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-1.0**

Title: Analysis of Soil or House Dust Samples Using Chlorpyrifos ELISA Test

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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Analysis of Soil or House Dust Samples Using Chlorpyrifos ELISA Test

1.0 Purpose and Applicability

1.1 This standard operating procedure (SOP) describes the procedures for analyzing both Stage II and Stage III soil and vacuum-cleaner collected house dust samples, and Stage III air samples using a commercially-available enzyme-linked immunosorbent assay test kit for quantification of the pesticide chlorpyrifos.

1.2 For Stage II soil and dust samples, this SOP provides the methods for extraction, analysis and calculation of dust concentration. For Stage III soil, dust, and air samples, GC/MS analysis is the primary analysis method and ELISA analysis is used for only a subset of the total samples for comparison purposes; this SOP provides the ELISA analysis method and necessary calculations for these samples used for comparison of analysis methods.

1.3 The extractions of Stage III soil, dust and air samples are covered in SOPs BCO-L-11 and L-14. At appropriate places in sample work-up, these SOPs direct the analyst to remove an aliquot of the extract and use that aliquot to begin ELISA analysis at a particular point in this SOP. This procedure will be applied to samples collected by SOPs UA-F-8.0, UA-F-9.0, and UA-F-14.

2.0 Definitions

2.1 Antibody-coupled magnetic particles: The solution of chlorpyrifos antibody, where the antibody molecules are covalently-bound to 1 mm diameter particles of a solid magnetic material.

2.2 Enzyme Conjugate: The solution of chlorpyrifos-bound horseradish peroxidase that is used to compete with the native chlorpyrifos for binding to the chlorpyrifos antibody.

2.3 Magnetic Separator: This device is used to apply a magnetic field to the tube in which the ELISA is carried out, this done as a separation/cleanup step in the assay. The assay is initiated with magnetic particles with covalently-bound antibodies. These particles are exposed to both the native pesticide and a fixed amount of an enzyme-pesticide conjugate. The two forms of the pesticide compete with each other for binding with the antibodies. After a fixed reaction time, the magnetic separator is used to retain the magnetic particles (which now

have an antibody-pesticide chain or an antibody-pesticide-enzyme chain) while unreacted material is decanted and washed from the tube.

3.0 References

3.1 Ohmicron, Corp. RaPID Assay product literature; Newtown, Pa. 1-800-544-8881.

3.2 J.M. VanEmon, "Immunoassay Technology", Environ. Testing and Anal., 18-21, July/August 1994.

3.3 E.J. Baum, "The Advantages of Field-Portable Enzyme Immunoassays", Environ. News, December/January 1991/1992.

3.4 "Lambda 4B UV/VIS Spectrophotometer," Operating Directions, Perkin Elmer, C099-1301 Rev. 1, July 1985.

4.0 Discussion

4.1 This method provides rapid detection and quantification for chlorpyrifos in soil, dust and air samples through the use of an antibody-specific ELISA protocol.

4.2 The dust or soil is extracted using sonification in acetone; air samples are Soxhlet-extracted in acetone. A small volume of the extract is diluted in a phosphate buffer and added to the assay. The native chlorpyrifos is in competition with enzyme-labelled chlorpyrifos for binding to the antibody. After a fixed reaction time, the unreacted material is washed out and a color reagent is added. A blue color develops from action of the enzyme conjugate with the color reagent; the intensity of color is inversely proportional to the chlorpyrifos concentration. After a fixed reaction time, the color development is halted with sulfuric acid, the blue color is converted to a yellow product which is measured spectrophotometrically. The concentration of chlorpyrifos is read from a log-linear calibration curve of chlorpyrifos concentration versus relative intensity.

5.0 Responsibilities

5.1 Field sampling and shipping to Battelle will be the responsibility of UA NHEXAS personnel.

5.2 Receipt and verification of samples at Battelle will be the responsibility of the Battelle NHEXAS sample custodian.

5.3 Extractions and ELISA analyses will be carried out at Battelle by trained laboratory staff. Data will be reviewed and checked for accuracy by the pesticide laboratory supervisor. Modifications to the protocol will be prepared by the supervisory scientist and submitted to UA. Laboratory analysts will be responsible for submitting the analysis results to the database coordinator. The laboratory analyst will be responsible for following this SOP, for reporting deviations and problems to the supervisor, and for implementing QA/QC actions as described here. The laboratory analyst will also be responsible for maintaining appropriate record keeping within the lab, as discussed later in this SOP.

6.0 Materials and Reagents

6.1 Materials

6.1.1 Ohmicron Magnetic Separation Rack; 60 position rack

6.1.2 Double-beam UV/VIS Spectrophotometer

6.1.3 Disposable 1 cm path length UV/VIS cuvettes (1 mL size)

6.1.4 Adjustable-volume Pipettor (10 µL) with disposable tips or syringes (10 µL)

6.1.5 Adjustable-volume Pipettor (1 mL) with disposable tips

6.1.6 Glass centrifuge tubes (15 mL) with Teflon-lined screw-top caps

6.1.7 Ultrasonic water bath (Bransonic 52)

6.1.8 Vortex Mixer (American Scientific Products)

6.1.9 Balance with 4 place accuracy (x.xxxx g)

6.1.10 Balance with 2 place accuracy (x.xx g)

6.1.11 Assorted sizes of silanized glass vials with Teflon-lined screw-top caps

6.1.12 Disposable glass test tubes (5 mL)

6.1.13 Large Kim-wipes (15 x 15 inches)

6.1.14 Bench-top centrifuge

6.1.15 Volumetric flasks of various sizes

6.1.16 Disposable latex rubber gloves

6.1.17 Tweezers and spatulas

6.1.18 Glass loaf pan (8" length x 4 " width x 4" height) filled 3/4 full with glass beads (4 mm diameter) and distilled water

6.2 Reagents

6.2.1 High Purity Acetone (HPLC grade or Distilled-in-Glass)

6.2.2 High Purity Methyl-t-Butyl Ether (HPLC grade or Distilled-in-Glass)

6.2.3 Distilled-deionized water

6.2.4 Reagent Chlorpyrifos (>99% pure)

6.2.5 Ohmicron Sample Diluent for Chlorpyrifos RaPID Assay

6.2.6 Ohmicron Chlorpyrifos RaPID Assay kit which includes antibody-coupled particles, enzyme conjugate, color reagent, washing solution and positive control

7.0 Procedure

7.1 Preparation of Calibration Solutions

7.1.1 Prepare stock solution of chlorpyrifos at 1 mg/mL. Weigh out accurately (to 4 places) approximately 0.0010 g (10 mg) (e.g. 0.0098 g; 9.8 mg) of chlorpyrifos in pre-cleaned silanized 20 mL glass vial.

7.1.2 Calculate the volume of solvent that needs to be added to give a 1 mg/mL solution for the amount weighed out:

solvent volume, mL= x mg weighed/1 mg ml^{-1}

7.1.3 Add this volume of methyl-t-butyl ether using a 10 mL syringe. Shake gently to mix.

7.1.4 Record the preparation of this standard in the NHEXAS pesticide sample prep lab notebook. Assign a 9 digit unique code (5 digit book number-2 digit page-2 digit line number) to this solution. Record the weight of chlorpyrifos, the amount of solvent added, the lot number of the solvent used, and the concentration.

7.1.5 Label the vial with the lab notebook number, analyte, concentration, solvent used, preparer's initials and date of preparation. Mark the volume with felt-tip pen on the side of the vial.

7.1.6 Prepare a dilution of the chlorpyrifos stock solution in acetone at 10 μg/mL. To do so, add 100 μL of 1 mg/mL stock to 100 mL volumetric flask and dilute to mark with acetone. Invert several times to mix, transfer to precleaned 100 mL screw-cap vial. Record this as in Step 7.1.4 and label as in Step 7.1.5.

7.1.7 Prepare chlorpyrifos calibration curve solutions at 0 ng/mL, 5 ng/mL, 50 ng/mL, and 250 ng/mL as detailed in the following table:

Chlorpyrifos conc, ng/mL	μL of 10 μg/mL solution	Final Volume, mL
0.0	0	100
5.0	50	100
50	500	100
250	2500	100

Prepare solutions in prelabeled 100 mL volumetric flasks and fill to volume with acetone. For each solution, invert several times to mix, and transfer to prelabeled screw-cap vial. Aliquot ~ 1 mL volumes of these solutions to individual 1.8 mL screw-cap vials that are also labeled. Store in -20°C freezer. Record these standard preparations as in Step 7.1.4 and label as in Step 7.1.5.

7.1.8 Discard all unused calibration curve solutions after 3 months and all stock solutions after 6 months and reprepare.

7.2 Extraction of Stage II House Dust Samples

7.2.1 Sign Chain of Custody form and receive the sample (stored in freezer) from the sample custodian.

7.2.2 Place zip-lock bag on lab bench for ~ 10 min to allow to come to room temperature.

7.2.3 Assemble samples (up to 20) for extraction. Assign unique code number in NHEXAS pesticides extraction lab notebook for each sample (9 digit code as in 7.1.4); enter field ID code next to lab ID number.

7.2.4 Put on gloves. Weigh out approximately 1 g of the dust into a 15 mL centrifuge tube using a 2-place balance and record the weight in the NHEXAS pesticide extraction lab notebook.

7.2.5 Add 5 mL of acetone to the dust, add cap and swirl to wet. Prop up to 20 sample tubes at an angle in the filled loaf pan (tip ends of tubes resting in the beads/water and side of tube just below the cap resting on the side of the pan).

7.2.6 Sonicate the pan of samples in an ultrasonic bath which contains 3"-deep water. Sonicate for 15 min. Remove the pan from the sonic bath and dry the outsides of the tubes.

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

7.2.8 Pipette 1 mL of each extract into a clean, prelabeled 1.8 mL screw-cap vial. [For Stage III samples (dust, soil, and air) where GC/MS analyses are the primary analysis method, the aliquot of sample extract provided at this point in the procedure for the ELISA analysis is 0.5 mL, rather than the 1.0 mL indicated above for Stage II samples; this difference in volume does not affect the method or procedure. Minor changes to final analyte concentration calculations are required as noted later in this SOP.]

7.2.9 Prepare a dilution of each nominal extract at 1:10. For the 1:10 dilution, add 100 µL of the nominal extract to 900 µL of acetone in a clean, prelabeled, 1.8 mL screw-cap vial. Store both nominal extract and 1:10 dilution in a -20°C freezer until analysis. After analysis has been completed, and data are verified and reported, retain the nominal extract in a -80°C freezer for archival purposes.

7.2.10 This step is not performed unless ELISA analyses of the nominal and 1:10 dilution samples indicate that chlorpyrifos levels exceed the calibration range. This step provides for greater dilutions to allow detection of the chlorpyrifos within the calibration range. Prepare 1:100 and 1:1000 dilutions of the nominal extract. For the 1:100 dilution, add

10 µL of the nominal dust extract to 990 µL of acetone in a clean, prelabeled, 1.8 mL screw-cap vial. For the 1:1000 dilution, add 10 µL of the 1:10 dilution to 990 µL of acetone in a second, clean, labeled, 1.8 mL screw-cap vial. Store these dilutions in a -20°C freezer until analysis.

7.2.11 Discard the dust and 4 mL volume of extract remaining in the extraction tube.

7.3 Extraction of Stage II Soil Samples

Prepare these samples for analysis in a manner identical to that used above for the dust samples (Steps 7.2.1-7.2.11), with the exception that soil extracts do not need to be centrifuged. The soil extracts are allowed to stand upright in the extraction tube for 10 min after extraction to allow soil particles to settle to the bottom of the tube.

7.4 ELISA Analysis of Dust and Soil Extracts

7.4.1 Add 250 µL of the chlorpyrifos assay sample diluent to a prelabeled 5 mL disposable test tube. Add 5 µL of the sample extract (or 5 µL of the 1:10 sample extract), and vortex 2 sec to mix. Add 250 µL of the enzyme conjugate solution (from the kit), 500 µL of the antibody-coupled magnetic particles (from the kit), and vortex 2 sec to mix.

7.4.2 Incubate at room temperature for 15 min.

7.4.3 Use the RaPID magnetic separator to decant and wash out the unreacted material twice using distilled-deionized water.

7.4.4 Add 500 µL of the color reagent (from the kit), and vortex 2 sec to mix.

7.4.5 Incubate at room temperature for 20 min.

7.4.6 Add 500 µL of the color-stopping solution (2 M sulfuric acid; from the kit).

7.4.7 Read the absorbance of the yellow-colored solution with a double-beam UV/VIS spectrophotometer (Lambda 4B UV/VIS Spectrophotometer, Perkin Elmer).

7.4.7.1 Turn instrument on. The switch is located in back of the instrument on the right side.

7.4.7.2 After the initialization procedure is completed, the main screen will appear on the CRT, with "700 nm" at the upper left, and absorbance at the upper right.

7.4.7.3 Type "450" and depress the "Go To l" key on the controller keypad.

7.4.7.4 Open the door exposing the cells compartment.

7.4.7.5 Fill two cuvettes with the wash solution from the ELISA kit, making sure you eliminate any bubbles stuck to the cuvette walls with gentle tapping.

7.4.7.6 Insert each cuvette into each of the two cell holders.

7.4.7.7 Close the door to the cells compartment.

7.4.7.8 Depress the "Auto Zero" key on the controller keypad.

7.4.7.9 Pour an ELISA sample from the test tube into a cuvette, making sure no bubbles stick to the cuvette walls.

7.4.7.10 Open the compartment door, remove the cuvette just read from the front cell, and insert an unread sample cuvette.

7.4.7.11 Close the compartment door, wait approximately 3 seconds, and record absorbance in the LRB.

7.4.7.12 Repeat Steps 7.4.7.9 - 7.4.11 until all the ELISA samples have been read.

7.4.8 Record the absorbance (B) of the sample extract. Using average B_0 value determined for the 0 ng/mL standard (see Step 7.5.), calculate the B/B_0 value for the sample solution.

7.4.9 For each sample, verify that the analysis of the 1:10 dilution yields a higher B/B_0 value than the B/B_0 value for the analysis of the nominal dilution. If this is not the case, reanalyze the sample, starting at Step 7.4.1 of this SOP.

7.4.10 From the analyses of the nominal extract and the 1:10 dilution of the nominal extract choose for final calculations the analysis that falls within the calibration range of the analyzed 5 and 250 ng/mL standards (amounts added to the assay from these standards are 25 and 1250 pg, respectively). Do not extrapolate beyond this calibration range as non-linearity of assay response occurs outside of these amounts in the assay.

7.4.11 For samples where both the nominal and 1:10 dilutions fall within the calibration range, choose the 1:10 dilution analysis for final calculation. For samples where the B/B_0 values of both the nominal and 1:10 dilutions are greater than the average B/B_0 value of the 5 ng/mL standard, then record that the chlorpyrifos concentration is less than the detection limits of the assay (see Tolerances and Detection Limits, Section 7.8). For samples where the B/B_0 values of both the nominal and 1:10 dilutions are less than the average B/B0 value of the 250 ng/mL standard, then repeat the analysis of this sample, starting at Step 7.4.1, using the 1:100 and 1:1000 dilutions of the original extract that are prepared using the procedure outlined in Step 7.2.10.

7.5 ELISA Analysis of Calibration Standards

7.5.1 For a calibration curve sample, add 250 µL of the chlorpyrifos assay sample diluent to a 5 mL disposable test tube. Add 5 µL of the calibration solution (prepare in duplicate for the 0, 5, 50, and 250 ng/mL calibration solutions), and vortex 2 sec to mix. Add 250 µL of the enzyme conjugate solution (from the kit), 500 µL of the antibody-coupled magnetic particles (from the kit), and vortex 2 sec to mix.

7.5.2 For a positive control sample, add 250 µL of the kit supplied positive control sample and 5 µL of acetone to a 5 mL disposable test tube and vortex to mix. Add 250 µL of the enzyme conjugate solution (from the kit), 500 µL of the antibody-coupled magnetic particles (from the kit), and vortex 2 sec to mix. Prepare this in duplicate.

7.5.3 Continue as described above in Steps 7.4.2 - 7.4.8.

7.5.4 Generate a linear calibration curve of the log of the amount (pg) of chlorpyrifos added to the assay (25, 250 and 1250 pg) (independent variable; x-axis) versus relative intensity (B/B_0) (dependent variable; y-axis) for these 5, 50, and 250 ng/mL solutions, where B= the absorbance of the individual standard solution and B_0= the average absorbance of the

two 0 ng/mL analyses. (see Tolerancs and Detection Limits, Section 7.8 for discussion of acceptable calibration data).

7.5.5 Using the linear regression, and QuattroPro spreadsheet or equivalent, input B/B_0 values from samples analyzed and obtain log pg values (the pg quantity of chlorpyrifos added to the assay). Using spreadsheet antilog function, convert log pg to pg. See Calculations section to convert from pg in assay to concentration in extract solution and concentration in dust.

7.5.6 Each test kit (100 tests) that is purchased is used for 2 experiment sets. Each experiment set consists of 50 analyses consisting of 20 samples (each analyzed at nominal level and 1:10 dilution), 8 calibration curve samples (4 levels in duplicate), 2 positive control samples (450 pg added to the assay). The 20 samples that are analyzed field samples, and QA/QC samples such as field blanks, field duplicates, lab duplicates, lab spikes and lab method blanks.

7.6 ELISA Analysis of QA/QC Samples

7.6.1 The preparation of a positive control sample for the ELISA analysis is detailed above in Step 7.5.2.

7.6.2 For field and lab blanks, field and lab duplicates and spikes, prepare and analyze the sample as described in Section 7.3 and Section 7.4 of this SOP.

7.7 Calculations

7.7.1 The following relationships are used to calculate the total ng in the sample extract:

	Total ng in Extract For:			
Sample Type	**Nominal Extract**	**1:10 dil.**	**1:100 dil.**	**1:1000 dil.**
Stage II soil/dust	(pg in assay x 5000 µL) / (5 µL x 1000 pg ng^{-1})	nominal x 10	nominal x 10^2	nominal x 10^3
Stage III soil/dust	(pg in assay x 5000 µL) / (5 µL x 0.9 x 1000 pg ng^{-1})	nominal x 10	nominal x 10^2	nominal x 10^3
Stage III air	(pg in assay x 5000 µL) / (5 µL x 1000 pg ng^{-1})	nominal x 10	nominal x 10^2	nominal x 10^3

where "pg in assay" is that amount calculated for a sample analysis from the calibration curve of log amount vs B/B_0; nominal refers to the relationship listed for the nominal extract.

7.7.2 The following relationships are used to calculate the amount of chlorpyrifos in the media sampled:

Sample Type	Concentration in Medium Sampled
Stage II soil/dust	total ng in extract/g of dust extracted= ng/g
Stage III soil/dust	total ng in extract/g of dust extracted= ng/g
Stage III air	total ng in extract/m^3 of air sampled= ng/m^3

7.7.3 The following equation is used to calculate the surface loading (ng/m^2) of chlorpyrifos in the carpet/floor dust:

surface loading, ng/m^2= (chlorpyrifos dust conc, ng/g) x (total g dust collected)/ surface area vacuumed, m^2

7.8 Quality Control

7.8.1 The linear quantification range of the assay is 25-1250 pg. Outside of this range, the assay exhibits non-linear response. The 5 ng/mL standard (25 pg in the assay) should have a B/B_0 value of 0.90±0.08. The 250 ng/mL standard (1250 pg in the assay) should have a B/B_0 value >0.20. If these tolerances are not met then the analysis must be repeated.

7.8.2 The calibration curve should have a correlation coefficient (R) of at least 0.990. If an obvious outlier is present, this one may be removed from the calibration. If the calibration curve is not sufficiently linear, then the analysis must be repeated.

7.8.3 The precision of replicate ELISA analyses of the same extract is at least 20%.

7.8.4 The precision of replicate analyses of the same dust sample (extraction and analysis) is at least 25%.

7.8.5 The accuracy of the ELISA analysis with respect to a standard analytical measurement is ±30%.

7.8.6 One extract will be analyzed in duplicate with each ELISA sample set to verify the precision of the assay.

7.8.7 Two control samples will be analyzed (chlorpyrifos but no soil or dust matrix) with each ELISA sample set to verify accuracy of the assay.

7.8.8 The quantification limits and range of the assay for two different dust sample sizes are shown below. The information is given for the smaller dust size becausse there may be some samples for which 1 g of dust is not available, and the extraction of a smaller dust quantity will change the detection limits.

	Dust/Soil		
		1 g extracted	**0.5 g extracted**
Quantification Limit		25 ppb	50 ppb
Detection Range	nominal	25-1250 ppb	50-2500 ppb
	1:10	250-12500 ppb	500-25000 ppb
	1:100	2.5-125 ppm	5.0-250 ppm
	1:1000	25-1250 ppm	50-2500 ppm

7.8.9 The quantification limits and range of the assay for four different air sample sizes are shown below. The information is given for the smaller sampling intervals because these will be used for various sampling scenarios (e.g. outdoor air sampled for 12 hr or 24 hr depending on dust levels; indoor air air sampled for 8 or 12 hr depending on presence of cigarette smoke; personal air sampled for 6 or 8 hr depending on presence of cigarette smoke). The shorter sampling times will change the detection limits.

	Air sampled at 4 L/min for:			
	24 hr	**12 hr**	**8 hr**	**6 hr**
Detection Limit	5 ng/m^3	10 ng/m^3	15 ng/m^3	20 ng/m^3
Range:nominal	5-250 ng/m^3	10-500 ng/m^3	15-750 ng/m^3	20-1000 ng/m^3
1:10	50-2500 ng/m^3	100-5000 ng/m^3	150-7500 ng/m^3	200-10,000 ng/m^3
1:100	0.5-25 $\mu g/m^3$	1-50 $\mu g/m^3$	1.5-75 $\mu g/m^3$	2-100 $\mu g/m^3$
1:1000	5-250 $\mu g/m^3$	10-500 $\mu g/m^3$	15-750 $\mu g/m^3$	20-1000 $\mu g/m^3$

8.0 Records

8.1 The chain-of-custody form will be signed and dated to verify receipt of the sample by the extraction laboatory. The data analysis form for a sample, which tracks the the sample by UA field code and BCO lab notebook number (NHEXAS pesticide extraction book, in this case) will be given to the Data Coordinator for entry into the database.

8.2 The data generated from a single experiment set will be recorded together in both the NHEXAS pesticides extraction notbook and in the spreadsheet used for calculations. The calibration curve and positive control data will be recorded, as well as solution concentrations of the samples analyzed. The calculations of chlorpyrifos dust concentration will also be recorded in that spreadsheet. The spreadsheet will be transferred to a 3.5" diskette for storage. Hard copies of the data will be stored with the laboratory notebook and in the NHEXAS analysis file maintained by the Data Coordinator.

8.3 Data will be maintained at Battelle for at least 5 yrs. Project lab notebooks will be kept in the laboratory where they are used, and will be transferred to the office of the Battelle co-PI at the conclusion of the program.