

Bayer Method DL-002-S10-03: An Analytical Method for the Determination of Residues of Trichlorfon and its Metabolites in Soil and Thatch Using LC/MS/MS

1.0 SUMMARY

An analytical method was developed to determine the residues of trichlorfon (Dimethyl (2,2,2-trichloro-1-hydroxyethyl)phosphonate) and its metabolites DDVP (2,2-dichloroethenyl dimethyl phosphate), desmethyl DDVP (2,2-dichloroethenyl methyl hydrogen phosphate) and DCA (dichloroacetic acid) in soil and thatch. In addition this analytical method provides details of LC/MS/MS conditions to monitor for the presence of the trichlorfon metabolite methyldichloroacetyl phosphate (MDAP).

This method consists of two separate extractions. In the first extraction, a soil/thatch sample is fortified with trichlorfon and DDVP. The samples are extracted with acidified methanol/water and shaken. An aliquot of the sample is diluted in acidified acetonitrile, amended with an isotopic internal standard and analyzed by LC/MS/MS for trichlorfon and DDVP. In the second extraction, a soil/thatch sample is fortified with desmethyl DDVP and DCA. The samples are extracted with ammonium bicarbonate and shaken. An aliquot of the sample is diluted in acidified acetonitrile/water, amended with an isotopic internal standard and analyzed by LC/MS/MS for desmethyl DDVP and DCA.

The method limit of quantitation (LOQ) for trichlorfon, DDVP, desmethyl DDVP and DCA is 0.25ppm in soil/thatch.

2.0 BACKGROUND

Trichlorfon is fast-acting insecticide registered by Bayer Environmental Science for control of surface feeding and soil insects including white grubs, mole crickets, sod webworms and cutworms. The analytical method presented in this report is designed to measure residues of trichlorfon and its metabolites DDVP, desmethyl DDVP and DCA using LC/MS/MS detection.

3.0 APPARATUS (functional equivalents may be substituted)

- Various general laboratory glassware and utensils
- MicroMan pipettors and tips (Rainin, M50, M250, and M1000)
- Imtakt Unison UK-C18 3 μ m 75 mm x 3.0 mm column with in-line column filter.
- SeQuant ZIC-HILIC 4.6 x 150 mm, 5 μ m, 200 Å, Part No. Q2712-155 with in-line column filter.
- API electrospray ionization (ESI) interface, Shimadzu HPLC pumps and a CTC PAL autosampler, and Analyst 1.4.1 data collection software (Applied Biosystems)
- Finnigan TSQ Quantum Ultra liquid chromatograph/mass spectrometer (LC-MS/MS) equipped with an electrospray interface, Surveyor HPLC pumps and autosampler, and XCalibur 1.4 data collection software (Thermo Electron Corporation)

4.0 REAGENTS AND CONSUMABLES (functional equivalents may be substituted)

- Methanol (HPLC Grade)
- Acetonitrile (HPLC Grade)
- Acetic Acid, 88% (Reagent Grade)
- Water (HPLC Grade or Millipore)
- Ammonium bicarbonate (chemical reagent)
- Hydrochloric acid (35-38% purity, trace metal grade)
- 1.0 M ammonium bicarbonate. Dissolve 79g of ammonium bicarbonate in 1L of millipore water
- Aqueous 0.1% acetic acid solution. Add 1mL of acetic acid to 1000mL of millipore water
- Acetonitrile/aqueous 0.1% acetic acid solution (50/50). Mix 500mL of aqueous 0.1% acetic acid solution with 500mL of acetonitrile and mix well
- Acetonitrile/aqueous 0.1% acetic acid solution (20/80). Mix 800mL of aqueous 0.1% acetic acid solution with 200mL of acetonitrile and mix well
- 0.5% aqueous hydrochloric acid solution. Transfer 995mL of deionized water into a measuring cylinder. Add 5ml of hydrochloric acid and mix well.
- Methanol/0.5% aqueous hydrochloric acid solution (50/50). Mix 500mL of 0.5% aqueous hydrochloric acid solution with 500mL of methanol and mix well.
- 1000mL plastic wide mouth bottles (Fisherbrand Catalog Number, 02 896F)
- HPLC vials and caps (2-mL, National Scientific, Part Nos. C4011-5W and C4011-55)

5.0 PREPARATION OF STANDARD SOLUTIONS

To prepare the standard solutions, standards of the analyte trichlorfon, and its metabolites DDVP, desmethyl DDVP, DCA and isotopic internal standard d_6 -DDVP and d_2 -DCA are necessary. These standards may be obtained from Bayer CropScience, 17745 South Metcalf, Stilwell, KS 66085. No analytical standards are available for methyldichloroacetyl phosphate (MDAP). Additional details about these chemicals are given in Appendix 1.

The toxicities of these chemicals have not been precisely determined. Thus, each chemical must be treated as a potential health hazard. Exposure to these chemicals should be reduced to the lowest reasonable level.

NOTE: The following procedure is an example description of how standard solutions may be prepared. Alternate or additional standards of appropriate weight and volume may be prepared as needed.

Class "A" volumetric glassware or calibrated pipets should be used in the preparation of all analytical standards. All standard solutions should be stored in a refrigerator in amber glass bottles when not in use. Solutions should be allowed to warm to room temperature prior to use. Corrections for standard purities should be applied when expressing standard concentrations.

5.1 Primary Stock Standard Solution

Prepare individual 100µg/mL stock solutions of trichlorfon or its metabolites DDVP, desmethyl DDVP and DCA by transferring about 0.0050 grams of analyte into a 50mL volumetric flask. Dilute to volume with acetonitrile except for desmethyl DDVP. Desmethyl DDVP is initially dissolved in 5.0mL of deionized water first and then bring the 50mL volumetric flask to volume with acetonitrile. Store at <-10°C when not in use.

5.2 Trichlorfon, DDVP, desmethyl DDVP and DCA Fortification Standard Solutions

Prepare individual 10.0µg/mL fortification standards solution of each analyte by taking a 10.0mL aliquot of each of the 100µg/mL stock solution and diluting to 100mL with acetonitrile. Refrigerate when not in use.

5.3 d₆-DDVP and d₂-DCA Isotopic Internal Standard Solutions

Prepare individual 50µg/mL stock solutions of d₆-DDVP and d₂-DCA by transferring 0.005 grams of each analyte into a 100mL volumetric flask. Dilute to volume with acetonitrile.

Prepare a 5µg/mL stock solution of d₆-DDVP by diluting 10mL of the 50µg/mL d₆-DDVP stock solution to 100mL with acetonitrile.

5.4 Intermediate Mix Standard Solutions

5.4.1 Trichlorfon and DDVP Intermediate Mix Standard Solutions

Prepare a 10µg/mL intermediate mix standard solution of trichlorfon and DDVP by pipetting 10mL each of 100µg/mL stock solutions of trichlorfon and DDVP to a 100mL volumetric flask. Bring the flask to volume with acetonitrile.

Prepare a 0.1µg/mL intermediate mix standard solution of trichlorfon and DDVP by pipetting 1.0mL of the 10µg/mL mixed stock solution of trichlorfon and DDVP into a 100mL volumetric flask. Bring the flask to volume with acetonitrile.

5.4.2 Desmethyl DDVP and DCA Intermediate Mix Standard Solutions

Prepare a 10µg/mL intermediate mix standard solution of desmethyl DDVP and DCA by pipetting 10mL each of 100µg/mL stock solutions of desmethyl DDVP and DCA into a 100mL volumetric flask. Bring the flask to volume with acetonitrile.

Prepare a 0.1µg/mL intermediate mix standard solution of desmethyl DDVP and DCA by pipetting 1.0mL of the 10µg/mL mixed stock solution of desmethyl DDVP and DCA into a 100mL volumetric flask. Bring the flask to volume with acetonitrile.

All solutions should be refrigerated when not in use.

5.5 Calibration Standard Solutions

5.5.1 Trichlorfon and DDVP Calibration Standard Solutions

Prepare a trichlorfon and DDVP calibration curve standard solutions in acetonitrile / 0.1% acetic acid aqueous (20/80, v/v) as shown in the table below:

| Standard Solution Concentration (µg/mL) | Volume Used (mL) | Internal Standard Solution Concentration (µg/mL) | Volume Internal Standard Used (mL) | Final Volume (mL) | Calibration Solution Concentration (ng/mL) | Internal Standard Solution Concentration (ng/mL) |
|---|------------------|--|------------------------------------|-------------------|--|--|
| 10.0 | 1.0 | 50.0 | 0.1 | 100.0 | 100 | 50 |
| 10.0 | 0.50 | 50.0 | 0.1 | 100.0 | 50 | 50 |
| 10.0 | 0.20 | 50.0 | 0.1 | 100.0 | 20 | 50 |
| 10.0 | 0.10 | 50.0 | 0.1 | 100.0 | 10 | 50 |
| 10.0 | 0.05 | 50.0 | 0.1 | 100.0 | 5.0 | 50 |
| 10.0 | 0.01 | 50.0 | 0.1 | 100.0 | 1.0 | 50 |
| 10.0 | 0.005 | 50.0 | 0.1 | 100.0 | 0.5 | 50 |

Additional solutions may be prepared as required. Refrigerate when not in use.

5.5.2 Desmethyl DDVP and DCA Calibration Standard Solutions

Prepare desmethyl DDVP and DCA calibration curve standard solutions in acetonitrile/0.1% acetic acid aqueous (50/50, v/v) as shown in the table below:

| Standard Solution Concentration (µg/mL) | Volume Used (mL) | Internal Standard Solution Concentration (µg/mL) | Volume Internal Standard Used (mL) | Final Volume (mL) | Calibration Solution Concentration (ng/mL) | Internal Standard Solution Concentration (ng/mL) |
|---|------------------|--|------------------------------------|-------------------|--|--|
| 10.0 | 1.0 | 50.0 | 0.1 | 100.0 | 100 | 50 |
| 10.0 | 0.50 | 50.0 | 0.1 | 100.0 | 50 | 50 |
| 10.0 | 0.20 | 50.0 | 0.1 | 100.0 | 20 | 50 |
| 10.0 | 0.10 | 50.0 | 0.1 | 100.0 | 10 | 50 |
| 10.0 | 0.05 | 50.0 | 0.1 | 100.0 | 5.0 | 50 |
| 10.0 | 0.01 | 50.0 | 0.1 | 100.0 | 1.0 | 50 |
| 10.0 | 0.005 | 50.0 | 0.1 | 100.0 | 0.5 | 50 |

Additional solutions may be prepared as required. Refrigerate when not in use.

All solutions are stable for one week.

6.0 PROCEDURE**6.1 Sample Extraction for Trichlorfon and DDVP**

- Step 1. Transfer the soil/thatch sample into a tared 1000mL stoppered container. Record the sample weight.
- Step 2. For the control and fortification samples weigh out three 200 ± 1 g samples. For fortifications at the method LOQ (250ng/g), fortify one sample with 5.0mL of the 10ug/mL trichlorfon fortification solution and a second sample with 5mL of the 10ug/mL DDVP fortification solution. (As trichlorfon readily degrades to DDVP it is recommended that mixed fortification solutions are not used.) The remaining sample is the untreated control sample. Let the fortified samples set for approximately 5 minutes.
- Step 3. Add 400mL of methanol/0.5% aqueous hydrochloric acid solution (50/50) to the container.
- Step 4. Shake the container for 20-25 minutes, remove from the shaker and let sit for 5 minutes.
- Step 5. Transfer a 0.5mL aliquot of the supernatant solution (typically 0.5mL but a smaller volume may be used if high concentrations trichlorfon and DDVP are expected) to a suitable container, add by pipet 0.1mL of the 5 μ g/mL d₆-DDVP isotopic internal standard and dilute to 10.0mL with acetonitrile/0.1% acetic acid (20:80).
- Step 6. Transfer an aliquot to an LC vial and inject the solution directly to the LC/MS/MS system.

NOTE: If the peak response for any of the analytical targets is outside the range of the calibration curve any further dilution should be performed using an aliquot of the solution from Step 4, with all dilutions performed using methanol/0.5% aqueous hydrochloric acid solution (50/50) and then proceed to step 5.

6.2 Sample Extraction for Desmethyl DDVP and Dichloroacetic Acid

- Step 1. Transfer the soil/thatch sample into a tared 1000mL stoppered container. Record the sample weight
- Step 2. For the control and fortification samples weigh out three 200 ± 1 g samples. For fortifications at the method LOQ (250ng/g), fortify one sample with 5.0mL of the 10ug/mL desmethyl DDVP fortification solution and a second sample with 5mL of the 10ug/mL DCA fortification solution. (As desmethyl DDVP readily degrades to DCA it is recommended that mixed fortification solutions are not used.) The remaining sample is the untreated control sample. Let set 5 minutes.

- Step 3. Add 400mL of 1.0 M ammonium bicarbonate aqueous solution to the container.
- Step 4. Shake the container for 20-25 minutes, remove from the shaker and let it sit for 5 minutes.
- Step 5. Transfer a 1.0mL aliquot of the supernatant solution (typically 1.0mL but a smaller volume may be used if high concentrations of desmethyl DDVP and DCA are expected) to a suitable container, add by pipet 250µL of the to 5µg/mL d₂-DCA solution and dilute to 25.0mL with acetonitrile/0.1% acetic acid (50:50)
- Step 6. Inject the solution directly to the LC/MS/MS system.

NOTE: If the peak response for any of the analytical targets is outside the range of the calibration curve further dilutions should be performed using an aliquot of the solution from Step 5, with all dilutions performed using acetonitrile/0.1% acetic acid (50:50) and then proceed to step 6. If additional dilutions are performed ensure that the concentration of d₂-DCA in the final sample is 50ng/mL.

7.0 ANALYSIS BY LC-MS/MS

7.1 Analytical Procedure

- Step 1. Using the recommended procedures listed below, analyze an aliquot of the 0.5, 1.00, 5.00, 10.0, 50.0, 100.0 and 200.0ng/mL standard solutions (these are calibration solution analyses).
- Step 2. Analyze an aliquot of the analytical samples.
Note: Up to 25 sample analyses can be made after the analysis of the standard solutions.
- Step 3. Again analyze an aliquot of the 0.5, 1.00, 5.00, 10.0, 50.0, 100.0 and 200.0ng/mL calibration standard solutions.
- Step 4. When necessary, analyze additional samples and standard solutions. Always finish the procedure with the analysis of a set of standard solutions.

NOTE: For the desmethyl DDVP and DCA analysis using the HILIC column, it is recommended that the seven calibration standard solutions are injected a minimum of three times each prior to starting the analysis and the analyst should ensure that these solutions give a consistent response prior to injecting the samples

7.2 LC/MS/MS Conditions

Variations in equipment or sample characteristics may require slight modifications in the chromatographic or detector conditions listed in order to obtain adequate chromatographic peak shapes or sensitivity. Therefore, the given LC/MS/MS parameters listed below are

guidelines and may be modified. These parameters should be optimized for the instrument and column actually used. Also, instrument parameters and mobile phase may be adjusted to improve separation from any observed interfering peaks.

7.2.1 Trichlorfon and DDVP HPLC Conditions

following recommended conditions were used on both a Thermo Finnigan Ultra and an Applied Biosystems API 4000 instrument:

Solvents: Aqueous 0.1% formic acid solution and acetonitrile

HPLC column: Imtakt Unison UK-C18 3 μ m 75 mm x 3.0 mm column

Injection volume: 15 μ L (Adjust for LC/MS/MS system being used)

HPLC gradient program:

| Time (min) | Acetonitrile | Flow rate μ L/min |
|------------|--------------|-----------------------|
| 0.0 | 25 | 300 |
| 2.0 | 25 | 300 |
| 3.5 | 40 | 300 |
| 5.5 | 95 | 300 |
| 7.0 | 95 | 300 |
| 7.1 | 25 | 300 |
| 10 | 25 | 300 |

Retention Times:

| Analyte | Approximate Retention Time (minutes) |
|-------------|--------------------------------------|
| Trichlorfon | 3.2 |
| DDVP | 7.0 |

7.2.2 Desmethyl DDVP and DCA HPLC Conditions

The following recommended conditions were used on an Applied Biosystems API 4000 instrument:

Solvents: Aqueous 0.1% acetic acid solution and acetonitrile

HPLC column: SeQuant ZIC-HILIC 4.6 x 150 mm, 5 μ m, 200 Å, Part No. Q2712-155
Injection volume: 5 μ L (Adjust for LC/MS/MS system being used)

HPLC gradient program:

| Time (min) | Acetonitrile | Flow rate μ L/min |
|------------|--------------|-----------------------|
| 0.0 | 80 | 300 |
| 4.0 | 65 | 300 |
| 4.1 | 50 | 300 |
| 5.0 | 50 | 300 |
| 5.1 | 80 | 300 |
| 10.0 | 80 | 300 |

Retention Times

| Analyte | Approximate Retention Time (minutes) |
|----------------|--------------------------------------|
| Desmethyl DDVP | 3.1 |
| DCA | 3.6 |

7.2.3 MDAP HPLC Conditions

The following recommended conditions were used on an Applied Biosystems API 4000 instrument:

Solvents: Aqueous 0.1% acetic acid solution and acetonitrile

HPLC column: SeQuant ZIC-HILIC 4.6 x 150 mm, 5 μ m, 200 Å, Part No. Q2712-155
Injection volume: 10 μ L (Adjust for LC/MS/MS system being used)

HPLC gradient program:

| Time (min) | Acetonitrile | Flow rate μ L/min |
|------------|--------------|-----------------------|
| 0.0 | 90 | 300 |
| 20.0 | 40 | 300 |
| 30.0 | 40 | 300 |
| 31.0 | 90 | 300 |

Retention Times:

| Analyte | Approximate Retention Time (minutes) |
|---------|--------------------------------------|
| MDAP | 17 |

7.2.4 Trichlorfon and DDVP Mass Spectrometer Conditions

The following recommended conditions were used on a Thermo Finnigan Ultra and an API 4000 LC/MS/MS system.

| Instrument ID: Thermo Electron Finnigan TSQ Quantum Ultra | | | |
|---|--------------------|-------------|---------------------------|
| Component: | <u>Trichlorfon</u> | <u>DDVP</u> | <u>d₆-DDVP</u> |
| Parent Ion: | 257 | 221 | 227 |
| Product Ion: | 109 | 109 | 115 |
| Ionization Mode: | ESI | ESI | ESI |
| Polarity: | Positive | Positive | Positive |
| Spray Voltage(V): | 4000 | 4000 | 4000 |
| Sheath Gas Pressure(psi): | 60 | 60 | 60 |
| Aux Gas Pressure(psi): | 25 | 25 | 25 |
| Ion Sweep Gas Pressure (psi): | 1.0 | 1.0 | 1.0 |
| API Temp(C): | 270 | 270 | 270 |
| Resolution for Q1MS(v): | 0.7 | 0.7 | 0.7 |
| Skimmer offset(v): | 18 | 18 | 18 |
| Collision Energy (v): | 25 | 21 | 21 |
| Q2 Collision Gas Pressure(mT): | 1.3 | 1.3 | 1.3 |
| Resolution for Q3MS(v): | 0.7 | 0.7 | 0.7 |

| Instrument ID: Applied Biosystems API4000 | | | |
|---|--------------------|-------------|---------------------------|
| Component: | <u>Trichlorfon</u> | <u>DDVP</u> | <u>d₆-DDVP</u> |
| Parent Ion: | 257 | 221 | 227 |
| Product Ion: | 109 | 109 | 115 |
| Ionization Mode: | ESI | ESI | ESI |
| Polarity: | Positive | Positive | Positive |
| Declustering Potential (DP): | 65 | 65 | 65 |
| Entrance Potential (EP): | 10 | 10 | 10 |
| Collision Cell Exit Potential (CXP): | 10 | 10 | 10 |
| Collision energy (CE) | 27 | 27 | 27 |
| Curtain Gas (CUR) | 15 | 15 | 15 |
| Ion Source Gas 1 (GS1) | 50 | 50 | 50 |
| Ion Source Gas 1 (GS2) | 50 | 50 | 50 |
| Source Temp (TEM) | 150 | 150 | 150 |
| Interface Heater (IHE) | On | On | On |
| Ion Transfer Voltage (IS) | 3000 | 3000 | 3000 |

7.2.5 Desmethyl DDVP and DCA Mass Spectrometer Conditions

The following conditions were used on an API 4000 LC/MS/MS system.

| Instrument ID: Applied Biosystems API4000 | | | |
|---|-----------------------|------------|--------------------------|
| Component: | <u>Desmethyl DDVP</u> | <u>DCA</u> | <u>d₂-DCA</u> |
| Parent Ion: | 205 | 127 | 128 |
| Product Ion: | 111 | 83 | 84 |
| Ionization Mode: | ESI | ESI | ESI |
| Polarity: | Negative | Negative | Negative |
| Declustering Potential (DP): | -50 | -32 | -32 |
| Entrance Potential (EP): | -10 | -10 | -10 |
| Collision Cell Exit Potential (CXP): | -4 | -6 | -6 |
| Collision energy (CE) | -26 | -14 | -14 |
| Curtain Gas (CUR) | 40 | 40 | 40 |
| Collision Gas (CAD) | 9.0 | 9.0 | 9.0 |
| Ion Source Gas 1 (GS1) | 40 | 40 | 40 |
| Ion Source Gas 1 (GS2) | 40 | 40 | 40 |
| Source Temp (TEM) | 150 | 150 | 150 |
| Interface Heater (IHE) | On | On | On |
| Ion Transfer Voltage (IS) | -3500 | -3500 | -3500 |

7.2.6 MDAP Mass Spectrometer Conditions

The following conditions were used on an API 4000 LC/MS/MS system.

| Instrument ID: Applied Biosystems API4000 | |
|---|------------------|
| Component: | <u>MDAP</u> |
| Scan Type | Q1 multiple Ions |
| Q1 | 223, 225 |
| Ionization Mode: | ESI |
| Polarity: | Negative |
| Declustering Potential (DP): | -32 |
| Entrance Potential (EP): | -10 |
| Curtain Gas (CUR) | 40 |
| Ion Source Gas 1 (GS1) | 40 |
| Ion Source Gas 1 (GS2) | 10 |
| Source Temp (TEM) | 150 |
| Interface Heater (IHE) | On |

| | |
|---------------------------|-------|
| Ion Transfer Voltage (IS) | -3500 |
|---------------------------|-------|

8.0 CALCULATION OF RESULTS

The example calculation displayed below was used by the laboratory developing this method. Alternate calculation procedures appropriate to the reporting requirements may be substituted.

Inject samples using the procedures outlined in Section 7.

Trichlorfon and DDVP residues are quantified using external standard linear regression analysis and desmethyl DDVP and DCA by internal standard linear regression analysis. A separate calibration curve was produced for each set of samples analyzed on the LC/MS/MS. A calibration curve was generated by linear regression of the standard peak area versus the standard concentrations in ng/mL for Trichlorfon and DDVP (or by the ratio of standard peak/internal standard peak areas for desmethyl DDVP and DCA) using Applied Biosystems Analyst Software (Version 1.4.1) or Finnigan LCQuan (Version 2.5), computer-programmed data capturing system. The software uses the MS/MS standard responses to calculate the regression coefficients for slope, M, and intercept, B, for each analytical set.

The standards were fit to the linear equation: $Y = MX + B$

where: X is the concentration of the reference standard in ng/mL^a
 M is the calibration line slope
 B is the calibration line intercept
 Y is the native peak area for Trichlorfon and DDVP or the native peak area:isotopic peak area ratio for desmethyl DDVP and DCA

The calibration points are weighted 1/x to provide better fit near the limit of detection.

After regression coefficients were calculated, the residue in parts per billion was determined. The ng/g of analyte in the sample is calculated using the following equation,

$$\text{Residue found (ng/g)} = \frac{(Y-B) \times D}{M}$$

$$\text{Where Dilution Factor (D)} = \frac{\text{Initial volume (V}_1\text{)}}{\text{Initial sample wt. (W)}} \times \frac{\text{Final volume (V}_4\text{)}}{\text{Aliquot (V}_3\text{)}}$$

Where: W = 200g
 V₁ = 400mL
 V₃ = (0.5mL Trichlorfon and DDVP/ 1mL for desmethyl DDVP and DCA)
 V₄ = (10.0mL Trichlorfon and DDVP/ 25mL for desmethyl DDVP and DCA)

Analyst or Xcalibur software was used to calculate the amount of analyte in ng/g for each

sample and the percent recovery for the spiked samples.

8.1 Fortification Experiments

Note: Fortification experiments may be performed as needed to monitor method efficiency and reproducibility, but are not required when analysis of samples is performed for tolerance enforcement. Fortification experiments are intended to be used for data collection methods or establishing & validating method efficiency.

With each sample set, analyze an untreated control sample and one or more fortified control samples. Calculate recoveries using the following equation:

$$\text{Recovery (\%)} = \frac{(R - S)}{T} \times 100$$

Where: R = $\mu\text{g/g}$ of target analyte found in fortified sample
S = $\mu\text{g/g}$ of target analyte found in control sample, real or apparent
T = theoretical $\mu\text{g/g}$ in fortified sample

Recoveries are determined by analyzing fortified control samples alone or in conjunction with a sample set. Samples may be fortified prior to extraction at the LOQ of 0.25 $\mu\text{g/g}$ or other appropriate level with fortification solutions. Calculate the final residue R for the control (S) and fortified control (R) samples.

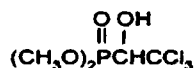
NOTE: As stated in section 6 of this method, due to the instability of these molecules degradation of trichlorfon to DDVP and of desmethyl DDVP to DCA may occur during the sample extraction process.

If the calculated recoveries of trichlorfon and desmethyl DDVP are less than 70%, the analyst should determine if the trichlorfon fortification solution contains significant concentrations of DDVP and if the desmethyl DDVP solution contains DCA.

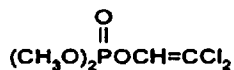
If so, the trichlorfon and DDVP recoveries may be combined after calculating the DDVP concentration in trichlorfon equivalents, likewise the desmethyl DDVP and DCA recoveries may be combined after calculating the DCA recoveries as desmethyl DDVP equivalents.

Appendix 1 Test and Reference Substances

Code Name: Trichlorfon
 CAS Name: Dimethyl (2,2,2-trichloro-1-hydroxyethyl)phosphonate
 Molecular Formula: C₄H₈Cl₃O₄P
 Molecular Weight: 257



Code Name: DDVP
 CAS Name: 2,2-dichloroethenyl dimethyl phosphate
 Molecular Formula: C₄H₇Cl₂O₄P
 Molecular Weight: 221



Code Name: Desmethyl DDVP
 CAS Name: 2,2-dichloroethenyl methyl hydrogen phosphate
 Molecular Formula: C₃H₅Cl₂O₄P
 Molecular Weight: 207



Code Name: DCA
 CAS Name: Dichloroacetic acid
 Molecular Formula: C₂H₂Cl₂O₂
 Molecular Weight: 129



Code Name: d₂-DCA
 CAS Name: d₂-Dichloroacetic acid
 Molecular Formula: C₂D₂Cl₂O₂
 Molecular Weight: 131



The toxicities of these chemicals have not been precisely determined. Thus, each chemical must be treated as a potential health hazard. Exposure to these chemicals should be reduced to the lowest reasonable level.