METHOD 4015

SCREENING FOR 2,4-DICHLOROPHENOXYACETIC ACID BY IMMUNOASSAY

1.0 SCOPE AND APPLICATION

1.1 Method 4015 is a procedure for screening soils and aqueous matrices to determine whether 2,4-dichlorophenoxyacetic acid (2,4-D) (CAS Registry 94-75-7) is likely to be present at concentrations above 0.1, 0.5, 1.0 or 5.0 mg/kg in soil, and in aqueous matrices above 10 mg/L (the toxicity characteristic regulatory action level) and 10 µg/L (ground water monitoring). Method 4015 provides an estimate for the concentration of 2,4-D by comparison against standards.

1.2 Using the test kit from which this method was developed, ≥95% of aqueous samples confirmed to have concentrations of 2,4-D below detection limits will produce a negative result in the 10 ppm test configuration.

1.3 In cases where the exact concentration of 2,4-D is required, additional techniques (i.e., gas chromatography, Method 8151, or high performance liquid chromatography, Method 8321) should be used.

1.4 This method is restricted to use by or under the supervision of trained analysts. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 Test kits are commercially available for this method. The manufacturer's directions should be followed.

2.2 In general, the method is performed using an extract of a soil sample, or directly on an aqueous sample. Filtered extracts may be stored cold, in the dark. An aliquot of the aqueous sample or extract and an enzyme-2,4-D conjugate reagent are added to immobilized 2,4-D antibody. The enzyme-2,4-D conjugate "competes" with 2,4-D present in the sample for binding to 2,4-D antibody. The enzyme-2,4-D conjugate bound to the 2,4-D antibody then catalyzes a colorless substrate to a colored product. The test is interpreted by comparing the color produced by a sample to the response produced by a reference reaction.

3.0 INTERFERENCES

3.1 Compounds that are chemically similar may cause a positive test (false positive) for 2,4-D. The data for the lower limit of detection of these compounds are provided in Tables 1A and 1C. Consult the information provided by the manufacturer of the kit used for additional information regarding cross reactivity with other compounds.

Solutions of Silvex alone, and Silvex/2,4-D mixtures, were prepared in TCLP buffer to demonstrate the potential effect of a structurally similar, environmentally significant cross-reactant on the immunoassay screening results. At one-half of the action level for 2,4-D (5ppm), 200 ppm of Silvex are required to be present to generate a false positive response. These results are summarized in Table 1B.

3.2 Storage and use temperatures may modify the method performance. Follow the manufacturer's directions for storage and use.

4.0 APPARATUS AND MATERIALS

Immunoassay test kit: 2,4-D RaPID$_{TM}$ Assay kit (Ohmicron), EnviroGard$_{TM}$ 2,4-D in Soil (Millipore, Inc.), or equivalent. Each commercially available test kit will supply or specify the apparatus and materials necessary for successful completion of the test.

5.0 REAGENTS

Each commercially available test kit will supply or specify the reagents necessary for successful completion of the test.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 See the introductory material to this chapter, Organic Analytes, Sec. 4.1.

6.2 Soil samples may be contaminated, and should therefore be considered hazardous and handled accordingly.

7.0 PROCEDURE

Follow the manufacturer's instructions for the test kit being used. Those test kits used must meet or exceed the performance specifications indicated in Tables 2-9.

8.0 QUALITY CONTROL

8.1 Follow the manufacturer's instructions for the test kit being used for quality control procedures specific to the test kit used. Additionally, guidance provided in Method 4000 and Chapter One should be followed.

8.2 Use of replicate analyses, particularly when results indicate concentrations near the action level, is recommended to refine information gathered with the kit.

8.3 Do not use test kits past their expiration date.

8.4 Do not use tubes or reagents designated for use with other test kits.

8.5 Use the test kits within their specified storage temperature and operating temperature limits.

8.6 Method 4015 is intended for field or laboratory use. The appropriate level of quality assurance should accompany the application of this method to document data quality.

9.0 METHOD PERFORMANCE

9.1 Sensitivity of the EnviroGard™ 2,4-D in Soil Test Kit was determined by establishing the "noise" level expected from matrix effects encountered in negative soil samples and determining the corresponding 2,4-D concentration by comparison to the analyte-specific response curve. Eight different soils which did not contain 2,4-D were assayed. Each of these soils was extracted in triplicate and each extract was assayed in three different assays. The mean and the standard deviation of the resulting %Bo's (%Bo = [(OD_{sample}/$OD_{negative\ control}$)x100]) were calculated and the sensitivity was estimated at two standard deviations below the mean. The sensitivity for Method 4015 was determined to be 80% Bo at a 95% confidence interval. Based on the average assay response to 2,4-D, this corresponds to 0.16 ppm 2,4-D. These data are shown in Table 2.

9.2 The effect of water content of the soil samples was determined by assaying three different soil samples which had been dried and subsequently had water added to 30% (w/w). Aliquots of these samples were then fortified with 2,4-D. Each soil sample was assayed three times, with and without added water, and with and without 2,4-D fortification. It was determined that water in soil up to 30% had no detectable effect on the method. These data are shown in Table 3.

9.3 The effect of the pH of the soil extract was determined by adjusting the soil pH of three soil samples. Soil samples were adjusted to pH 2 - 4 using 6N HCl and pH 10 - 12 using 6N NaOH. Aliquots of the pH adjusted soil samples were fortified with 2,4-D. Each soil sample was assayed unadjusted and with pH adjusted to 2-4 and 10-12, both unfortified and fortified. It was determined that soil samples with pH ranging from 3 to 11 had no detectable effect on the performance of the method. These data are shown in Table 4.

9.4 The method bias was estimated by fortifying three different soil samples at two different concentrations (0.3 and 2 ppm 2,4-D). Each fortified sample was extracted three times and each extract was assayed three times. Recovery for individual determinations ranged from 27% to 151%. Average recovery for each individual extract ranged from 70% to 120%. Overall average recovery for all samples was 99.7%. These data are summarized in Table 5.

9.5 The probabilities of generating false negative and false positive results at a 10 ppm action level are shown in Table 6.

9.6 The results obtained from spiking 2,4-D into TCLP leachates and other aqueous samples are reported in Table 7. Each matrix was diluted 1:1000 and tested by immunoassay 5 times. The results are reported as positive (+) or negative (-). Municipal water results are based on a 52 ppb cutoff to determine positive from negative, and were diluted 1:7.

9.7 Comparison of the results from immunoassay and GC (Method 8151) testing of aqueous samples are presented in Table 8.

9.8 A field trial was undertaken to evaluate the ability of the EnviroGard™ 2,4-D in Soil Test Kit to identify 2,4-D contaminated soil at a remediation site. A total of 30 soil samples were evaluated by both the immunoassay and Method 8151. Interpretation of the results at 200 µg/kg resulted in 0/32 (0%) false negatives and 1/32 (3%) false positives. This corresponds to specificity 95% and sensitivity of 100%. These data are shown in Table 9.

10.0 REFERENCES

1. 2,4-D RaPID™ Assay kit Users Guide, Ohmicron.

2. EnviroGard™ 2,4-D in Soil Test Kit Guide, Millipore, Inc.

3. Lawruk, T.S., Hottenstein, C.S., Fleeker, J.R., Hall, J.C., Herzog, D.P., Rubio, F.M., "Quantitation of 2,4-D and Related Chlorophenoxy Herbicides by A Magnetic Particle-Based ELISA"' 1993, (manuscript submitted for publication).

4. Hayes, M.C., Jourdan, S.W., Lawruk, T.S., and Herzog, D.P., "Screening of TCLP Extracts of Soil and Wastewater for 2,4-D by Immunoassay", USEPA Ninth Annual Waste Testing and Quality Assurance Symposium, 1993.

TABLE 1A

CROSS-REACTIVITY[a] OF CHLOROPHENOXY COMPOUNDS AND STRUCTURALLY UNRELATED PESTICIDES

Compound	Concentration Giving a Positive Result (ppm TCLP Leachate)	Percent Cross-Reactivity
2,4-D	10	100
2,4-D propylene glycol ester	0.52	1900
2,4-D ethyl ester	0.54	1850
2,4-D isopropyl	0.96	1040
2,4-D methyl ester	1.09	917
2,4-D sec-butyl ester	1.40	714
2,4-D butyl ester	1.60	625
2,4-D butoxyethyl ester	2.00	500
2,4,5-T methyl ester	12.0	86
2,4-D iso-octyl ester	20.0	50
2,4-D butoxy-propylene ester	20.6	49
2,4-DB	95	11
MCPA	110	9
2,4,5-T	130	8
Silvex methyl ester	665	1.5
4-Chlorophenoxyacetic acid	815	1.2
MCPB	980	1.0
Silvex (2,4,5-TP)	1375	0.7
Dichlorophenol	2380	0.4
Dichloroprop	5000	0.2
Triclopyr	>10,000	<0.1
MCPP	>10,000	<0.1
Mecoprop	>10,000	<0.1
Pentachlorophenol	>10,000	<0.1
Picloram	>10,000	<0.1

TABLE 1A (cont.)

CROSS-REACTIVITY[a] OF CHLOROPHENOXY COMPOUNDS AND STRUCTURALLY UNRELATED PESTICIDES

Compound	Concentration Giving a Positive Result (ppm TCLP Leachate)	Percent Cross-Reactivity
Alachlor	>10,000	<0.1
Aldicarb	>10,000	<0.1
Aldicarb sulfate	>10,000	<0.1
Aldicarb sulfoxide	>10,000	<0.1
Atrazine	>10,000	<0.1
Benomyl	>10,000	<0.1
Butylate	>10,000	<0.1
Captan	>10,000	<0.1
Captofol	>10,000	<0.1
Carbaryl	>10,000	<0.1
Carbofuran	>10,000	<0.1
Dicamba	>10,000	<0.1
1,3-Dichloropropene	>10,000	<0.1
Dinoseb	>10,000	<0.1
Metolachlor	>10,000	<0.1
Metribuzin	>10,000	<0.1
Simazine	>10,000	<0.1
Terbufos	>10,000	<0.1
Thiabendazol	>10,000	<0.1

[a] 2,4-D RaPID™ Assay kit

TABLE 1B

CROSS-REACTIVITY[a] OF 2,4-D WITH SILVEX

Silvex Concentration (ppm)	2,4-D Concentration (ppm)	Screening Result
0	0	-
0.5	0	-
1.0	0	-
2.0	0	-
100	0	-
200	0	-
0	5.0	-
0.5	5.0	-
1.0	5.0	-
2.0	5.0	-
100	5.0	-
200	5.0	+

[a] 2,4-D RaPID™ Assay kit

TABLE 1C

CROSS REACTIVITY[a]

Compound	Concentration Required for Positive Interpretation (ppm)
2,4-D Acid	0.2
2,4-D butyl ester	0.025
2,4-D Dichlorophenol	1.5
2,4-D isobutyl ester	0.2
2,4-D isopropyl ester	0.2
2,4-D methyl ester	0.1
2,4-DB	0.2
2,4-DB butyl ester	0.9
Dichloroprop	6.0
Diclofop	42.5
MCPA	0.8
2,4,5-T acid	7.0

[a] EnviroGard™ 2,4-D in Soil Test Kit (Millipore Corporation)

TABLE 2

Sensitivity of the EnviroGard™ 2,4-D in Soil Test Kit

Part 1 - Average Response with Negative Soils			
Soil#	Soil Type	Average %Bo (n=9)	Standard Deviation
S1	LOAM	90.0	1.7
S2	LOAM	89.6	2.3
S3	SAND/LOAM	89.3	2.1
S4	CLAY	86.3	1.9
S5	CLAY	90.0	2.3
S6	LOAM/SAND	86.9	2.6
S7	SAND	88.8	2.8
	LOAM	86.9	2.9
Average		88.5	6.5

Part 2 - Average Response with 2,4-D Calibrators		
2,4-D Calibrator Concentration (ppm)	Average Absorbance	Average %Bo
0	1.442	N/A
0.1	1.186	82.2
0.5	0.776	53.8
1.0	0.600	41.7
5.0	0.301	20.9

Part 3 - Method Sensitivity

Based on Part 1 and Part 2 Above:

Average %Bo - 2 SD = 75.6 which is equivalent to 0.16 ppm 2,4-D

Average %Bo - 3 SD = 69.1 which is equivalent to 0.23 ppm 2,4-D

(%Bo = [(OD_{sample}/$OD_{negative\ control}$)x100])

TABLE 3

Effect of Water Content of Soil Samples on the EnviroGard™
2,4-D in Soil Test Kit

Soil	% Water	Fortified?	Rep. 1	Rep. 2	Rep. 3	Mean	Std. Dev.	± 2 SD Range
S1	0	No	98.7*	99.9	102.9	100.5	2.2	96.1 - 105
S1	30	No	96.0	95.4	93.7	95.0	1.2	92.6 - 97.4
S1	0	Yes	61.4	62.0	73.1	65.5	6.6	52.3 - 78.7
S1	30	Yes	63.1	59.9	69.4	64.1	4.8	54.5 - 73.7
S2	0	No	98.5	90.7	97.8	95.7	4.3	87.1 - 104
S2	30	No	96.0	95.4	96.8	96.1	0.7	94.7 - 97.5
S2	0	Yes	47.6	47.0	46.0	46.9	0.8	45.3 - 48.5
S2	30	Yes	37.6	37.7	40.0	38.4	1.3	35.8 - 41.0
S3	0	No	98.7	94.1	105.2	99.4	5.6	88.2 - 111
S3	30	No	97.3	97.2	95.9	96.8	0.8	95.2 - 98.4
S3	0	Yes	41.0	39.3	48.8	43.1	5.1	32.9 - 53.3
S3	30	Yes	43.1	40.4	47.4	43.6	3.5	36.6 - 50.6

* All values shown are $\%Bo = [(OD_{sample}/OD_{negative\ control})x100]$

TABLE 4

Effect of pH of Soil Samples on the EnviroGard™ 2,4-D in Soil Test Kit

Soil	pH Adj.	Fortified?	Rep. 1*	Rep. 2	Rep. 3	Mean	Std. Dev.	± 2 SD Range
S1	None	No	95.5	92.5	88.7	92.2	3.4	85.4 - 99.0
S1	Acidic	No	102	105	93.1	100	6.2	87.6 - 112
S1	Basic	No	96.8	98.3	79.4	91.5	10.5	70.5 - 113
S1	None	Yes	46.0	47.5	48.6	47.4	1.3	44.8 - 50.0
S1	Acidic	Yes	50	51.9	43.7	48.5	4.3	39.9 - 57.1
S1	Basic	Yes	43.0	52.4	39.1	44.8	6.8	31.2 - 58.4
S2	None	No	94.3	90.6	90.8	91.9	2.1	87.7 - 96.1
S2	Acidic	No	91.7	95.8	85.9	91.1	5.0	81.1 - 101
S2	Basic	No	89.7	94.2	81.0	88.3	6.7	74.9 - 102
S2	None	Yes	50.5	52.6	50.2	51.1	1.3	48.5 - 53.7
S2	Acidic	Yes	56.3	58.1	44.3	52.9	7.5	37.9 - 67.9
S2	Basic	Yes	46.9	54.2	46.4	49.1	4.4	40.3 - 57.9
S3	None	No	82.2	92.0	85.4	86.5	5.0	76.5 - 96.5
S3	Acidic	No	95.0	85.1	86.9	89.0	5.3	78.4 - 99.6
S3	Basic	No	86.1	84.4	103	91.2	10.4	70.4 - 112
S3	None	Yes	52.2	63.6	49.4	55.1	7.5	40.1 - 70.1
S3	Acidic	Yes	55.2	59.5	66.6	60.4	5.8	48.8 - 72.0
S3	Basic	Yes	59.4	54.3	54.9	56.2	2.8	50.6 - 61.8

* All values shown are $\%Bo = [OD_{sample}/OD_{negative\ control})x100]$

TABLE 5

Bias of the EnviroGard™ 2,4-D in Soil Test Kit

Soil#	Fortification (ppm)	Extraction#	Recovered (ppm)*	% Recovery
S1	0.3	1	0.21	70.0
S1	0.3	2	0.24	80.0
S1	0.3	3	0.23	76.6
S1	2	1	1.87	93.5
S1	2	2	2.12	106
S1	2	3	2.40	120
Average >>>				91.0
S2	0.3	1	0.29	96.7
S2	0.3	2	0.29	96.7
S2	0.3	3	0.30	100
S2	2	1	2.05	102
S2	2	2	1.89	94.5
S2	2	3	2.22	111
Average >>>				100
S3	0.3	1	0.31	103
S3	0.3	2	0.31	103
S3	0.3	3	0.31	103
S3	2	1	2.28	114
S3	2	2	2.30	115
S3	2	3	2.24	112
Average >>>				108

Overall Average %Recovery = 99.7

TABLE 6

Probability of False Negative and False Positive Results for 2,4-D RaPID™ Assay Kit at a 10 ppm Action Level in TCLP Extract from Organic Soil

Spike Concentration 2,4-D (ppm)	Probability of False Positive (%)	Probability of False Negative (%)
5	0	N/A
7.5	70	N/A
10	N/A	0
15	N/A	0

Results were based on ten replicate spiked samples. Cutoff levels were established using 30 replicates of each solution tested in 3 immunoassay batch runs.

N/A = No false positives possible above/below the action limit.

TABLE 7

2,4-D SPIKING RESULTS ON AQUEOUS ENVIRONMENTAL MATRICES[a]

ID #	Matrix+Spike	R1	R2	R3	R4	R5	%POS	%NEG
1	TCLP Buffer	-	-	-	-	-	-	-
2	TCLP Buffer + 15 ppm	+	+	+	+	+	+	+
3	TCLP Buffer + 10 ppm	+	+	+	+	+	+	+
4	TCLP Buffer + 5 ppm	-	-	-	-	-	-	-
5	Sandy Extract[b]	-	-	-	-	-	-	-
6	Sandy Extract + 15 ppm	+	+	+	+	+	+	+
7	Sandy Extract + 10 ppm	+	+	+	+	+	+	+
8	Sandy Extract + 5 ppm	-	-	-	-	-	-	-
9	Organic Extract[c]	-	-	-	-	-	-	-
10	Organic Extract + 15	+	+	+	+	+	+	+
11	Organic Extract + 10	+	+	+	+	+	+	+
12	Organic Extract + 5	-	-	-	-	-	-	-
13	Effluent #1	-	-	-	-	-	-	-
14	Effluent #1 + 15 ppm	+	+	+	+	+	+	+
15	Effluent #1 + 10 ppm	+	+	+	+	+	+	+
16	Effluent #1 + 5 ppm	-	-	-	-	-	-	-
17	Effluent #2	-	-	-	-	-	-	-
18	Effluent #2 + 15 ppm	+	+	+	+	+	+	+
19	Effluent #2 + 10 ppm	+	+	+	+	+	+	+
20	Effluent #2 + 5 ppm	-	-	-	-	-	-	-
21	Runoff	-	-	-	-	-	-	-
22	Runoff + 15 ppm	+	+	+	+	+	+	+
23	Runoff + 10 ppm	+	+	+	+	+	+	+
24	Runoff + 5 ppm	-	-	-	-	-	-	-
25	Municipal Water	-	-	-	-	-	-	-
26	Municipal Water + 140 ppb	+	+	+	-	-	-	-

TABLE 7 (cont.)

2,4-D SPIKING RESULTS ON AQUEOUS ENVIRONMENTAL MATRICES[a]

ID #	Matrix+Spike	R1	R2	R3	R4	R5	%POS	%NEG
27	Municipal Water + 70 ppb	+	+	+	-	-	-	-
28	Municipal Water + 35 ppb	-	-	-	-	-	-	-

[a] 2,4-D RaPID$_{TM}$ Assay kit

[b] Sandy Soil TCLP Extract

[c] Organic Soil TCLP Extract

TABLE 8

2,4-D Spiking Results
2,4-D RaPID™ Assay Kit vs. Method 8151

ID#	Matrix/Spike	Immunoassay Results	Method 8151 2,4-D (ppm)	Correlation IA vs. GC
1	TCLP Buffer	5/5 Negative	nd	Yes
2	TCLP Buffer + 15 ppm	5/5 Positive	13.0	Yes
3	TCLP Buffer + 10 ppm	5/5 Positive	11.0	Yes
4	TCLP Buffer + 5 ppm	5/5 Negative	5.6	Yes
5	Sandy Extract[a]	5/5 Negative	nd	Yes
6	Sandy Extract + 15 ppm	5/5 Positive	*	*
7	Sandy Extract + 10 ppm	5/5 Positive	5.9, 5.2	No
8	Sandy Extract + 5 ppm	5/5 Negative	*	*
9	Organic Extract[b]	5/5 Negative	nd	Yes
10	Organic Extract + 15 ppm	5/5 Positive	*	*
11	Organic Extract + 10 ppm	5/5 Positive	10.0, 9.5	Yes
12	Organic Extract + 5 ppm	5/5 Negative	*	*
13	Effluent #1	5/5 Negative	*	*
14	Effluent #1 + 15 ppm	5/5 Positive	*	*
15	Effluent #1 + 10 ppm	5/5 Positive	11.0, 7.8	Yes
16	Effluent #1 + 5 ppm	5/5 Negative	3.6	Yes
17	Effluent #2	5/5 Negative	*	*
18	Effluent #2 + 15 ppm	5/5 Positive	11.0	Yes
19	Effluent #2 + 10 ppm	5/5 Positive	8.8, 9.5	Yes
20	Effluent #2 + 5 ppm	5/5 Negative	*	*
21	Runoff	5/5 Negative	nd	Yes
22	Runoff + 15 ppm	5/5 Positive	*	*
23	Runoff + 10 ppm	5/5 Positive	9.7, 8.6	Yes
24	Runoff + 5 ppm	5/5 Negative	5.5	Yes
25	Municipal Water	5/5 Negative	nd	N/A

TABLE 8 (cont.)

ID#	Matrix/Spike	Immunoassay Results	Method 8151 2,4-D (ppm)	Correlation IA vs. GC
26	Municipal Water + 140 ppb	5/5 Positive	*	N/A
27	Municipal Water + 70 ppb	5/5 Positive	58.59 (ppb)	N/A
28	Municipal Water + 35	5/5 Negative	*	N/A

[a] Sandy Soil TCLP Extract

[b] Organic Soil TCLP Extract

nd non-detectable

N/A Not applicable to wastewater regulatory limit

* No analysis with Method 8150

TABLE 9

Comparison of the EnviroGard™ 2,4-D in Soil Test Kit to Method 8151
Interpretation of Results at 200 µg/kg

Sample #	Method 8151, µg/kg	Immunoassay Result	Agrees?
1	<200	NEGATIVE	YES
2	<200	NEGATIVE	YES
3	220	POSITIVE	YES
4	<200	NEGATIVE	YES
5	<200	NEGATIVE	YES
6	330	POSITIVE	YES
7	<200	POSITIVE	FALSE POSITIVE
8	<200	NEGATIVE	YES
9	830	POSITIVE	YES
10	<200	NEGATIVE	YES
11	310	POSITIVE	YES
12	350	POSITIVE	YES
13	<200	NEGATIVE	YES
14	<200	NEGATIVE	YES
15	200	POSITIVE	YES
16	<200	NEGATIVE	YES
17	<200	NEGATIVE	YES
18	440	POSITIVE	YES
19	560	POSITIVE	YES
20	380	POSITIVE	YES

TABLE 9 (cont.)

Sample #	Method 8151, µg/kg	Immunoassay Result	Agrees?
21	<200	NEGATIVE	YES
22	360	POSITIVE	YES
23	<200	NEGATIVE	YES
24	<200	NEGATIVE	YES
25	<200	NEGATIVE	YES
26	<200	NEGATIVE	YES
27	<200	NEGATIVE	YES
28	<200	NEGATIVE	YES
29	<200	NEGATIVE	YES
30	<200	NEGATIVE	YES