL), number of injections per sample (1 - 4), number of sample pre-washes (0 - 10), and number of solvent post-washes (0 - 10) are selected through the front panel of the controller unit. Samples are loaded into the tray module in the order in which they are to be analyzed.

### 7.1.2 Daily GC/MS Tuning and Standardization

7.1.2.1 Once daily, the GC/MS system must be tuned according to manufacturer's instructions, to verify that acceptable performance criteria are achieved.

7.1.2.2 To tune the GC/MS, FC-43 is introduced directly into the ion source via the molecular leak. The instrumental parameters (i.e., lens voltages, resolution, etc.) are adjusted to give documented, standard relative abundances as well as acceptable resolution (i.e. baseline mass resolution) and Gaussian peak shape. If the instrument fails to tune under auto-tune conditions, then the ion source will require cleaning as per the manufacturer's instructions, or other corrective (cleaning) issues must be considered and carried out.

7.1.2.3 After tuning is complete, output one spectrum of FC-43 from the calibration analyses and store this spectrum in the instrument calibration file folder in the MS laboratory.

### 7.1.3 Initial Calibration of the GC/MS System

7.1.3.1 Before analyzing a sample set on a new column, or after the instrument has been vented for cleaning or maintenance,

calibration runs are performed with the Calibration Standards, under the same conditions used to analyze the field samples.

7.1.3.2 Calibration standards encompass five levels, plus a zero level, that bracket the expected concentration range of interest.

7.1.3.3 For the present purposes, a linear response corresponds to a correlation coefficient >0.98 for a linear least squares fit of the concentration versus relative response (peak area of the target ion of the analyte divided by the peak area of the target ion of the IS; $A_S/A_{IS}$) data.

7.1.3.4 Once response linearity has been demonstrated, samples can be analyzed as described below.

## 7.2 Preparation of Samples for Analysis

7.2.1 Arrange the sample vials in sets of 20 samples with six standards (one standard vial from each concentration level; see BCO-L-21.0) in the following order: standard, 4 samples, standard, 4 samples, etc, until all are used.

7.2.2 Inspect each vial. If any defects, such as low volume with respect to the marked volume line are observed, note them in the "Comments" column of the Data Sheet.

7.2.3 For samples with low volume, dilute to the indicated line with mtbe. Recap and analyze.

## 7.3 Sample Extract Analysis

7.3.1 Sample analysis is accomplished using a 60 m x 0.25 mm id, 0.25 µm film thickness, DB-5 fused silica capillary column. Optimum analytical results are achieved with this column by temperature-programming the GC oven from 90 °C to 180 °C at 10 °/min, 180 °C to 210 °C at 2 °/min, then 210 °C to 300 °C at 20 °/min. The injection port is held at 250 °C.

7.3.2 Using the above temperature program rate, the first standard is analyzed to determine the retention time of all target analytes, surrogate standards, and internal standard. For the GC/MSD, the SIM windows (RT ± 5 s) are determined and set according to the operator's manual.

7.3.3 After the final target compound elutes from the column, terminate the acquisition. Hold at the upper temperature for 10-15 min to bake off residual material from the GC column.

7.3.4 Once a stable baseline has been achieved, the system may be readied for the next analysis.

7.3.5 Data processing involves (1) generating a calibration curve for each pesticide and SRS; (2) calculating a solution concentration from the calibration curve using the ChemStation software; and (3) manually reviewing each data file to ensure that identified compounds meet acceptance criteria for co-maximized ion peaks , correct retention time, and correct target-to-qualifier ion ratio.

7.3.6. The characteristic ions for each analyte include the "target ion", which is used for quantification, and the "qualifier ion", which is used to verify detection on the basis of correct intensity (± 25%) relative to the target ion intensity. These diagnostic ions are listed below with their respective relative intensities.

| Compound | Target Ion (Intensity) | Qualifier Ion (Intensity; Acceptable Range) |
|---|---|---|
| Diazinon | 179 (100%) | 304 (50%; 37 - 63%) |
| Chlorpyrifos | 314 (100%) | 316 (73%; 55 - 91%) |
| Malathion | 173 (100%) | 158 (54%; 40 - 68%) |
| Fenchlorphos (SRS) | 285 (100%) | 287 (65%; 49 - 81%) |
| DDT | 235 (100%) | 237 (65%; 49 - 81%) |
| $^{13}C_{12}$-DDT | 247 (100%) | 249 (65%; 49 - 81%) |
| DDE | 318 (100%) | 320 (50%; 40 - 68%) |
| DDD | 235 (100%) | 237 (65%; 49 - 81%) |
| γ-Chlordane | 373 (100%) | 375 (90%; 80 – 100%) |
| $^{13}C_{12}$-DDE | 330 ( 80%) | 258 (100%; 80 - 100%) |

7.3.7 For sample extracts where pesticide levels exceed the calibration range of standards, prepare dilutions at 1:10 and 1:100 for reanalysis.

7.3.7.1 For 1:10 dilutions, add 890 µL of hexane to a muffled/silylated GC vial, add 9 µL of the IS spiking solution, and 100 µL of the sample extract. Cap, invert several times to mix, label, and analyze as per 7.3.1-7.3.6.

7.3.7.2 For 1:100 dilutions, add 980 µL of hexane to a muffled/silylated GC vial, add 10 µL of the IS spiking solution, and 10 µL of the sample extract. Cap, invert to mix, label and analyze as per 7.3.1-7.3.6

## 7.4 Calculations

### 7.4.1 Extract Concentrations

7.4.1.1 The relative area ($A_S/A_{IS}$) of the target ions and analyte concentration values ($C_s$) are analyzed using a standard linear regression analysis routine (e.g., QuattroPro, Excel) to calculate the slope (m) and the intercept (b) from the equation $y = mx+b$, where y is the relative area ($A_S/A_{IS}$), and x is the analyte concentration in µg/mL. These data are taken from the standards that were analyzed concurrently with a given sample set.

7.4.1.2 Once the slope and intercept have been established for each analyte, then these values can be input into the same equation ($y = mx+b$), together with the $A_S/A_{IS}$ (y) value from a sample, and thus obtain the corresponding concentration value (x).

7.4.1.3 For diluted samples, multiply the solution concentration obtained by the appropriate factor (10 or 100) to obtain the concentration of the original sample extract.

### 7.4.2 Calculation of Target Compound Content of Field Samples

7.4.2.1 For dermal wipe samples, multiply the analyte concentration (µg/mL) by the volume (1.0 mL) to obtain the amount (µg) of analyte in the hand wipe.

7.4.2.2 For dust or soil samples, multiply the analyte concentration (µg/mL) by the volume (1.0 mL); then divide by the amount of material extracted (g). This gives the dust or soil concentration (µg/g or ppm). To obtain the surface loading of dust samples (µg/m$^2$), multiply the analyte concentration (µg/mL) by the extract volume (1.0 mL) and the total collected mass of dust <62 µm; then divide by the amount of dust <62 µm extracted, the total area vacuumed (m$^2$). The ratio of total collected mass of dust <62 µm to amount of dust <62 µm extracted is used to account for the split of dust between metals and pesticide analyses.

7.4.2.3 For air samples, multiply the analyte concentration (μg/mL) by the extract volume (1.0 mL), and divide by the air volume sampled (typically 5.76 $m^3$ for sampling 24 h at 4 L/min) to obtain the air concentration (μg/$m^3$). Multiply this air concentration by 1,000 to obtain air concentrations as ng/$m^3$.

7.4.2.4 To determine the recovery of each surrogate recovery compound, multiply its concentration in the extract (μg/mL) by the volume of the extract (1.0 mL); divide this value by the amount spiked (μg) and multiply by 100%.

### 7.4.3 Limit of Detection

7.4.3.1 The limit of detection LOD for a target compound is obtained from the above data. It is defined as:

$$LOD = A + 3.3\sigma \quad 1$$

where $A$ = intercept (coefficient) from the least squares fit to the calibration curve; and $s$ = standard deviation of the lowest concentration measurements.

## 7.5 Quality Control

### 7.5.1 Instrument Tuning and Standardization

7.5.1.1 Refer to Section 7.1.2. These procedures provide a means of monitoring MS performance characteristics over time, and permanent records of the information are kept in the laboratory.

### 7.5.2 Calibration for Quantitative Analysis

7.5.2.1 Refer to the quantitative analysis calibration procedures in Sections 7.1.3 and 7.4. If quantitative responses (in area counts) of the lowest level standard mixture fall below the detection limits, the instrument and/or GC column and injector must be checked for performance degradation. The injector and/or ion source should be cleaned or the first 0.5 m of the column should be removed.

7.5.2.2 Those samples which were analyzed during the period when low level standards were not detected will be reanalyzed.

7.5.2.2 Those samples which were analyzed during the period when low level standards were not detected will be reanalyzed.

## 8.0 Records

8.1 All operations, maintenance and performance calibration data are stored in each instrument's log book.

8.2 All analytical results are entered into the appropriate results template, approved by the laboratory director, and automatically imported into the sample results database.

8.3 Hardcopy output of chromatograms and data reports are available after each run. From each analysis set, one file folder will be used to hold/archive the hardcopy output of the chromatograms (samples and standards), the calibration curve, and the data summary of concentrations. The calibration curve sheet lists the response factor and fit ($r^2$) for the data. The data summary sheet lists the file name and sample name together with the calculated concentration. The sample name here includes the 9 digit laboratory notebook number (for internal tracking) and the UA field ID number. These hardcopy files will be kept in the pesticide extraction laboratory for reference and comparison of instrument performance until the end of the program.

8.4 All data files are stored on ZIP disks for permanent record. The disks are stored in the pesticide extraction laboratory together with the hardcopy files until the completion of the program, at which time they will be stored in the office of the Battelle PI.

8.5 Final calculations of the data are performed in Excel spreadsheets and stored on removable diskettes. All data are entered into the NHEXAS Border project database and sent to UA after one-over-one review of the data.

8.6 A Laboratory Data Sheet is shown in Figure 1. One sheet is filled out and filed with the hard copy output for the sample set.

**Laboratory Data Sheet - NHEXAS Pesticides ITMS Analyses**

Date _____

Analyst _____

Standards LRB #s _____

Helium Gas Cylinder ID # _____

Date Received _____

GC Column ID _____

Serial # _____

Date Installed _____

GC Carrier Gas Flow _____

GC Temperature Program _____

_____

_____

_____

Auto Sampler Settings

# injections/sample _____

injection volume (uL) _____

# syringe pre-rinse _____

# syringe post-rinse _____

ITMS Settings

Multiplier _____

B Value _____

Emission Current _____

# of Samples Analyzed _____

# of Samples Recorded _____

Comments:

Figure 1. Example of laboratory data sheet for analysis of pesticides by GC/MS.