METHOD 4041

SOIL SCREENING FOR CHLORDANE BY IMMUNOASSAY

1.0 SCOPE AND APPLICATION

1.1 Method 4041 is a procedure for screening soils to determine whether chlordane (CAS Registry 57-74-9) is present at concentrations above 20, 100 or 600 µg/kg. Method 4041 provides an estimate for the concentration of chlordane by comparison against standards.

1.2 In cases where the exact concentration of chlordane is required, additional techniques [i.e., gas chromatography (Method 8081) or gas chromatography/mass spectrometry (Method 8270)] should be used.

1.3 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. Filtered extracts may be stored cold, in the dark. An aliquot of the extract and an enzyme-chlordane conjugate reagent are added to immobilized chlordane antibody. The enzyme-chlordane conjugate "competes" with chlordane present in the sample for binding to chlordane antibody. The enzyme-chlordane conjugate bound to the chlordane 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 chlordane. The test kit used to develop this method was evaluated for interferences. The data for the lower limit of detection of these compounds are provided in Table 1. Consult the information provided by the manufacturer of the kit used for additional information regarding cross reactivity with other compounds.

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

4.1 Immunoassay test kit: EnviroGard™ Chlordane 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 2-5.

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 4041 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 Method sensitivity was determined by establishing the "noise" level expected from matrix effects encountered in negative soil samples and determining the corresponding Chlordane concentration by comparison to the analyte-specific response curve. 8 different soils which did not contain Chlordane 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 ($\%Bo = [(OD_{sample}/OD_{negative\ control})x100]$) was calculated and the sensitivity was estimated at two standard deviations below the mean. The sensitivity for Method 4041 was determined to be 80% Bo at a 95% confidence interval. Based on the average assay response to Chlordane, this corresponds to 6.4 µg/kg Chlordane. 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 Chlordane (100 µg/kg). Each soil sample was assayed three times, with and without added water, and with and without Chlordane 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 Chlordane (100 µg/kg). 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 A field trial was undertaken to evaluate to ability of the EnviroGard™ Chlordane in Soil Test Kit to identify chlordane contaminated soil at a remediation site. A total of 32 soil samples were evaluated by both Method 4041 and Method 8080. Interpretation of the results at a 1 mg/kg cutoff resulted in 2/32 (6.3%) false negatives and 0/32 (0%) false positives. Interpretation of the results at a cutoff of 10 mg/kg resulted in 0/32 (0%) false negatives and 2/32 (6.3%) false positives. These data are shown in Table 5.

10.0 REFERENCES

1. EnviroGard™ Chlordane in Soil Test Kit Guide, Millipore, Inc.

TABLE 1

CROSS REACTIVITY

Compound	Concentration Required for Positive Interpretation (µg/kg)
Chlordane	5
Endrin	3
Endosulfan I	3
Endosulfan II	3
Dieldrin	3
Heptachlor	3
Aldrin	10
Toxaphene	100
gamma-BHC (Lindane)	300
alpha-BHC	1000
delta-BHC	1000
The following compounds were found to yield a negative result for concentrations up to 200,000 µg/kg: Gasoline Pentachlorophenol PCB (Aroclor 1248) Trinitrotoluene	

TABLE 2

METHOD SENSITIVITY

Part 1 - Average Response with Negative Soils

Soil No.	Soil Type	Average %Bo (n = 8)	Standard Deviation
S1	Loam/sand	92.8	2.0
S2	Loam	86.2	1.0
S3	Clay	85.5	8.8
S4	Clay	95.4	1.1
S5	Clay	83.9	2.6
S6	Loam/sand	88.5	1.8
S7	Sand	81.4	2.7
S8	Sand	95.8	0.8
AVERAGE		88.7	4.5

Part 2 - Average Response with Chlordane Calibrators

Chlordane Concentration (µg/kg)	Average Absorbance	Average %Bo
0	1.043	N/A
5	0.882	84.4
25	0.598	57.2
125	0.322	30.8
500	0.159	15.2

Part 3 - Method Sensitivity

Based on Part 1 and Part 2 Above:

Average %Bo - 2 SD = 79.7 which is equivalent to 6.4 µg/kg Chlordane

Average %Bo - 3 SD = 75.2 which is equivalent to 8.6 µg/kg Chlordane

TABLE 3

EFFECT OF WATER CONTENT IN SOIL SAMPLES

Soil	% Water	Fortified?	Rep. 1	Rep. 2	Rep. 3	Mean	Std. Dev.	± 2 SD Range
S1	0	No	95.2	101	94.5	97.0	3.7	89.6 - 104
S1	30	No	96.0	99.2	96.0	97.1	1.8	93.5 - 101
S1	0	Yes	40.5	38.5	35.9	38.3	2.3	33.7 - 42.9
S1	30	Yes	42.2	43.0	43.0	42.8	0.5	41.8 - 43.8
S2	0	No	85.8	87.1	85.5	86.1	0.9	84.3 - 87.9
S2	30	No	78.7	84.9	79.8	81.1	3.3	74.5 - 87.8
S2	0	Yes	37.7	39.5	40.6	39.3	1.5	36.3 - 42.3
S2	30	Yes	39.8	38.8	37.0	38.5	1.4	35.7 - 41.3
S3	0	No	76.6	76.6	73.0	75.4	2.1	71.2 - 79.6
S3	30	No	87.4	88.7	85.7	87.3	1.5	84.3 - 90.3
S3	0	Yes	40.0	40.2	35.7	38.7	2.5	33.7 - 43.7
S3	30	Yes	40.8	37.1	38.7	38.9	1.9	35.1 - 42.7

TABLE 4

EFFECT OF pH OF SOIL SAMPLES

Soil	pH Adj.	Fortified?	Rep. 1*	Rep. 2	Rep. 3	Mean	Std. Dev.	± 2 SD Range
S1	None	No	97.5	87.8	94.8	93.4	5.0	83.4 - 103
S1	Acidic	No	97.4	114	94.7	102	10.7	80.8 - 124
S1	Basic	No	107	114	93.8	105	10.1	84.7 - 125
S1	None	Yes	43.6	47.5	38.5	43.2	4.5	34.2 - 52.2
S1	Acidic	Yes	43.6	51.8	34.1	43.2	8.8	25.6 - 60.8
S1	Basic	Yes	44.8	50.8	32.0	42.5	9.6	23.3 - 61.7
S2	None	No	87.8	86.3	87.6	87.3	0.8	85.7 - 88.9
S2	Acidic	No	94.2	108	80.5	94.1	13.5	67.1 - 121
S2	Basic	No	89.3	99.3	77.9	88.8	10.7	67.4 - 110
S2	None	Yes	43.9	48.9	33.9	42.2	7.7	26.8 - 57.6
S2	Acidic	Yes	44.6	55.9	41.5	47.4	7.6	32.2 - 62.6
S2	Basic	Yes	42.3	59.2	36.5	46.0	11.8	22.4 - 69.6
S3	None	No	72.3	74.5	79.3	75.4	3.6	68.2 - 82.6
S3	Acidic	No	85.3	105	75.7	88.8	15.1	58.6 - 119
S3	Basic	No	89.4	83.8	85.9	86.4	2.8	80.8 - 92.0
S3	None	Yes	44.5	49.5	32.6	42.2	8.7	24.8 - 59.6
S3	Acidic	Yes	40.5	52.1	34.7	42.4	8.9	24.6 - 60.2
S3	Basic	Yes	40.6	37.1	43.9	40.5	3.4	33.7 - 47.3

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

TABLE 5

CORRELATION TO METHOD 8081

Test Interpretation at 1 mg/kg

Sample ID	Method 8081 (mg/kg)	Immunoassay (mg/kg)	Results Agree?
co-ss-2	45	POSITIVE	YES
co-ss-3	4.9	POSITIVE	YES
co-ss-4	25	POSITIVE	YES
co-ss-5	1.4	NEGATIVE	FALSE NEGATIVE
co-ss-6	2.7	POSITIVE	YES
co-ss-7	2.5	POSITIVE	YES
co-ss-8	<1.0	NEGATIVE	YES
co-ss-9	7.9	POSITIVE	YES
co-ss-10	6.0	POSITIVE	YES
co-ss-13	5.2	POSITIVE	YES
co-ss-14	2.9	POSITIVE	YES
co-ss-15	2.1	POSITIVE	YES
co-ss-17	<1.0	NEGATIVE	YES
co-ss-20	2.8	NEGATIVE	FALSE NEGATIVE
co-ss-21	<1.0	NEGATIVE	YES
co-ss-22	<1.0	NEGATIVE	YES
co-ss-23	<1.0	NEGATIVE	YES
co-ss-24	51	POSITIVE	YES
co-ss-25	1.4	POSITIVE	YES
co-ss-26	<1.0	NEGATIVE	YES
co-ss-27	<1.0	NEGATIVE	YES
co-ss-28	9.6	POSITIVE	YES
co-ss-28-17D	2.6	POSITIVE	YES
co-ss-29	14	POSITIVE	YES
co-ss-30	1.8	POSITIVE	YES
co-ss-31	<1.0	NEGATIVE	YES
co-ss-32	<1.0	NEGATIVE	YES
co-ss-33	2.9	POSITIVE	YES
co-ss-34	4.2	POSITIVE	YES
co-ss-35	<1.0	NEGATIVE	YES
co-ss-36	5.9	POSITIVE	YES
co-ss-41	<1.0	NEGATIVE	YES

TABLE 5 (cont.)

Test Interpretation at 10 mg/kg

Sample ID	Method 8081 (mg/kg)	Immunoassay (mg/kg)	Results Agree?
co-ss-2	45	POSITIVE	YES
co-ss-3	4.9	NEGATIVE	YES
co-ss-4	25	POSITIVE	YES
co-ss-5	1.4	NEGATIVE	YES
co-ss-6	2.7	NEGATIVE	YES
co-ss-7	2.5	NEGATIVE	YES
co-ss-8	<1.0	NEGATIVE	YES
co-ss-9	7.9	POSITIVE	FALSE POSITIVE
co-ss-10	6.0	POSITIVE	FALSE POSITIVE
co-ss-13	5.2	NEGATIVE	YES
co-ss-14	2.9	NEGATIVE	YES
co-ss-15	2.1	NEGATIVE	YES
co-ss-17	<1.0	NEGATIVE	YES
co-ss-20	2.8	NEGATIVE	YES
co-ss-21	<1.0	NEGATIVE	YES
co-ss-22	<1.0	NEGATIVE	YES
co-ss-23	<1.0	NEGATIVE	YES
co-ss-24	51	POSITIVE	YES
co-ss-25	1.4	NEGATIVE	YES
co-ss-26	<1.0	NEGATIVE	YES
co-ss-27	<1.0	NEGATIVE	YES
co-ss-28	9.6	NEGATIVE	YES
co-ss-28-17D	2.6	NEGATIVE	YES
co-ss-29	14	POSITIVE	YES
co-ss-30	1.8	NEGATIVE	YES
co-ss-31	<1.0	NEGATIVE	YES
co-ss-32	<1.0	NEGATIVE	YES
co-ss-33	2.9	NEGATIVE	YES
co-ss-34	4.2	NEGATIVE	YES
co-ss-35	<1.0	NEGATIVE	YES
co-ss-36	5.9	NEGATIVE	YES
co-ss-41	<1.0	NEGATIVE	YES