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Determination of Residues of Triclopyr and Trichloropyridinol in Water Using Magnetic Particle-Based Immunoassay Test Kits

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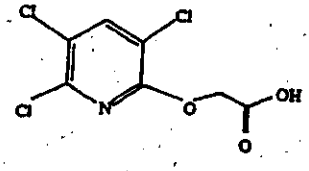
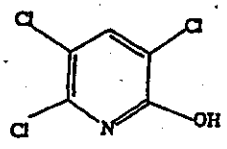
A. Scope

This method is applicable for the quantitative determination of residues of triclopyr and its metabolite, 3,5,6-trichloro-2-pyridinol (trichloropyridinol, TCP) in water. The concentration range for analysis of triclopyr is 0.10 to 300 ng/mL, with a validated limit of quantitation of 0.10 ng/mL. The concentration range for analysis of trichloropyridinol is 0.50 to 300 ng/mL, with a validated limit of quantitation of 0.50 ng/mL.



Triclopyr
CAS Number: 55335-06-3



3,5,6-Trichloro-2-pyridinol
CAS Number: 6515-38-4

B. Principle

The Triclopyr and Trichloropyridinol RaPID Assay test kits apply the principles of enzyme-linked immunosorbent assay (ELISA) (1, 2) for the determination of residues in water samples. An aliquot of each sample is pipeted into a disposable test tube. Enzyme-conjugated triclopyr or trichloropyridinol and paramagnetic particles coated with specific antibodies are seqnentially added to the tube. During an incubation period, the sample residue and the enzyme conjugate compete for antibody sites on the magnetic particles. At the end of the incubation period, a magnetic field is applied to the particles. The sample residue and enzyme conjugate bound to the antibodies on the particles are held in the tube by the magnetic field while the unbound reagents are decanted. After decanting, the particles are washed to remove unbound enzyme conjugate. The presence of triclopyr or trichloropyridinol is detected by adding the enzyme substrate (hydrogen peroxide) and a chromogen (3,3',5,5'-tetramethylbenzidine; TMB), generating a colored product. After another incubation period, the reaction is stopped and stabilized by the addition of acid. Since the enzyme conjugate is in competition with the sample residue for the antibody sites, the level of color development is inversely proportional to the concentration of triclopyr or trichloropyridinol in the sample (i.e., lower concentrations result in greater color development). The absorbance at 450 nm is measured in each tube using the RPA-1 RaPID Analyzer. A calibration curve is generated and the residue concentration in unknown samples is calculated from the regression equation using the preprogrammed software capabilities of the RPA-1 RaPID Analyzer.

C. Safety Precautions

1. Each analyst must be acquainted with the potential hazards of the reagents and products used in this method before commencing laboratory work. SOURCES OF INFORMATION INCLUDE: MATERIAL SAFETY DATA SHEETS, PRODUCT LITERATURE, AND OTHER RELATED DATA. Safety information on non-DowElanco products should be requested from the supplier. Disposal of reagents must be in compliance with local, state, and federal laws and regulations.

2. Avoid contact of the Stopping Solution (0.5% sulfuric acid) with skin and mucous membranes. Wear protective clothing and proper eye protection when working with this material. If this reagent comes in contact with skin, flush the exposed area with water.

3. Volatile and flammable organic solvents such as acetone and methanol must be used in well-ventilated areas away from ignition sources.

D. Equipment (Note N.1.)

1. Balance, analytical, Mettler, Model AE50, Mettler Instrument Corporation, Hightstown, NJ 08520.

2. Magnetic Separator Rack and Base, 60-position, catalog number A00004, Strategic Diagnostics Inc., Newtown, PA 18940.

3. Mixer, Vortex-Genie, catalog number 12-812, Fisher Scientific, Pittsburgh, PA 15238.

4. Photometer, fixed wavelength spectrophotometer RPA-1 RaPID Analyzer, catalog number A00003, Strategic Diagnostics Inc.

5. Pipetter, Eppendorf, 2-20 µL, catalog number 21-381-201, Fisher Scientific.

6. Pipetter, Eppendorf, repeater, catalog number 21-380-8, Fisher Scientific.

7. Pipetter, Eppendorf, tri-volume, (100-µL, 200-µL, 250-µL), catalog number 21-278-38, Fisher Scientific.

8. Timer, minutes and seconds with alarm, catalog number 14-649-14, Fisher Scientific.

E. Glassware and Materials (Note N.1.)

1. Bottles, 8-oz. (237-mL) graduated, with PTFE-lined caps, catalog number 03-320-11G, Fisher Scientific.

2. Culture tubes, disposable glass, 16 x 100 mm, catalog number 14-962-10B, Fisher Scientific.

3. Cylinders, mixing, 50-mL, graduated, with stoppers, catalog number 08-56C, Fisher Scientific.

4. Pipet tips, Eppendorf Combitip for repeater pipet, 12.5 mL, catalog number 21-380-8C, Fisher Scientific.

5. Pipet tips, Eppendorf disposable, 0.1 mL-1.0 mL, catalog number 21-372-4, Fisher Scientific.

6. Pipet tips, Eppendorf disposable, 1-100 µL, catalog number 21-381-303, Fisher Scientific.

7. Pipets, 1-mL disposable, catalog number 13-678-25B, Fisher Scientific.

8. Pipets, 5-mL disposable, catalog number 13-678-25D, Fisher Scientific.

9. Pipets, 10-mL disposable, catalog number 13-678-31J, Fisher Scientific.

10. Vials, 40 mL glass, catalog number 03-339-5C, Fisher Scientific.

F. Reagents and Prepared Solutions (Note N.1.)

1. Reagents

a. Acetone, Omnisolv, catalog number AX0116-1, EM Science, Gibbstown, NJ 08027.

b. Methanol, ChromAR HPLC grade, catalog number 3041-09, Mallinckrodt Specialty Chemicals Company, Paris, KY 40361.

c. Trichloropyridinol RaPID Assay Test Kit, catalog number A00208, Strategic Diagnostics Inc. Kit contents include (Note N.2.):

(1) Trichloropyridinol Antibody, coupled to paramagnetic particles
(2) Enzyme Conjugate
(3) Calibration Standards (0.50 ng/mL, 2.5 ng/mL and 6.0 ng/mL)
(4) Quality Control Sample
(5) Diluent/Zero Standard
(6) Color Solution
(7) Stopping Solution
(8) Washing Buffer
(9) Test Tubes

d. Triclopyr RaPID Assay Test Kit, catalog number A00171, Strategic Diagnostics Inc. Kit contents include (Note N.2.):

(1) Triclopyr Antibody, coupled to paramagnetic particles
(2) Enzyme Conjugate
(3) Calibration Standards (0.10 ng/mL, 1.0 ng/mL and 3.0 ng/mL)
(4) Quality Control Sample
(5) Diluent/Zero Standard
(6) Color Solution
(7) Stopping Solution
(8) Washing Buffer
(9) Test Tubes

e. Sample Diluent: trichloropyridinol, catalog number A00210; triclopyr, catalog number A00173, Strategic Diagnostics Inc. (Note N.2.)

f. Standard:

Obtain triclopyr and TCP analytical standards from Test Substance Coordinator, DowElanco, 9330 Zionsville Rd., Building 306, Indianapolis, IN 46268-1053.

g. Water, OmniSolv HPLC grade, catalog number WX001-4, EM Science.

G. Preparation of Fortification Stock Solutions (Note N.3.)

1. Preparation of Trichloropyridinol Fortification Solutions

a. Weigh 0.050 g of the TCP analytical standard using an analytical balance. Quantitatively transfer to a 100-mL volumetric flask and dissolve in acetone. Dilute to volume with acetone to obtain a stock solution containing 500 µg/mL of TCP.

b. Dilute 10.0 mL of the above 500-µg/mL TCP stock solution with acetone in a 100-mL volumetric flask to obtain a 50.0-µg/mL stock solution.

c. Dilute 10.0 mL of the above 50.0-µg/mL TCP solution with acetone in a 100-mL volumetric flask to obtain a 5.0-µg/mL stock solution.

d. Dilute 10.0 mL of the above 5.0-µg/mL TCP solution with acetone in a 100-mL volumetric flask to obtain a 0.50-µg/mL stock solution.

e. Prepare the following fortification stock solutions in either Trichloropyridinol Sample Diluent or distilled water. Use the TCP solutions described in G.1.b., G.1.c., and G.1.d. These solutions should be prepared immediately before use.

Original Concentration µg/mL	Original Volume mL	Diluent Volume mL	Final Volume mL	Final Concentration µg/mL
50.0	6.0	14.0	20.0	15.0
50.0	1.0	19.0	20.0	2.5
5.00	1.0	19.0	20.0	0.25
0.50	1.0	19.0	20.0	0.025
0.50	0.5	19.5	20.0	0.0125

f. Use the fortification stock solutions from G.1.e. for preparation of the fortified water samples as follows. The samples should be prepared immediately before use.

Stock Solution Concentration µg/mL	Stock Volume mL	Final Volume mL	Final Concentration µg/mL
15.0	1.0	50	0.3
2.5	1.0	50	0.05
0.25	1.0	50	0.005
0.025	1.0	50	0.0005
0.0125	1.0	50	0.00025

2. Preparation of Triclopyr Fortification Solutions

a. Weigh 0.050 g of the triclopyr analytical standard using an analytical balance. Quantitatively transfer to a 100-mL volumetric flask and dissolve in methanol. Dilute to volume with methanol to obtain a stock solution containing 500 µg/mL of triclopyr.

b. Dilute 2.0 mL of the above 500-µg/mL triclopyr solution with methanol in a 100-mL volumetric flask to obtain a 10.0-µg/mL stock solution.

c. Dilute 10.0 mL of the above 10.0-µg/mL triclopyr solution with methanol in a 100-mL volumetric flask to obtain a 1.0-µg/mL stock solution.

d. Dilute 10.0 mL of the above 1.0-µg/mL triclopyr solution with methanol in a 100-mL volumetric flask to obtain a 0.10 µg/mL stock solution.

e. Prepare the following fortification stock solutions in either Triclopyr Sample Diluent or distilled water. Use the triclopyr solutions described in G.2.c. and G.2.d. These solutions should be prepared immediately before use.

Original Concentration µg/mL	Original Volume mL	Diluent Volume mL	Final Volume mL	Final Concentration µg/mL
1.0	3.0	17.0	20.0	0.150
1.0	1.0	19.0	20.0	0.050
0.10	2.5	17.5	20.0	0.0125
0.10	1.0	19.0	20.0	0.005
0.10	0.5	19.5	20.0	0.0025

f. Use the fortification stock solutions from G.2.b. and G.2.e. for preparation of the fortified water samples as follows. The samples should be prepared daily.

Stock Solution Concentration µg/mL	Stock Volume mL	Final Volume mL	Final Concentration µg/mL
10.0	1.5	50	0.3
10.0	0.75	50	0.15[a]
10.0	0.375	50	0.075
10.0	0.125	50	0.025
1.0	0.25	50	0.005
0.15	1.0	50	0.003
0.05	2.0	50	0.002
0.05	1.0	50	0.001
0.05	0.5	50	0.0005
0.0125	1.0	50	0.00025
0.005	1.0	50	0.00010
0.0025	1.0	50	0.00005

[a] Use 0.15 µg/mL as non-specific binding reagent (NSB).

H. Instrument Settings

1. To obtain results from the Trichloropyridinol RaPID Assay, use the following parameter settings on the RPA-1 RaPID Analyzer:

Parameter	RPA-1 Abbreviation	Setting
Protocol Name	Protocol Name	TCP
Data Reduction	Data Reduct	Linear Regression
Transformation	Xformation	Ln/LgtB
Number of Calibrators	# of Calibrator	4
Number of Calibrator Replicates	# of Reps:	2
Calibrator #1 Concentration	Cal #1 Conc	0.00
Calibrator #2 Concentration	Cal #2 Conc	0.50
Calibrator #3 Concentration	Cal #3 Conc	2.50
Calibrator #4 Concentration	Cal #4 Conc	6.00
Minimum Correlation	Correlation Flag	0.990
Maximum Concentration (pg/mL)	Normal Range Hi	6.00
Minimum Concentration (pg/mL)	Normal Range Low	0.25
Number of Controls	# of Controls	1
Number of Control Replicates	Ctrl Replicates	2
Number of Reagent Blanks	# Rgt Blk	0
Wavelength	Wavelength	450 nm
Read Mode	Read Mode	Absorbance
Units	Units	ng/mL
Precision of Calibrators	Rep %CV Flag	100

2. To obtain results from the Triclopyr RaPID Assay, use the following parameter settings on the RPA-1 RaPID Analyzer:

Parameter	RPA-1 Abbreviation	Setting
Protocol Name	Protocol Name	Triclopr
Data Reduction	Data Reduct	Linear Regression
Transformation	Xformation	Lo/LgtB
Number of Calibrators	# of Calibrator	4
Number of Calibrator Replicates	# of Reps:	2
Calibrator #1 Concentration	Cal #1 Conc	0.00
Calibrator #2 Concentration	Cal #2 Conc	0.10
Calibrator #3 Concentration	Cal #3 Conc	1.00
Calibrator #4 Concentration	Cal #4 Conc	3.00
Minimum Correlation	Correlation Flag	0.990
Maximum Concentration (pg/mL)	Normal Range Hi	3.00
Minimum Concentration (pg/mL)	Normal Range Low	0.05
Number of Controls	# of Controls	1
Number of Control Replicates	Ctrl Replicates	2
Number of Reagent Blanks	# Rgt Blk	1
Wavelength	Wavelength	450 nm
Read Mode	Read Mode	Absorbance
Units	Units	ng/mL
Precision of Calibrators	Rep %CV Flag	100

I. Determination of Recovery of Triclopyr and Trichloropyridinol in Water

1. Preparation of Recovery Samples

a. Allow the samples to warm to room temperature. Measure 50.0-mL portions of the control water samples into 8-oz glass bottles using volumetric graduated cylinders. For laboratory recovery samples, add the appropriate spiking solution from Section G. An unfortified control sample, if available, should be carried through the method with each sample set.

b. Vortex mix the samples well before removing the analysis aliquot.

c. Assay each sample according to the procedure described in Section I.2.

d. If the sample contains more than 3.0 ng/mL of triclopyr or 6.0 ng/mL of trichloropyridinol, perform an additional dilution of the sample from Step I.1.b. prior to assay (e.g., for a 1:10 dilution, pipet 4.5 mL of Sample Diluent or distilled water into a culture tube, add 0.5 mL of the sample from Step I.1.b. to the tube and vortex to mix). After vortexing, wait at least 5 minutes before proceeding with the assay. Assay the diluted aliquot as described in Section I.2.

2. Assay Procedure

Conduct each test in an individual test tube. The average of duplicate analyses of a sample or standard constitute a single result. A standard curve and the appropriate control and recovery samples must be included in each analytical batch. For further details, consult the Triclopyr or Trichloropyridinol RaPID Assay Kit Inserts.

Remove all kit reagents from refrigerated storage and allow them to equilibrate to room temperature prior to use. A minimum of 30 minutes is recommended for warming.

a. Turn on the RPA-I Photoanalyzer at least 30 minutes prior to measuring absorbance in the completed assay.

b. Label test tubes for standards, controls, and samples. Place the tubes in the proper rack position. Be sure that the rack is removed from the Magnetic Separator.

c. Critical step: Use the indicated pipeting technique in this step to obtain accurate and precise data. Add the standard, quality control solution, non-specific binding (NSB) reagent (see *G.2.f.*) or sample to each test tube using an Eppendorf pipetter (250 µL with the TCP kit, 200 µL with the triclopyr kit). Pipet each sample or standard directly to the bottom of the tube; avoid liquid adhering to the sides of the test tube. Use a fresh pipet tip for each standard and sample.

d. Using an Eppendorf repeater pipet equipped with a 12.5-mL Combitip, add 0.25 mL (Dial Setting = 1) of Enzyme Conjugate down the inside wall of each tube.

e. Before use, thoroughly mix the Antibody Coupled Paramagnetic Particles by swirling the bottle. Avoid vigorous shaking and foaming.

f. Using a repeater pipet equipped with a 12.5-mL Combitip, add 0.50 mL (Dial Setting = 2) of the Antibody Coupled Paramagnetic Particles down the inside wall of each tube.

g. When dispensing of the magnetic particles has been completed, mix the samples by gently vortexing (Vortex setting = 3-4) each tube for 1-2 seconds.

h. Incubate at room temperature (20 minutes for analysis of TCP, 30 minutes for analysis of triclopyr).

i. After the incubation period, combine the rack and the magnetic base. Seat all tubes by pressing them into the base. Allow 2 minutes for the particles to separate.

j. Do not separate the tube rack from the magnetic base. Using a smooth motion, invert the combined rack assembly over a collection container and pour out the tube contents. Keep the rack inverted and gently blot the test tube rims on several layers of paper towels. Do not shake or bump the rack as the magnetic particles may fall out of the tubes.

k. Using a repeater pipet equipped with a 12.5-mL Combitip, add 1 mL (Dial Setting = 4) of Washing Buffer to each tube. Wait 2 minutes. Do not separate the tube rack from the magnetic base. Using a smooth motion, invert the combined rack assembly over a collection container and pour out the tube contents. Keep the rack inverted and gently blot the test tube rims on several layers of paper towels.

l. Repeat the wash step described in Step L2.k. an additional time.

m. Remove the tube rack from the magnetic separator and then add 0.50 mL of Color Solution to each tube using a repeater pipet equipped with a 12.5-mL Combitip (Dial Setting = 2).

n. Gently vortex each tube for 1-2 seconds.

o. Incubate for 20 minutes at room temperature. During this incubation, pour approximately 1 mL of Washing Buffer into a clean tube for use as an instrument blank.

p. At the end of the incubation period, add 0.5 mL of Stopping Solution to each tube using a repeater pipet equipped with a 12.5-mL Combitip (Dial Setting = 2).

q. Analyze each tube using the RPA-1 RaPID Analyzer within 15 minutes after adding the Stopping Solution.

3. Operating Procedure for the RPA-1 RaPID Analyzer

The RPA-1 RaPID Analyzer is pre-programmed with the protocols for several RaPID Assay procedures. The following steps describe how to set up and run the analyzer to measure absorbance in the tubes for the Triclopyr or Trichloropyridinol RaPID Assay.

a. Switch on the instrument and allow it to warm up at least 30 minutes prior to use. The RPA-1 RaPID Analyzer will perform a self test. If all parameters are satisfactory, the "SELECT COMMAND" prompt will appear.

b. At the "SELECT COMMAND" prompt, press "RUN".

c. At the "RUN PROTOCOL" prompt, scroll through the protocols using the arrow keys until "TCP" appears for trichloropyridinol analysis or "Triclopr" appears for triclopyr analysis. Press "ENTER".

d. At the "SPL. REPLICATES" (sample replicates) prompt, press "2" to indicate the number of replicates for each sample, then press "ENTER".

e. At the "BLANK TUBE/INSERT TUBE" prompt, insert the tube containing approximately 1 mL of Washing Buffer. The display will briefly read "EVALUATING TUBE" then "REMOVE TUBE" and the instrument will produce

an audible beep indicating that the absorbance has been measured. After hearing the beep, remove the tube.

f. At the "CAL. #1 REP. #1/INSERT TUBE" prompt, insert the first replicate of the first standard/calibrator (0.00 ng/mL). Remove the tube after the beep.

g. Follow the prompts on the instrument display until all of the standards have been measured. The tube order is important. The RPA-1 RaPID Analyzer has been programmed to evaluate the standards in ascending order, in duplicate, starting with 0.00 ng/mL.

h. After all of the standards have been evaluated, the instrument will report the equation of the line, the transformed data and the standards data.

i. Insert the quality control tubes at the "CNTRL. #1 REP. #1" and "CNTRL. #1 REP. #2" prompts. The instrument will report the calculated values for each replicate of the quality control sample.

j. Evaluate the results for the standard curve and the quality control sample. At the "EDIT CALIBRATORS YES/NO" prompt, press "NO".

k. At the "SPL. #1 REP #1/INSERT TUBE" prompt, insert the first sample tube. Remove the tube after the beep.

l. Continue sample analysis following the prompts on the instrument display. Press "STOP" after all the samples have been evaluated and the results have been reported by the RPA-1 RaPID Analyzer.

J. Calculations

1. Calibration Curve

The RPA-1 RaPID Analyzer contains preprogrammed data reduction capabilities which calculate a calibration curve for each analytical batch using the absorbances of the standards supplied with the kit. The calibration curve is constructed by linear regression after performing a ln/Logit data transformation of the concentration and absorbance values, respectively.

The regression equation is :

$$\text{Logit}\ \frac{B}{B_0} = [\text{slope} \times \ln(\text{Conc})] + Y\ \text{intercept}$$

Where:

$$\text{Logit}\ \frac{B}{B_0} = \ln \frac{B/B_0}{1 - B/B_0}$$

B = the absorbance measured at a specific concentration
B_0 = the absorbance measured for the 0.00 ng/mL standard
Conc = the concentration of the standard

An example of a calibration curve is presented in Figure 1.

2. Calculation of Trichloropyridinol or Triclopyr in Samples

The RPA-1 RaPID Analyzer will calculate the concentration of trichloropyridinol or triclopyr in each sample using the preprogrammed data reduction parameters (Note N.4.). It will report the absorbance value and calculated concentration for each sample tube as well as the mean absorbance, the mean sample concentration and the percent coefficient of variation (%CV) of the duplicate measurements for each sample. The mean values are the final result for each sample.

To calculate sample concentration, use the following equation:

Measured Concentration = e^a

Where:

$$a = \left(\frac{\text{Logit} \frac{B}{B_0} - \text{Y intercept}}{\text{slope}} \right)$$

Example:

Water sample fortified at 0.10 ng/mL, rack positions 35 and 36 (Figure 2):

Mean absorbance (450 nm) (B) = 0.905
Mean absorbance, 0 calibrator (B_0) = 1.213

$$\frac{B}{B_0} = \frac{0.905}{1.213}$$

$$= 0.746$$

Therefore:

$$\text{Logit} \frac{B}{B_0} = \ln \frac{0.746}{1 - 0.746}$$

$$= \ln (2.937)$$

$$= 1.077$$

$$\text{Measured Concentration} = e^{\left(\frac{1.077 - (-0.482)}{-0.655}\right)}$$

$$= 0.093 \text{ ng/mL}$$

$$= 0.000093 \text{ µg/mL}$$

3. Calculation of the Method Factor and Gross Sample Concentration

Method Factor = Dilution Factor

Thus, for a sample receiving a 1:10 additional dilution:

Gross Sample Concentration = 0.000093 µg/mL x 10

Gross Sample Concentration = 0.00093 µg/mL

4. Calculation of Percent Recovery

The percent recovery for the fortified samples is calculated as follows:

$$\text{Recovery} = \frac{\text{Concentration Found}}{\text{Concentration Added}} \times 100\%$$

From J.4.:

$$\text{Recovery} = \frac{0.000093 \text{ µg/mL}}{0.00010 \text{ µg/mL}} \times 100\%$$

Recovery = 93%

5. Correction for Recovery

For those analyses that require correction for method recovery, use the average recovery of all the recovery samples in a given analytical batch to correct for method efficiency as follows:

$$\text{Sample Concentration (corrected µg/mL)} = \text{Sample Concentration (gross µg/mL)} \times \frac{100}{\text{Average \% Recovery}}$$

K. Determination of Triclopyr or Trichloropyridinol in Water

1. Prepare treated samples, a system (reagent) blank, fortified recovery samples, and an untreated control as described in Section I.1.

2. Use the RPA-1 RaPID Analyzer to calculate a standard curve and determine the gross concentration in each sample as described in Section J.

L. Quality Control

1. Analytical Batch Definition

An analytical batch of samples is defined as a group of 60 tubes. The size of the batch is based on the capacity of the magnetic separator rack. An analytical batch of less than 60 tubes can be analyzed. The first 10 tubes (rack positions 1-10) are used for duplicate analysis of the four standards and the quality control solution. Following the quality control solution, up to 25 samples (recovery or study samples) may be analyzed in duplicate (2 tubes). If more samples are to be analyzed than can be accommodated in one rack, the remaining samples should be analyzed as a different analytical batch with a new standard curve, control, and recovery samples.

2. Quality Control Solution

A quality control solution containing 3.0 ng/mL of trichloropyridinol or 2.0 ng/mL of triclopyr (supplied with the kit; Section F.1.c.(4) or F.1.d.(4), respectively, should be analyzed at the beginning of every batch of samples (rack positions 9 and 10). The quality control solution should be assayed in the same manner as all other samples. Additional fortified matrix recovery samples should be analyzed to further ensure proper execution of the method.

3. Study Samples

Study samples should be assayed in duplicate. If the concentration of the sample exceeds the range of the assay, dilute with Sample Diluent (typically a 10-fold dilution is performed) and then assay the diluted sample aliquot. Multiply the result by the appropriate method factor to obtain the final result.

4. Criteria for Acceptance of an Analytical Batch

The correlation coefficient for the linear regression of the calibration curve should be greater than 0.990. The absorbance %CV should be less than 10% for each duplicate pair of standards. The concentration %CV should be less than 20% for the quality control sample, and the recovery value should be within ±20% of the expected value (i.e., 2.4-3.6 ng/mL for trichloropyridinol or 1.6-2.4 ng/mL for triclopyr). If the data fail to meet these performance criteria, the analyst should evaluate the results, determine the potential source of the variation, and repeat the analysis if necessary. An example of calibration and quality control data are graphed and summarized in Figure 1.

5. Interferences

Thirty-seven pesticides, seventeen organic/inorganic compounds and four solvents were tested for the potential to interfere with conjugate binding in the trichloropyridinol assay (3) (Table I). Forty-five pesticides, seventeen organic/inorganic compounds and four solvents were tested for the potential to interfere with conjugate binding in the triclopyr assay (4) (Table II). None of the pesticides exhibited an I_{50} concentration below 10 ng/mL in either test kit. The I_{50} concentration is the concentration which results in a 50% inhibition of conjugate binding to the available antibodies, and it is a commonly used reference value for expressing cross reactivity and determining the extent of interference. In comparison, the I_{50} for triclopyr in the Triclopyr RaPID Assay test kit is approximately 0.78 ng/mL and 2.31 ng/mL for trichloropyridinol in the Trichloropyridinol RaPID Assay test kit.

6. Specificity/Sensitivity

The metabolites of triclopyr, 2-methoxy-3,5,6-trichloropyridine and 3,5,6-trichloro-2-pyridinol, have been tested to determine whether the Triclopyr RaPID Assay test kit will detect their presence in a water sample (3). The Triclopyr RaPID Assay test kit is sensitive to the methoxypyridine metabolite (I_{50} = 4 ng/mL) but not to the trichloropyridinol metabolite (I_{50} >10,000 ng/mL). The Trichloropyridinol RaPID Assay test kit is not sensitive to either triclopyr or methoxypyridine metabolite at an I_{50} concentration below 10 µg/mL.

7. Modifications and Uses

Modifications to the assay procedure are not recommended. This procedure is valid only when using reagents manufactured by SDI. This procedure is for use on water samples. Gross particulate matter should be removed prior to analysis by allowing the sample to settle, by centrifugation, or by filtration through a glass fiber filter. Validation of the method for analysis of other sample matrices would be required prior to implementing this method for sample analysis.

4. Confirmatory Method

The detection and/or quantitation of trichloropyridinol and triclopyr can be confirmed by capillary gas chromatography with mass selective detection, using method GRM 95.18, "Determination of Residues of Triclopyr, 3,5,6-Trichloro-2-pyridinol, and 2-Methoxy-3,5,6-trichloropyridine in Water by Capillary Gas Chromatography with Mass Selective Detection" (8).

5. Assay Time

The time required to analyze a typical analytical batch (25 samples or recoveries, four standards and the quality control sample, in duplicate) is from 2 to 3 hours.

N. Notes

1. Equipment, glassware, materials, reagents, and chemicals equivalent to those specified may be substituted with the understanding that their performance must be confirmed by appropriate tests. Common laboratory supplies are assumed to be readily available and are not listed here. Other immunochemical reagents should not be substituted.

2. Refrigerate all kit reagents and sample diluent at 2-8 °C. Do not freeze. Reagents may be used until the expiration date printed on the labels. The test tubes require no special storage conditions and may be stored separately from the kit reagents.

3. Standard solutions can be prepared at other concentrations by making appropriate dilutions. Store the solutions in a location that is protected from light.

4. The following information may appear as part of the raw data report:

"x.xx nd" (e.g., 0.80nd) indicates that the calculated concentration is below the minimum concentration programmed in the RPA-1 RaPID Analyzer parameters (20 pg/mL). The result should be reported "not detected" and the minimum concentration value should be noted.

"nd " indicates the absorbance measured is greater than or equal to the absorbance of the 0.00 ng/mL standard; therefore, a concentration cannot be calculated. The result should be reported as "not detected".

"x.xx Hi " (e.g., 3.82Hi) indicates that the calculated concentration exceeds the maximum concentration programmed in the RPA-1 RaPID Analyzer parameters (3.6 ng/mL). The sample should be diluted (e.g., 1:10) with Sample Diluent and then re-analyzed.

O. References

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Table I. Compounds Tested for the Potential to Interfere in the Trichloropyridinol RaPID Assay Test Kit

Pesticides	Solvents (Max Tolerated)	Organic/Inorganic compounds
Alachlor	Acetone (15%)	Calcium (chloride dihydrate)
Aldicarb	Acetonitrile (5%)	Copper (chloride)
Ametryn	DMF (>20%)	Humic Acid
Atrazine	Methanol (10%)	Iron (chloride hexahydrate)
Azinphos methyl		Magnesium (chloride hexahydrate)
Benomyl		Manganese (chloride)
Carbaryl		Mercuric (chloride)
Carbendazim		Nickel (sulfate hexahydrate)
Carbofuran		Nitrate (sodium)
Chlorpyrifos		Peroxide (hydrogen)
Chlorpyrifos methyl		Phosphate (sodium, heptahydrate)
Clopyralid		Silicates (sodium meta-)
2,4-D		Sodium chloride
Diazinon		Sulfate (sodium)
Dinoseb		Sulfite (sodium)
Fenitrothion		Thiosulfate (sodium, pentahydrate)
Fluroxypyr		Zinc (chloride)
Glyphosate		
Lindane		
Malathion		
MCPA		
Methamidophos		
Metolachlor		
Methomyl		
Oxamyl		
Parathion		
Parathion methyl		
Phosmet		
Picloram		
Pirimicarb		
Pirimiphos-ethyl		
Pirimiphos-methyl		
Profenfos		
Propachlor		
Terbufos		
Thiophanate-methyl		
Triclopyr		

Table II. Compounds Tested for the Potential to Interfere in the Triclopyr RaPID Assay Test Kit

Pesticides	Solvents (Max Tolerated)	Organic/Inorganic compounds
Alachlor	Acetonitrile (2%)	Calcium (chloride dihydrate)
Aldicarb	Acetone (1%)	Copper (chloride)
Aldicarb Sulfoxide	DMF (0.5%)	Humic Acid
Aldicarb Sulfone	Methanol (10%)	Iron (chloride hexahydrate)
Atrazine		Magnesium (chloride hexahydrate)
Benomyl		Manganese (chloride)
Butachlor		Mercuric (chloride)
Butylate		Nickel (sulfate hexahydrate)
Captan		Nitrate (sodium)
Carbaryl		Peroxide (hydrogen)
Carbendazim		Phosphate (sodium, heptahydrate)
Carbofuran		Silicates (sodium meta-)
Chlorpyrifos		Sodium chloride
Chlorpyrifos methyl		Sulfate (sodium)
Clopyralid		Sulfite (sodium)
2,4-D		Thiosulfate (sodium, pentahydrate)
2,4,5-T		Zinc (chloride)
Diazinon		
Dicamba		
Dichloropropene		
Dinoseb		
Disulfoton		
Fenitrothion		
Fenoxaprop		
Fluroxypyr		
Glyphosate		
Hexazinone		
Imazapyr		
Isofenfos		
Lindane		
MCPA		
Methamidophos		
Metolachlor		
Metribuzin		
Metsulfuron		
Pentachlorophenol		
Picloram		
Pirimicarb		
Pirimiphos-ethyl		
Pirimiphos-methyl		
Profenfos		
Propachlor		
Terbufos		
Thiabendazole		
Thiophanat-methyl		