METHOD 4010A

SCREENING FOR PENTACHLOROPHENOL BY IMMUNOASSAY

1.0 SCOPE AND APPLICATION

1.1 Method 4010 is a procedure for screening solids such as soils, sludges, and aqueous media such as waste water and leachates for pentachlorophenol (PCP) (CAS Registry No. 87-86-5).

1.2 Method 4010 is recommended for screening samples to determine whether PCP is likely to be present at defined concentrations (i.e., kits are available which give positive results at 0.005 mg/L for aqueous samples, and at 0.5, 10 or 100 mg/kg in solid samples). Method 4010 provides an estimate for the concentration of PCP by comparison with a standard.

1.3 Using the test kits from which this method was developed, 95% of aqueous samples containing 2 ppb or less of PCP will produce a negative result in the 5 ppb configuration. Also, 95% of soil samples containing 125 ppb or less of PCP will produce a negative result in the 5000 ppb test configuration.

1.4 In cases where the exact concentration of PCP is required, additional techniques (i.e., gas chromatography (Methods 8141, 8151) or gas chromatography/mass spectrometry (Method 8270)) should be used.

1.5 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 a water sample or an extract of a water sample. The sample/extract and an enzyme conjugate reagent are added to immobilized antibody. The enzyme conjugate "competes" with PCP present in the sample for binding to immobilized anti-PCP antibody. The test is interpreted by comparing the response produced by testing a sample to the response produced by testing standard(s) simultaneously.

3.0 INTERFERENCES

3.1 Compounds that are chemically similar may cause a positive test (false positive) for PCP. The test kits used in preparation of this method were evaluated for interferences. Tables 1A and 1B provide the concentration of compounds which will give a false positive test at the indicated concentration.

3.2 Other compounds have been tested for cross reactivity for PCP and have been demonstrated not to interfere with the specific kits tested. Consult the information provided by the manufacturer of the kit used for additional information regarding cross reactivity with other compounds.

3.3 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: PENTA RISc™ (EnSys, Inc.), EnviroGard™ PCP 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 2-10.

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 4010 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 This method has been applied to a series of groundwater, process water, and wastewater samples from industries which use PCP, and the results compared with GC/MS determination of PCP (Method 8270). These results are provided in Table 2. These results represent determinations by two laboratories using the PENTA RISc™ test kit.

9.2 This method has been applied to a series of soils from industries which use PCP and the results compared with GC/MS determination of PCP via method 8270. These results are provided in Table 3. These results represent determinations by two laboratories using the PENTA RISc™ test kit.

9.3 Sensitivity of the EnviroGard PCP in Soil Test Kit was determined by establishing the "noise" level expected from matrix effects encountered in negative soil samples and determining the corresponding PCP concentration by comparison to the analyte-specific response curve. Eight different soils which did not contain PCP were assayed. Each of these soils was extracted in triplicate and each extract was analyzed in three different assays. The mean and the standard deviation of the resulting %Bo's (%Bo = $[(OD_{sample}/OD_{negative\ control})x100]$, see definitions in Method 4000) were calculated and the sensitivity was estimated at two standard deviations below the mean. The sensitivity for Method 4010 was determined to be 80% Bo at a 95% confidence interval. Based on the average assay response to PCP, this corresponds to 2 ppm PCP. These data are shown in Table 4.

9.4 The effect of water content of the soil samples on the EnviroGard™ PCP in Soil test kit 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 PCP. Each soil sample was assayed three times, with and without added water, and with and without home heating oil (HHO) fortification. It was determined that water in soil up to 30% had no detectable effect on the method. These data are shown in Table 5.

9.5 The effect of the pH of the soil extract on the EnviroGard™ PCP in Soil test kit 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 PCP and the unfortified and fortified samples were extracted. These extracts were assayed three times. 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 6.

9.6 The bias of the EnviroGard™ PCP in Soil test kit was estimated by fortifying three different soil samples at two different concentrations (10 and 100 ppm PCP). Each fortified sample was extracted three times and each extract was assayed three times. Recovery for individual determinations ranged from 60% to 125%. Average recovery for each individual extract ranged from 72% to 101%. Overall average recovery for all samples was 86%. These data are summarized in Table 7.

9.7 The effect of co-contamination of soil samples with oil on the EnviroGard™ PCP in Soil test kit was investigated. Three soil samples were adulterated with diesel oil and aliquots were fortified with PCP. The samples were extracted and the extracts each assayed three times. It was determined that no interference was detected in samples with up to 10% oil contamination. The data from samples adulterated at 10% are shown in Table 8.

9.8 A field trial was conducted at a contaminated site using the EnviroGard™ PCP in Soil test kit. Method 4010 was used to identify soil which had been contaminated with PCP from wood

treatment operations. A total of 33 samples were analyzed including 5 field duplicates. For the field duplicates, the reference method demonstrated an average coefficient of variation of 16%. For Method 4010 average coefficient of variation was 31%. Since Method 4010 is not quantitative, quantitative values were estimated. These data are shown in Table 9. At the 10 ppm cutoff, there were 0/33 (0%) false negatives and 0/33 (0%) false positives. At the 100 ppm cutoff, there was 1/33 (3%) false negatives and 1/33 (3%) false positives. These data are shown in Table 10.

10.0 REFERENCES

1. J.P. Mapes, K.D. McKenzie, L.R. McClelland, S. Movassaghi, R.A. Reddy, R.L. Allen, and S.B. Friedman, "Rapid, On-Site Screening Test for Pentachlorophenol in Soil and Water - PENTA-RISc™", Ensys Inc., Research Triangle Park, NC 27709

2. J.P. Mapes, K.D. McKenzie, L.R. McClelland, S. Movassaghi, R.A. Reddy, R.L. Allen, and S.B. Friedman, "PENTA-RISc™ - An On-Site Immunoassay for Pentachlorophenol in Soil", Ensys Inc., Research Triangle Park, NC 27709

3. PENTA-RISc™ Instructions for Use, Ensys Inc.

4. EnviroGard™ PCP in Soil Test Kit Guide, Millipore, Inc.

TABLE 1A

Cross Reactivity for PCP PENTA RISc™ Test Kit

Compound[a]	Concentration (mg/Kg) in Soil to Cause a False Positive for PCP at 0.5 mg/Kg	Concentration (µg/L) in Water to Cause a False Positive for PCP at 5 µg/L
2,6-Dichlorophenol	700	600
2,3,4-Trichlorophenol	400	600
2,4,6-Trichlorophenol	16	100
2,4,5-Trichlorophenol	100	500
2,3,5,6-Tetrachlorophenol	1.2	7
Tetrachlorohydroquinone	500	>1500

[a] Compounds assayed at 3.75 µM (molar equivalent of PCP at 1000 µg/L), except where noted.

TABLE 1B

Cross Reactivity for PCP EnviroGard™ PCP in Soil Test Kit

Compound	Lower Limit of Detection (mg/kg)
Pentachlorophenol	10
2,5-Dichlorophenol	1000
2,6-Dichlorophenol	1000
2,3,4-Trichlorophenol	1000
2,3,5-Trichlorophenol	500
2,3,6-Trichlorophenol	500

TABLE 1B (cont.)

Compound	Lower Limit of Detection (mg/kg)
2,4,5-Trichlorophenol	500
2,4,6-Trichlorophenol	500
The following compounds were tested and found to yield negative results at 1,000 ppm:	
2,3,5,6-Tetrachloronitrobenzene 3,5-Dichlorophenol 2,4-Dichlorophenol 2,3-Dichlorophenol 4-Chlorophenol	PCB (Aroclor 1248) TNT DDT PAHs Chlordane

TABLE 2

Comparison of PENTA RISc™ Test Kit with GC/MS - Aqueous Matrix

Sample Type	Screening Results (ppm)							Concentration measured by GC/MS (ppm)	AGREEMENT[a] Y, FP, FN
	0.005	0.05	0.1	0.5	1	5	50		
groundwater					>	>	<	3.5	FP
			>	<				0.35	Y
	>	<						<0.1	Y
						>	<	8.2	Y
	>	>			>	<		2.8	Y
	>	>			>	<		2.9	Y
process water	>	>	<	<				0.21	FN
	>	>	<	<				0.17	Y
			>	<				0.12	Y
wastewater		>	>	<	<			0.6	FP
					>	<		1.4	Y
	>	>	<					<0.1	Y
			>	<				0.17	Y
run-off	>	>	<					<0.1	Y
	>	<						0.034	Y
		>			<			0.098	Y
		>			<			0.084	Y
		>			<			0.086	Y

TABLE 2 (cont.)

Sample Type	Screening Results (ppm)							Concentration measured by GC/MS (ppm)	AGREEMENT[a] Y, FP, FN
	0.005	0.05	0.1	0.5	1	5	50		
						>		2.1	FP
run-off (cont.)		>			<			0.073	Y
		>			<			0.026	FP
	>	<						0.006	Y
					>			0.169	FP
		>			<			0.239	Y
					>			0.190	FP
					>			0.114	FP
			>			<		0.346	Y
						>		1.1	Y
						>		19	Y
					>		<	4.3	Y

[a] Y = Yes, FN = False Negative, FP = False Positive

TABLE 3

Comparison of PENTA RISc™ Test Kit with GC/MS
Soil Matrix

Concentration measured by GC/MS (ppm)	Screening Results (ppm)			AGREEMENT[a] Y, FP, FN
	0.5	5	50	
1100	>	>	>	Y
88	>	>	<	FN
0.31	<	<	<	Y
0.72	<	<	<	FN
315	>	>	>	Y
1.5	>	<	<	Y
6.4	>	>	<	Y
9	>	>	<	Y
1.9	>	<	<	Y
46	>	>	>	FP
<1	>	<	<	Y
21	>	>	<	Y
3.3	>	<	<	Y
4	>	>	<	FP
11	>	>	<	Y
18	>	>	<	Y
33	>	>	<	Y
54	>	>	>	Y
65	>	>	>	Y
74	>	>	>	Y
83	>	>	>	Y
1.1	>	<	<	Y
14.3	>	>	<	Y
<1	<	<	<	Y
<1	<	<	<	Y

TABLE 3 (cont.)

Concentration measured by GC/MS (ppm)	Screening Results (ppm)			AGREEMENT[a] Y, FP, FN
	0.5	5	50	
<1	<	<	<	Y
3.9	>	<	<	Y
<1	<	<	<	Y
1.4	>	<	<	Y
48	>	>	>	FP
<1	<	<	<	Y
142	>	>	>	Y
108	>	>	<	FN
117	>	>	>	Y
56	>	>	>	Y
2.5	>	<	<	Y
3.5	>	>	<	FP
143	>	>	>	Y
nd	<	<	<	Y
0.02	<	<	<	Y
5	>	<	<	Y

Y = Yes, FN = False Negative, FP = False Positive.

TABLE 4

EnviroGard™ PCP in Soil Test Kit Sensitivity

Part 1 - Average Response with Negative Soils

Soil#	Soil Type	Average %Bo (n = 9)	Standard Deviation
S1	LOAM	97.6	3.0
S2	CLAY	100.1	1.4
S3	SAND	101.4	2.8
S4	LOAM	99.4	4.9
S5	SAND	100.2	3.1
S6	CLAY	97.4	2.7
S7	LOAM/SAND	102.6	0.3
S8	SAND/LOAM	97.5	3.6
AVERAGE		99.5	5.2

Part 2 - Average Response with Pentachlorophenol Calibrators

PCP Concentration (ppm)	Average Absorbance	Average %Bo
0	1.142	N/A
5	0.828	72.6
20	0.556	48.7
50	0.382	33.4
200	0.162	14.1

Part 3 - Method Sensitivity

Based on Part 1 and Part 2 Above:

Average %Bo - 2 SD = 89.2 which is equivalent to 1.6 ppm PCP

Average %Bo - 3 SD = 84.0 which is equivalent to 2.3 ppm PCP

(%Bo = [(ODsample/ODnegative control)x100])

TABLE 5

EFFECT OF WATER CONTENT IN SOIL SAMPLES[a]

Soil	% Water	Fortified?	Rep. 1	Rep. 2	Rep. 3	Mean	Std. Dev.	± 2 SD Range
S1	0	No	104.5*	106.5	99.7	103.6	3.5	96.6 - 111
S1	30	No	101.9	106.3	95.2	101.1	5.6	89.9 - 112
S1	0	Yes	38.9	47.2	40.2	42.1	4.4	33.3 - 50.9
S1	30	Yes	49.2	51.1	48.2	49.5	1.5	46.5 - 52.5
S2	0	No	97.8	105.7	96.7	100.1	4.9	90.3 - 110
S2	30	No	105.1	109.7	93.9	102.9	8.1	86.7 - 119
S2	0	Yes	40.2	47.5	42.7	43.5	3.7	36.1 - 50.9
S2	30	Yes	48.8	47.2	44.8	46.9	2.0	42.9 - 50.9
S3	0	No	98.3	107.1	99.7	101.7	4.7	92.3 - 111
S3	30	No	111.5	103.1	95.1	103.2	8.2	86.8 - 120
S3	0	Yes	43.3	47.2	43.2	44.6	2.3	40.0 - 49.2
S3	30	Yes	46.5	49.8	48.0	48.1	1.7	44.7 - 51.5

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

[a] EnviroGard™ PCP in Soil (Millipore, Inc.)

TABLE 6

EFFECT OF pH OF SOIL SAMPLES[a]

Soil	pH Adj.	Fortified?	Rep. 1*	Rep. 2	Rep. 3	Mean	Std. Dev.	± 2 SD Range
S1	None	No	103.1	98.6	98.6	100.1	2.6	94.9 - 105
S1	Acidic	No	88.7	96.9	100.2	95.3	5.9	83.5 - 107
S1	Basic	No	85.2	90.9	98.0	91.3	6.4	78.5 - 104
S1	None	Yes	52.7	44.8	45.8	47.8	4.3	39.2 - 56.4
S1	Acidic	Yes	57.1	44.6	45.2	48.9	7.0	34.9 - 62.9
S1	Basic	Yes	44.6	41.6	45.9	44.0	2.2	39.6 - 48.4
S2	None	No	105.6	93.9	102.5	100.7	6.1	88.5 - 113
S2	Acidic	No	104.4	91.3	105.8	100.5	8.0	84.5 - 117
S2	Basic	No	93.4	87.7	105.8	95.6	9.3	77.0 - 114
S2	None	Yes	47.8	45.1	44.3	45.7	1.8	42.1 - 49.3
S2	Acidic	Yes	51.4	44.4	54.1	50.0	5.0	40.0 - 60.0
S2	Basic	Yes	43.3	40.7	44.0	42.7	1.8	39.1 - 46.3
S3	None	No	92.3	101.8	100.4	98.2	5.2	87.8 - 109
S3	Acidic	No	96.6	91.9	98.5	95.7	3.4	88.9 - 103
S3	Basic	No	87.7	99.8	96.3	94.6	6.2	82.2 - 107
S3	None	Yes	55.2	49.5	55.9	53.6	3.5	46.6 - 60.6
S3	Acidic	Yes	55.3	48.3	42.0	48.5	6.7	35.1 - 61.9
S3	Basic	Yes	44.3	39.3	48.0	43.9	4.4	35.1 - 52.7

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

[a] EnviroGard™ PCP in Soil (Millipore, Inc.)

TABLE 7

TEST KIT[a] BIAS

Soil#	Fortification(ppm)	Extraction#	Recovered(ppm)*	% Recovery
S1	10	1	9	91
S1	10	2	9	86
S1	10	3	9	88
S1	100	1	84	84
S1	100	2	78	78
S1	100	3	76	76
Average >>>				84
S2	10	1	10	100
S2	10	2	8	76
S2	10	3	8	76
S2	100	1	101	101
S2	100	2	98	98
S2	100	3	88	88
Average >>>				90
S3	10	1	7	72
S3	10	2	8	76
S3	10	3	8	81
S3	100	1	95	95
S3	100	2	90	90
S3	100	3	87	87
Average >>>				84

Overall Average %Recovery = 86

[a] EnviroGard™ PCP in Soil (Millipore, Inc.)

TABLE 8

EFFECT OF CO-CONTAMINATION WITH DIESEL OIL[a]

Soil#	Adulterated	Fortified	Rep.#1	Rep.#2	Rep.#3	Mean
S1	NO	NO	103.2*	92.5	99.8	98.5
S1	YES	NO	93.4	99.4	106.2	99.7
S1	NO	YES	52.7	44.8	45.8	47.8
S1	YES	YES	50.9	49.7	44.6	48.4
S2	NO	NO	103.1	98.3	102.3	101.2
S2	YES	NO	85.4	95.1	99.9	93.5
S2	NO	YES	47.8	45.1	44.3	45.7
S2	YES	YES	44.6	50.8	49.0	48.1
S3	NO	NO	98.9	95.4	108.1	100.8
S3	YES	NO	103.8	99.7	101.4	101.6
S3	NO	YES	55.2	49.5	55.9	53.6
S3	YES	YES	50.4	50.6	56.7	52.6

* Figures are %Bo = $[(OD_{sample}/OD_{negative\ control})*100]$

[a] EnviroGard™ PCP in Soil (Millipore, Inc.)

TABLE 9

FIELD DUPLICATES[a]

	Sample ID				
	059	073	074	086	087
Method 8270					
Determination #1	9600	74.8	836	6.59	34.0
Determination #2	10300	78.2	1520	6.88	51.8
Average	9950	76.5	1178	6.74	42.9
Standard Deviation	495	2.4	484	0.20	12.6
% Coefficient of Variation	5.0	3.1	41	3.0	29
Immunoassay*					
Determination #1	4480	79.5	604	2.4	36.0
Determination #2	3370	122	421	5.0	24.0
Average	3920	101	512	3.7	30.0
Standard Deviation	785	30.0	129	1.8	8.5
% Coefficient of Variation	20	30	25	50	28

* For the purpose of this comparison, quantitative values were calculated for the immunoassay.

[a] EnviroGard™ PCP in Soil (Millipore, Inc.)

TABLE 10

IMMUNOASSAY[a] COMPARED TO METHOD 8270
TEST INTERPRETATION AT 10 PPM PCP

Sample ID	Method 8270	Immunoassay	Concurrence?
059	9600	>10	YES
059D	10300	>10	YES
060	1010	>10	YES
061	2740	>10	YES
063	1610	>10	YES
064	1980	>10	YES
065	1580	>10	YES
066	57.8	>10	YES
067	110	>10	YES
068	47.7	>10	YES
069	798	>10	YES
070	2890	>10	YES
071	289	>10	YES
072	326	>10	YES
073	74.8	>10	YES
073D	78.2	>10	YES
074	836	>10	YES
074D	1520	>10	YES
075	3690	>10	YES
076	4590	>10	YES
077	2040	>10	YES
078	1720	>10	YES
079	792	>10	YES
080	2550	>10	YES
081	125	>10	YES
082	2400	>10	YES
083	270	>10	YES
084	1140	>10	YES
085	57.7	>10	YES
086	6.59	<10	YES
086D	6.88	<10	YES
087	34.0	>10	YES
087D	51.8	>10	YES

[a] EnviroGard™ PCP in Soil (Millipore, Inc.)

TABLE 10 (cont.)

Sample ID	Method 8270	Immunoassay	Concurrence?
059	9600	>100	YES
059D	10300	>100	YES
060	1010	>100	YES
061	2740	>100	YES
063	1610	>100	YES
064	1980	>100	YES
065	1580	>100	YES
066	57.8	<100	YES
067	110	>100	YES
068	47.7	<100	YES
069	798	>100	YES
070	2890	>100	YES
071	289	>100	YES
072	326	>100	YES
073	74.8	<100	YES
073D	78.2	>100	False Positive
074	836	>100	YES
074D	1520	>100	YES
075	3690	>100	YES
076	4590	>100	YES
077	2040	>100	YES
078	1720	>100	YES
079	792	>100	YES
080	2550	>100	YES
081	125	<100	False Negative
082	2400	>100	YES
083	270	>100	YES
084	1140	>100	YES
085	57.7	<100	YES
086	6.59	<100	YES
086D	6.88	<100	YES
087	34.0	<100	YES
087D	51.8	<100	YES

[a] EnviroGard™ PCP in Soil (millipore, Inc.).