Determination of Organophosphorous Pesticides in Water Samples

1.0 Scope and Application

1.1 This is a modified EPA Method 8141A and describes the sample preparation and quantitative analysis of trace level organophosphorous pesticides in surface, municipal and wastewater using liquid-liquid extraction and high resolution gas chromatography with Flame Photometric Detector (FPD) in phosphorous mode and Thermionic Bead Specific Detector (TSD). The following target analytes can be determined by this method:

Target Analyte	CAS Registry No.
Aspon	3244-90-4
Azinphos ethyl	2642-71-9
Azinphos methyl	86-50-0
Bolstar (Sulprofos)	35400-43-2
Carbophenothion	786-19-6
Chlorfenvinphos	470-90-6
Chlorpyrifos	2921-88-2
Chlorpyrifos methyl	5598-13-0
Ciodrin (Crotoxyphos)	7700-17-6
Coumaphos	56-72-4
Demeton-s	126-75-0
Diazinon	333-41-5
Dichlofenthion	97-17-6
Dichlorvos	62-73-7
Dicrotophos	141-66-2
Dimethoate	60-51-5
Dioxathion	78-34-2
Disulfoton	298-04-4
Ethion	563-12-2
Ethoprop	13194-48-4
Famphur	52-85-7
Fenchlorphos (Ronnel)	299-84-3
Fenitrothion	122-14-5
Fensulfothion	115-90-2
Fenthion	55-38-9

Fonofos (Dyfonate)	944-22-9
Leptophos	21609-90-5
Malathion	121-75-5
Merphos	150-50-5
Methidathion	950-37-8
Mevinphos (Phosdrin)	7786-34-7
Molinate	2212-67-1
Naled (Dibrom)	300-76-5
Parathion, Ethyl	56-38-2
Parathion, Methyl	298-00-0
Phorate	298-02-2
Phosmet	732-11-6
Phosphamidon	13171-21-6
Sulfotep	3689-24-5
Terbufos	13071-79-9
Tetrachlorvinphos	22248-79-9
Thiobencarb	28249-77-6
Thionazin	297-97-2
Tokuthion	34643-46-4
Trichlorfon	52-68-6
Trichloronate	327-98-0
Triphenyl phosphate (surrogate)	115-86-6

1.2 The estimated detection limit for each analyte is listed in Table 1. The actual MDL may differ from those listed, depending upon the nature of interferences in the sample matrix. Validation of the target analytes produced recoveries greater than 70 percent (Appendix I) for most analytes. Some target compounds are widely accepted as having lower recoveries, as listed in Section 9.3.3. The range of percent recoveries for each analyte is also included in Table 1.

1.3 If possible, unknowns in the sample will be qualitatively confirmed for compound identification by Gas Chromatography with a Mass Spectrometer – Ion Trap Detector (GC/MS-ITD).

Table 1. Organophosphorous pesticides analyzed, their Minimum Detection Limits (MDL), Reporting Limits (RL) and Range of Percent Recovery in water.

Target Analytes	MDL (µg/l)	RL (µg/l)	Recovery Range (%)*
Aspon	0.030	0.050	85 – 105
Azinphos ethyl	0.030	0.050	95 – 110
Azinphos methyl (Guthion)	0.030	0.050	50 – 90
Bolstar (Sulprofos)	0.030	0.050	80 – 95
Carbophenothion	0.030	0.050	90 – 100
Chlorfenvinphos	0.030	0.050	80 – 100
Chlorpyrifos	0.020	0.050	80 – 100
Chlorpyrifos methyl	0.020	0.050	95 – 110
Ciodrin (Crotoxyphos)	0.030	0.050	90 – 110
Coumaphos	0.040	0.050	50 – 90
Demeton-s	0.040	0.050	30 – 80
Diazinon	0.005	0.020	95 – 110
Dichlofenthion	0.030	0.050	95 – 105
Dichlorvos	0.030	0.050	85 – 105
Dicrotophos	0.030	0.050	20 – 70
Dimethoate	0.030	0.050	90 – 100
Dioxathion	0.030	0.050	50 – 90
Disulfoton	0.010	0.050	80 – 95
Ethion	0.020	0.050	80 – 105
Ethoprop	0.030	0.050	80 – 100
Famphur	0.030	0.050	90 – 105
Fenchlorphos (Ronnel)	0.030	0.050	90 – 105
Fenitrothion	0.030	0.050	90 – 110
Fensulfothion	0.030	0.050	40 – 80
Fenthion	0.030	0.050	80 – 100
Fonofos (Dyfonate)	0.020	0.050	85 – 110
Leptophos	0.030	0.050	80 – 100
Malathion	0.030	0.050	95 – 105
Merphos	0.030	0.050	85 – 110
Methidathion	0.030	0.050	95 – 105
Mevinphos (Phosdrin)	0.030	0.050	80 – 90
Molinate	0.100	0.200	65 – 100
Naled (Dibrom)	0.030	0.050	40 – 80
Parathion, Ethyl	0.030	0.050	85 – 110
Parathion, Methyl	0.010	0.050	90 – 105
Phorate	0.030	0.050	80 – 95

Phosmet	0.030	0.050	80 – 100
Phosphamidon	0.030	0.050	85 – 100
Sulfotep	0.030	0.050	95 – 110
Terbufos	0.030	0.050	85 – 100
Tetrachlorvinphos	0.030	0.050	80 – 105
Thiobencarb	0.100	0.200	90 – 110
Thionazin	0.040	0.050	95 – 110
Tokuthion	0.030	0.050	85 – 105
Trichlorfon	0.030	0.050	90 – 115
Trichloronate	0.030	0.050	80 – 105
Triphenyl phosphate (surrogate)	0.030	0.050	90 – 105

* Recoveries fall within 75-125% accept as discussed in Section 9.3.3.

2.0 Summary of Method

2.1 A measured volume of sample (1000 ml) is extracted with methylene chloride (DCM) using a separatory funnel. The DCM extract is dried with sodium sulfate, evaporated using Kuderna-Danish (K-D) and solvent exchanged into petroleum ether. The extract is concentrated with micro-snyder (micro K-D) apparatus to approximately 1 ml and adjusted to 2.0 ml with iso-octane. The extracts are analyzed by gas chromatography using conditions which permit the separation and measurement of the target analytes in the extracts by FPD and TSD detection.

2.2 Interferences in analyses may be encountered in very dirty samples and cleanup may be needed to aid in the elimination or reduction of these interferences. Florisil column cleanup or Gel Permeation Chromatography (GPC) procedures will be followed.

3.0 Interferences

3.1 Solvents, reagents, glassware, and other sample processing hardware may cause GC artifacts and/or elevated baselines, resulting in the misinterpretation of chromatograms. All materials should be demonstrated to be free from interferences under the conditions of the analysis by running method blanks initially and with each sample lot. Specific selection of reagents and purification of solvents by distillation in all-glass systems are required. High-purity distilled-in-glass solvents are commercially available.

An effective way of cleaning laboratory glassware is by rinsing with polar and non-polar solvents before use. The cleaning procedure used must be tested by analyzing procedural blanks prior to analyzing samples.

3.2 Phthalates are common laboratory contaminants that are used widely as plasticizers. Sources of phthalate contamination include plastic lab-ware, plastic tubing, plastic gloves, plastic coated glassware clamps, and have been found as a contaminant in Na_2SO_4.

Polytetrafluoroethylene (PTFE) can be used instead of polypropylene or polyethylene to minimize this potential source of contamination. However, use of PTFE lab-ware will not necessarily preclude all phthalate contamination. Na_2SO_4 can be solvent rinsed to eliminate contaminants.

3.3 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source. A Florisil or GPC cleanup procedure can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4.0 Apparatus and Laboratory Supplies

4.1 Separatory funnel. 2000-ml, with TFE-fluorocarbon stopcock, ground glass or TEF stopper.

4.2 Automatic shaker designed to fit 2 liter separatory funnels with rpm and timer controls.

4.3 Beakers. Borosilicate glass, 400 mL

4.4 Chromatographic Column. 300 cm x 22 cm borosilicate glass chromatography column with Teflon stopcock.

4.5 Glass wool. Pyrex - solvent washed prior to use.

4.6 Kuderna-Danish (K-D) Apparatus.

4.6.1 Concentrator tube. 15 mL, graduate (Kontes K0570012-0500, or equivalent). A ground stopper, 19/22 joint, is used to prevent evaporation of extracts.

4.6.2 Evaporation flask. 500 mL (Kontes K-570050-0500, or equivalent), attached to concentrator tube with blue clamp (Kontes K-662750-0012).

4.6.3 Snyder column. Three ball (Kontes K-503000-0121, or equivalent).

4.6.4 Micro-Snyder column. Alltech 9058 or equivalent.

4.6.5 Boiling chips. Hengar granules, high purity amphoteric alundum - extracted with acetone and petroleum ether. Note that boiling chips can be a significant source of contamination if not properly cleaned.

4.7 Water bath. Blue M, 115 V, thermostatically controlled with stainless steel cover to fit K-D apparatus, installed in a fume hood.

4.8 GC vials. GC autosampler vials, borosilicate glass, 2 mL with PTFE-lined screw cap.

4.9 Analytical balance. Capable of weighing 0.1 mg.

4.10 Drying oven.

4.11 Disposable Pasteur Pipettes. 2 mL, rinsed with solvents before use.

4.12 Glass filter funnel. Fluted, 75 mm or larger.

4.13 Graduated cylinder. 1000 ml, 250 mL and 100 mL.

4.14 Culture tubes. 13 x 100 mm with PTFE lined screw cap.

4.15 Analytical systems

4.15.1 Gas chromatograph. **Agilent 6890** equipped with dual FPD detectors with phosphorous filters, split-splitless injector in pulsed splitless mode with EPC, a **7683** autosampler and dual capillary columns (J&W Scientific) connected to a single injection port using a "Y" press fit connector. Section 9 describes the acquisition and analysis procedures while Table 2 lists the operating parameters.

4.15.2 Gas chromatograph. **Varian 3600**, equipped with dual Thermionic Specific Detectors (TSD), direct and Septum Programmable Injector (SPI), a **8200** autosampler and dual megabore columns (J&W Scientific). Section 9 describes the acquisition and analysis procedures while Table 3 lists the operating parameters.

4.15.3 Data System. Hewlett-Packard, to collect and record GC data, generates reports, computes and records response factors for multi-level calibrations. Data system should be capable of calibrating a method using a minimum of 5 concentrations of analytical standards and calculating in external standard mode.

Table 2 Operating parameters for Agilent 6890 GC/FPD

Gases
- Carrier: Helium, 1 mL/min
- Makeup: Nitrogen, 1 mL/min
- Flame: Air and Hydrogen

Columns
- DB5, 30 m x 0.32 mm I.D. x 0.25 µm film thickness
- DB17, 30 m x 0.32 mm I.D. x 0.25 µm film thickness

Inlet
- Isocratic temp: 200 ºC

Oven
- Initial temperature: 90 ºC Initial time: 1.00 min
- Ramp 1: 8.0 deg/min, final temp 220 ºC, hold time 5.00 min
- Ramp 2: 20.0 deg/min, final temp 250 ºC, hold time 13.00 min

Detectors (FPD)
- Temperature: 225 ºC

Injection Volume: 3 µL

Table 3 Operating parameters for Varian 3600 GC/TSD

Gases
- Carrier: Helium
- Makeup: Nitrogen
- Flame: Air and Hydrogen

Columns
- DB5, 15 m x 0.53 mm I.D. x 1.5 µm film thickness
- DB17, 15 m x 0.53 mm I.D. x 1.5 µm film thickness

Inlet

Isocratic temp: 190 ºC

Oven
Initial temperature: 190 ºC Initial time: 3.00 min
Ramp 1: 5.0 deg/min, final temp 250 ºC, hold time 10.00 min

Detectors (TSD)
Temperature: 225 ºC

Injection Volume: 3 µL

5.0 Reagents, materials, gases and standards

5.1 Reagent water is defined as water in which an interferent is not observed at method detection limit of each parameter of interest. Deionized (DI) water was used for method validation and as method blank.

5.2 Petroleum ether (PE), acetone, methylene chloride (DCM), diethyl ether, isooctane. Pesticide residue quality or equivalent.

5.3 Sodium sulfate. Anhydrous granular reagent grade, rinsed with PE prior to use.

5.4 Nitrogen. Ultra-pure (99.99999%) for GC/FPD/TSD

5.5 Helium. Ultra-pure (99.99999%) for GC/FPD/TSD

5.6 Air. Compressed, breathing quality for GC/FPD/TSD

5.7 Hydrogen. Ultra high purity for GC/FPD/TSD

5.8 Stock standards. Individual stock standards (100 µg/ml) are purchased as certified solutions from ChemService as well as premixed solutions of 8140 and 8141A, as shown in Table 4. Additional compounds analyzed are prepared as WPCL solution “OP Mix C”

Table 4 Organophosphorous analyte spiking solutions and standard curves.

EPA 8140 Analytes	EPA 8141A Analytes	OP Mix C Analytes
Azinphos methyl(Guthion)	Aspon	Dimethoate
Bolstar (Sulprofos)	Azinphos ethyl	Malathion
Chlorpyrifos	Carbophenothion	Methidathion

Coumaphos
Demeton-s
Diazinon
Dichlorvos
Disulfoton
Ethoprop (Prophos)
Fenchlorphos (Ronnel)
Fensulfothion
Fenthion
Merphos
Mevinphos (Phosdrin)
Naled (Dibrom)
Parathion, Methyl
Phorate
Tetrachlorvinphos
Tokuthion
Trichloronate
Chlorfenvinphos
Chlorpyrifos methyl
Ciodrin (Crotoxyphos)
Dichlofenthion
Dicrotophos
Dioxathion
Ethion
Famphur
Fenitrothion
Fonofos (Dyfonate)
Leptophos
Phosmet (Imidan)
Phosphamidon
Terbufos
Thionazin (Zinophos)
Trichlorfon (Dylox)
Molinate
Parathion,Ethyl
Sulfotep
Thiobencarb

6.0 Sample Collection, Preservation, and Storage

6.1 Samples are collected in one liter amber glass bottles and iced or refrigerated at 4 °C from time of collection until extraction.

6.2 All samples must be extracted within 7 days and completely analyzed within 40 days of extraction.

7.0 Sample Extraction

7.1 Remove water samples from refrigerator and transfer contents to a pre-cleaned 2-liter separatory funnel. Immediately add 1.0 ml of the 200 ppb OP pesticide surrogate solution to every sample. For Method Blank, add 1.0 ml of the 200 ppb OP pesticide surrogate solution (TPP) to 1 liter DI water. For laboratory control spike (LCS) and matrix spikes (MS/MSD) also add 1.0 ml of 200 ppb OP pesticide spiking solution for each mix (8140, 8141A and Mix C)

7.2 Add 60 ml of methylene chloride (DCM) to the empty bottle, replace the cap and rinse the bottle. Pour the DCM into the separatory funnel and repeat with another 60 mL aliquot of DCM. Extract the sample by shaking the funnel for 5 minutes on the auto-shaker with periodic venting to release excess pressure. Allow organic layer to separate from the water phase for a minimum of 10 minutes. Collect the methylene chloride extract in a 400 ml beaker.

7.3 Add a second 120 ml volume of methylene chloride to the separatory funnel and repeat the extraction procedure a second time, combining the extracts in the beaker.

7.4 Set up and label pre-cleaned K-D flasks with concentrator tubes and attached with a blue clamp on ring stands in the fume hood. Add 0.5 ml iso-octane as “keeper” and a solvent rinsed micro-boiling chip to each K-D concentrator tube. Place a filter funnel containing a plug of pre-cleaned glass wool in the bottom of the funnel and place the funnel in the top of the K-D flask. Add about two inches of solvent rinsed sodium sulfate to the funnel.

7.5 Pour the combined extracts from the beaker through sodium sulfate into the K-D flask. Rinse the beaker with about 10 mL of DCM and add this rinse to the sodium sulfate. Repeat with another 10 mL DCM rinse. Rinse the sodium sulfate with an additional portion of DCM (~10-20 mL).

7.6 Place a Snyder column on the K-D flask, clamp with a green clamp and place the flask on the hot water bath set at 78-82 ^{o}C. Evaporate solvent on the hot water bath. When the apparent volume of solvent in the

concentrator tube is 5-10 mL, add 20-30 mL of petroleum ether through the top of the Snyder column. Repeat this procedure when the apparent volume is again at 5-10 mL. When the reflux line falls below the top of the Snyder column, the K-D apparatus should be removed from the hot water bath. Dry outer KD apparatus with a Kimwipe to prevent condensation water from entering the concentrator tube. Upon cooling, remove the concentrator tube from the K-D apparatus.

7.7 Place a clean micro-Snyder column on the concentrator tube with a blue clamp, add a new micro boiling chip and place in a 400 mL beaker containing water heated to approximately 78 ^{o}C on a hot plate. If the solvent does not begin to boil, remove the tube from the bath immediately, allow it to cool slightly, add a new micro boiling stone to prevent it from bumping and place it back in the bath.

7.8 When the solvent has been evaporated to 0.5-1 mL remove the tube from the bath and allow it to cool in a test tube rack. Dry outer KD apparatus with a Kimwipe to prevent condensation water from entering the concentrator tube. Remove the micro-Snyder column and add iso-octane to the concentrator tube to reach a final volume of 2.0 mL. Mix the tube contents by tapping the bottom of the tube causing a vortex which will rinse the sides of the tube. A Vortex Genie mixer may be used for this step.

7.9 Transfer the solution from the concentrator tube to a culture tube and cap with a Teflon faced cap. Place extracts in a refrigerator for storage until analysis or cleanup, if necessary.

7.10 When ready for analysis, transfer extract to labeled GC vials and cap.

8.0 Cleanup Procedure

8.1 Cleanup of dirty samples may be necessary due to interferences in the analysis of baseline or co-elution with target analytes of the sample extract. Follow the in-house SOP for Florisil® column or GPC method, as needed.

9.0 Analytical Procedure

9.1 The final extract will be analyzed on an Agilent 6890 GC/FPD and a Varian 3600 GC/TSD.

9.1.1 Chromatographic conditions for operating the Agilent 6890 GC/FPD are found in Table 2.

9.1.2 Chromatographic conditions for operating the Varian 3600 GC/TSD are found in Table 3.

9.2 GC acquisition

9.2.1 Pour several isooctanes into GC vials using the same lot as used for samples with each GC run.

9.2.2 Pour standard curves into GC vials using 20, 50, 100, 200 and 500 ppb Std 8140 and 8141A and 50, 100, 200 and 500 ppb OP Mix C in isooctane. Pour extra vials of a midlevel concentration for use as CCV (to be analyzed every 20 samples or less).

9.2.3 Create sequence file and sequence table on computer. Use the WPCL login number for “Data Subdirectory” and “Save As” sequence name.

9.2.4 Acquire data and recap each vial daily to preserve sample integrity.

9.3 Analysis

9.3.1 Recalibrate OP curves and analyze samples in external standard mode. Add a printed chromatogram and report for each standard and sample to folder.

9.3.2 Certain analytes will coelute on a given column. However, using two columns with different polarities will allow for confirmation of target analytes.

9.3.3 EPA Method 8141A cites the following common analytical difficulties encountered for target analytes:

9.3.3.1 The water solubility of Dichlorvos (DDVP) is 10 g/L at 20EC, and recovery is poor from aqueous solution.

9.3.3.2 Naled is converted to Dichlorvos (DDVP) on column by debromination. This reaction may also occur during sample workup. The extent of debromination will depend on the nature of the matrix being analyzed. The analyst must consider the potential for debromination when Naled is to be determined.

9.3.3.3 Trichlorfon rearranges and is dehydrochlorinated in acidic, neutral, or basic media to form Dichlorvos (DDVP) and hydrochloric acid. If this method is to be used for the determination of organophosphates

in the presence of Trichlorfon, the analyst should be aware of the possibility of rearrangement to Dichlorvos to prevent misidentification.

9.3.3.4 Demeton (Systox) is a mixture of two compounds; O,O-diethyl O-[2-(ethylthio)ethyl]phosphorothioate (Demeton-O) and O,O-diethyl S-[2-(ethylthio)ethyl]phosphorothioate (Demeton-S). Two peaks are observed in all the chromatograms corresponding to these two isomers. It is recommended that the early eluting compound (Demeton-S) be used for quantitation.

9.3.3.5 Dioxathion is a single-component pesticide. However, several extra peaks are observed in the chromatograms of standards. These peaks appear to be the result of spontaneous oxygen-sulfur isomerization. Because of this, Dioxathion is not included in composite standard mixtures.

9.3.3.6 Merphos (tributyl phosphorotrithioite) is a single-component pesticide that is readily oxidized to its phosphorotrithioate (Merphos oxone). Chromatographic analysis of Merphos almost always results two peaks (unoxidized Merphos elutes first). As the relative amounts of oxidation of the sample and the standard are probably different, quantitation based on the sum of both peaks may be most appropriate.

9.3.3.7 Many analytes will degrade on reactive sites in the chromatographic system. Analysts must ensure that injectors and splitters are free from contamination and are silanized. Columns should be installed and maintained properly.

9.3.3.8 Performance of chromatographic systems will degrade with time. Column resolution, analyte breakdown and baselines may be improved by column washing. Oxidation of columns is not reversible.

10.0 References

U.S. Environmental Protection Agency, Office of Water, EPA 821-R-92-002, April 1992, Methods For The Determination of Nonconventional Pesticides In Municipal And Industrial Wastewater, p. 227.

Method 622, *The Determination of Organophosphorous Pesticides in Municipal and Industrial Wastewater*.
Method 8141A, *Organophosphorous Compounds by Gas Chromatography: Capillary Column Technique.*

APPENDIX I: Validation of organophosphorous compounds in water.

1.0 Scope

A method validation for organophosphorous compounds in water was performed using a modified EPA 8141A method, as detailed in the **OP's in Water SOP**. Recoveries shall be 75-125% with RPE of less than 20 percent. Method detection and reporting limits were determined during the method development phase.

2.0 Setup

Perform nine replicate spiked extractions and analysis following the SOP for water extraction of OP's for **each** mix (8140, 8141A and OP-Mix C). Using 1 liter of DI water, add 1 mL of 200 ppb OP spike mix and 1 mL of 200 ppb TPP (surrogate) to each replicate. Also, prepare a method blank using 1 liter of DI water and 1 mL of 200 ppb TPP (surrogate).

3.0 Results

The following tables show the results for the OP method validation as well as statistical data. Table A-1 contains the values obtained while Table A-2 contains the results converted to percent recoveries.