STANDARD OPERATING PROCEDURES

DETERMINATION OF METHYL PARATHION IN WIPE SAMPLES BY IMMUNOASSAY

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1.0 SCOPE AND APPLICATION

The objective of this standard operating procedure is to provide guidance on the requirements for the analysis of methyl parathion in wipe samples using enzyme-linked immunoassay (ELISA).

These are standard (i.e., typically applicable) operating procedures with may be varied or changed as required, dependent upon matrix conditions, equipment limitations or limitations imposed by the procedure. In all instances, the ultimate procedures employed should be documented and associated with the final report.

Mention of trade names or commercial products does not constitute U.S. Environmental Protection Agency (U.S. EPA) endorsement or recommendation for use.

2.0 METHOD SUMMARY

The methyl parathion plate kit is designed for the laboratory detection of methyl parathion pesticide residues in surface wipe samples. The plate contains polyclonal antibodies on the inside of the ninety-six wells in the plate. These antibodies will bind both methyl parathion and the parathion-enzyme conjugate supplied with the kit. Wipe samples are extracted with methylene chloride, and then diluted. Diluted extracts are added to the plate, followed by the parathion-enzyme conjugate. After a wash step removes unbound compounds, a colorless substrate/chromoge solution is added which produces a blue color inversely proportional to the methyl parathion concentration.

3.0 SAMPLE PRESERVATION, CONTAINERS, HANDLING AND STORAGE

3.1 Sample Storage

From the time of receipt and after analysis, extracts and unused samples must be protected from light and refrigerated at 4°C (±2°C) for the periods specified by the Task Leader and/or Work Assignment Manager.

Samples, sample extracts, and standards must be stored separately in an atmosphere demonstrated to be free of all potential contaminants.

3.2 Immunoassay Kit Storage

All plate kit components should be refrigerated at 4°C (±2°C) when not in use. Allow reagents to come to room temperature before use. The substrate must never be exposed to sunlight.

3.3 Holding Times

Extraction and analysis of wipe samples shall be completed within seven days of sampling.

4.0 INTERFERENCES AND POTENTIAL PROBLEMS

Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or interferences to

sample analysis. All these materials must be shown to be free from interferences under the conditions of the analysis by analyzing method blanks. Specific selection of reagents and purification of solvents by distillation in an all glass system may be required.

This method does not distinguish between methyl parathion and the following compounds; ethyl parathion, paraoxon, methyl paraoxon, and fenitrothion.

5.0 EQUIPMENT/APPARATUS

1. Methyl Parathion plate kit (EnviroLogix, or equivalent), containing the following;
    - 8 strips of 12 antibody-coated wells each, in plate frame
    - 1 vial of Negative Control (methylene chloride)
    - 1 vial of 0.1 ppm Methyl Parathion Calibrator (in methylene chloride)
    - 1 vial of 1.0 ppm Methyl Parathion Calibrator (in methylene chloride)
    - 1 vial of 6.5 ppm Methyl Parathion Calibrator (in methylene chloride)
    - 1 bottle of Methyl Parathion-enzyme Conjugate
    - 1 bottle of Substrate
    - 1 bottle of Stop Solution
2. Glass vials for dilution
3. Positive-displacement pipetter with disposable tips and plungers for dilution of calibrators and sample extracts, capable of delivering 15 µL
4. Vortex mixer
5. Disposable tip, adjustable air-displacement pipette capable of delivering 50-150 µL
6. Tape or Parafilm
7. Marking pen (indelible)
8. Timer
9. Optical microplate reader; (Molecular Devices Emax Microplate Reader, coupled with Softmax software, Cat #0200-2011, or equivalent).
10. Twelve channel pipette that will measure 50 µL, 100 µL, and 150 µL

11. Racked dilution tubes for loading samples into the plate with a 12-channel pipette.

12. Orbital plate shaker

13. Spectrophotometer

6.0 REAGENTS

1. Deionized water

2. Methylene chloride (glass distilled, suitable for GC)

7.0 PROCEDURES

1. Calibrators must be prepared at 0 ppm, 0.1 ppm, 1 ppm, and 6.5 ppm methyl parathion in the extraction solvent. Calibrators and extracts must be diluted 1:1000 prior to analyzing in the ELISA: using a positive-displacement pipet, deliver 15 µL of each calibrator and sample extract into an appropriately labeled vial containing 15 mL of water. Mix thoroughly by vortexing.

2. Design a plate map incorporating duplicate wells for calibrators and samples. Each plate (or batch) of samples should contain the following;

    1. Duplicate wells of each of the three calibrators, 0.1 ppm, 1.0 ppm, and 6.5 ppm.

    2. Duplicate wells of the Method Blank, which is the extraction solvent at a 1:1000 dilution.

    3. Duplicate wells of all samples.

    4. Fill in any extra wells with more calibrators or dilutions of samples.

    5. Complete the sample log in the data file. Be sure "Sample ID" is correctly entered. This report will serve as the only sample analysis log.

3. Load calibrators/samples into the plate. Add 50 µL diluted calibrators and samples to duplicate wells of the plate, following the plate map designed in step 2.

4. Immediately add 150 µL of parathion-enzyme conjugate to each well of the plate, in the same order that calibrators and samples were applied. Mix the plate by gently swirling on a flat surface, then cover wells with tape or Parafilm and incubate for 1 hour.

5. Shake the well contents out into a sink or suitable container. Wash the wells by filling to overflowing with clean deionized water. Shake out as much water as possible. Repeat this wash procedure 4 times more, slapping the plate on a paper towel after the last one to remove as much water as possible.

6. Immediately add 100 µL substrate solution to each well. Mix gently as above, cover with new tape or Parafilm, and incubate for 30 minutes.

7. Add 100 µL stop solution to each well, turning the color from blue to yellow. Read the absorbance of the wells at a wavelength of 450 nanometers, subtracting the 650 (or 630) nanometer reading as a reference wavelength. Plot the concentration of the 3 non-zero calibrators on a log scale versus their mean absorbance (linear scale) to yield a semi-log curve. Multiply results by the volume of extraction solvent used in milliliters to convert data to concentration (µg/100 cm^2).

8. Sample analysis should be repeated if the values of the duplicate varies by more than 50% and the calculated final concentrations are close to the required Action Level.

8.0 CALCULATIONS

The microplate reader calculates a semi-log curve for the three calibrators and reports the methyl parathion concentration in µg/100 cm^2, so no calculation is required by the user. An example of the semi-log curve and the concentration output is found in Figure 1.

9.0 QUALITY ASSURANCE/QUALITY CONTROL

The limit of quantification (LOQ) for surface wipe samples is 0.1 ppm in the extracts, equivalent to 3 µg/100 cm^2, for a sample extracted with 30 mL of solvent. Samples with higher optical density than the lowest calibrator are reported as "<0.1 ppm", or < 3 µg/100 cm^2. Samples with lower optical density than the highest calibrator are reported as ">6.5 ppm", or > 195 µg/100 cm^2, and should be diluted and reanalyzed.

Alternate methods such as GC/MS should be used to confirm 10% of the results.

10.0 DATA VALIDATION

The accuracy of sample IDs, dilution factors, and the linearity of the standards should be reviewed and coutersigned by a peer, preferably one familiar with immunoassay analysis, before submittal of preliminary results.

11.0 HEALTH AND SAFETY

When working with potentially hazardous materials, refer to U.S. EPA, OSHA and corporate health and safety practices. More specifically, refer to ERT/SERAS SOP #3013, SERAS Laboratory Safety Program.

12.0 REFERENCES

Methyl-Parathion Plate Kit manual, EnviroLogix Inc. (see Attachment 1, Appendix B).