METHOD 4042

SOIL SCREENING FOR DDT BY IMMUNOASSAY

1.0 SCOPE AND APPLICATION

1.1 Method 4042 is a procedure for screening soils to determine whether 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane (DDT) (CAS Registry 50-29-3) and its breakdown products (DDD, DDE, and DDA) are present at concentrations above 0.2, 1.0 or 10 mg/kg. Method 4042 provides an estimate for the sum of concentrations of DDT and daughter compounds by comparison against standards.

1.2 In cases where the exact concentration of DDT 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-DDT conjugate reagent are added to immobilized DDT antibody. The enzyme-DDT conjugate "competes" with DDT present in the sample for binding to DDT antibody. The enzyme-DDT conjugate bound to the DDT 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 DDT. 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™ DDT 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 4042 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 DDT concentration by comparison to the analyte-specific response curve. Eight different soils which did not contain DDT 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 4042 was determined to be 81.4% Bo at a 95% confidence inteval. Based on the average assay response to DDT, this corresponds to 0.044 ppm DDT. 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 DDT (1.0 mg/kg). Each soil sample was assayed three times, with and without added water, and with and without DDT 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 DDT (1.0 mg/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 study was conducted at a contaminated site using a commercially available test kit (EnviroGard™ DDT in Soil Test Kit, Millipore Corp.). The immunoassay was used to identify soil which had been contaminated with DDT. The standard method (Method 8081) was performed at a certified laboratory and the results were compared to the immunoassay. When interpreting the results at a 0.2 ppm cutoff, the immunoassay yielded 0/32 (0%) false negatives and 2/32 (6.3%) false positives. When interpreting the results at a 1.0 ppm cutoff, the immunoassay yielded 1/32 (3.1%) false negatives and 2/32 (6.3%) false positives. These data are shown in Table 5.

10.0 REFERENCES

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

TABLE 1

CROSS REACTIVITY

Compound	Concentration Required for Positive Interpretation (ppm)
p,p'-DDT	0.04
p,p'-DDD	0.01
p,p'-DDE	0.18
o,p'-DDT	4.0
o,p'-DDD	0.4
o,p'-DDE	3.0
DDA	0.002
Chloropropylate	0.007
Chlorobenzilate	0.03
Dicofol	0.14
Chloroxuron	24
Monolinuron	25
Thiobencarb	5
Tebuconazole	7
Neburon	17
Tetradifon	1.2
Diclofop	70
PCB (Aroclor 1248)	90

The following analytes are not detected at or above 100 ppm:

2,4-D	4-Chlorophenoxyacetic acid	Chlordane
Pentachlorophenol	Chlorbromuron	Chlortoluron
Dicamba	Diflubenzuron	Diuron
Lindane	Linuron	MCPA acid
MCPB	Mecoprop	Gasoline
Diesel fuel	2,4,6-Trinitrotoluene	Toxaphene

TABLE 2

METHOD SENSITIVITY

Part 1 - Average Response with Negative Soils

Soil No.	Soil Type	Average %Bo (n = 9)	Standard Deviation
S1	Loam	87.0	7.5
S2	Clay	93.2	2.3
S3	Sand	97.2	2.6
S4	Loam	87.7	1.2
S5	Loam/sand	88.1	2.3
S6	Clay	100.8	2.1
S7	Loam/sand	103.6	0.3
S8	Sand/loam	89.6	4.5
AVERAGE		93.4	6.0

Part 2 - Average Response with DDT Calibrators

DDT Concentration (ppm)	Average Absorbance	Average %Bo
0	1.133	N/A
0.1	0.897	79.4
1.0	0.569	50.3
10.0	0.362	32.0
50.0	0.259	22.9

Part 3 - Method Sensitivity

Based on Part 1 and Part 2 Above:

Average %Bo - 2 SD = 81.4 which is equivalent to 0.044 ppm DDT

Average %Bo - 3 SD = 75.4 which is equivalent to 0.097 ppm DDT

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	79.7*	79.3	83.7	80.9	2.4	76.1 - 85.7
S1	30	No	89.1	84.0	85.9	86.4	2.6	81.2 - 91.6
S1	0	Yes	49.8	62.1	46.3	52.8	8.3	36.2 - 69.4
S1	30	Yes	55.8	59.9	58.0	57.9	2.1	53.7 - 62.1
S2	0	No	85.2	96.2	97.9	93.1	6.9	79.3 - 106.9
S2	30	No	94.8	94.3	95.0	94.7	0.3	94.1 - 95.3
S2	0	Yes	54.4	47.0	56.1	52.5	4.8	42.9 - 62.1
S2	30	Yes	56.3	53.8	60.2	56.8	3.2	50.4 - 63.2
S3	0	No	96.2	91.3	100.0	95.8	4.3	87.2 - 104.4
S3	30	No	95.6	90.5	96.4	94.2	3.2	87.8 - 100.6
S3	0	Yes	54.8	52.9	54.8	54.2	1.1	52.0 - 56.4
S3	30	Yes	59.4	55.0	54.5	56.3	2.7	50.9 - 61.7

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

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	91.4	91.3	78.3	87.0	7.5	72.0 - 102
S1	Acidic	No	79.7	87.0	86.8	84.5	4.1	76.3 - 92.7
S1	Basic	No	80.5	84.5	78.5	81.2	3.0	75.2 - 87.2
S1	None	Yes	57.5	60.3	55.1	57.6	2.6	52.4 - 62.8
S1	Acidic	Yes	54.2	60.6	55.2	56.7	3.4	49.9 - 63.5
S1	Basic	Yes	52.8	60.2	53.3	55.5	4.1	47.3 - 63.7
S2	None	No	94.7	90.6	94.5	93.2	2.3	88.6 - 97.8
S2	Acidic	No	87.8	100.1	100.9	96.3	7.3	81.7 - 111
S2	Basic	No	87.9	81.6	98.3	89.3	8.5	72.3 - 106
S2	None	Yes	51.7	56.9	48.3	52.3	4.3	43.7 - 60.9
S2	Acidic	Yes	52.2	61.0	55.2	56.1	4.5	47.1 - 65.1
S2	Basic	Yes	52.0	53.5	48.9	51.5	2.3	46.9 - 56.1
S3	None	No	99.1	94.2	98.2	97.2	2.6	92.0 - 102
S3	Acidic	No	86.4	84.3	85.5	85.4	1.1	83.2 - 87.6
S3	Basic	No	94.9	100.3	92.9	96.1	3.8	88.5 - 104
S3	None	Yes	56.2	54.3	52.8	54.4	1.7	51.0 - 57.8
S3	Acidic	Yes	54.5	53.5	53.9	54.0	0.5	53.0 - 55.0
S3	Basic	Yes	54.6	57.2	62.9	58.2	4.2	49.8 - 66.6

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

TABLE 5

COMPARISON TO METHOD 8081

Test Interpretation at 0.2 mg/kg

Sample ID	Method 8081 (mg/kg)	Immunoassay (mg/kg)	Results Agree?
co-ss-2	3.6	POSITIVE	YES
co-ss-3	0.55	POSITIVE	YES
co-ss-4	2.3	POSITIVE	YES
co-ss-5	<0.05	NEGATIVE	YES
co-ss-6	0.15	POSITIVE	FALSE POSITIVE
co-ss-7	0.3	POSITIVE	YES
co-ss-8	0.1	NEGATIVE	YES
co-ss-9	0.8	POSITIVE	YES
co-ss-10	0.23	POSITIVE	YES
co-ss-13	0.79	POSITIVE	YES
co-ss-14	0.58	POSITIVE	YES
co-ss-15	0.35	POSITIVE	YES
co-ss-17	<0.05	NEGATIVE	YES
co-ss-20	0.18	NEGATIVE	YES
co-ss-21	0.06	NEGATIVE	YES
co-ss-22	<0.05	NEGATIVE	YES
co-ss-23	<0.05	NEGATIVE	YES
co-ss-24	1.2	POSITIVE	YES
co-ss-25	0.12	NEGATIVE	YES
co-ss-26	<0.05	NEGATIVE	YES
co-ss-27	<0.05	NEGATIVE	YES
co-ss-28	0.16	NEGATIVE	YES
co-ss-28-17D	0.18	POSITIVE	FALSE POSITIVE
co-ss-29	0.69	POSITIVE	YES
co-ss-30	0.73	POSITIVE	YES
co-ss-31	0.68	POSITIVE	YES
co-ss-32	<0.05	NEGATIVE	YES
co-ss-33	0.32	POSITIVE	YES
co-ss-34	0.23	POSITIVE	YES
co-ss-35	0.52	POSITIVE	YES
co-ss-36	1.0	POSITIVE	YES
co-ss-41	<0.05	NEGATIVE	YES

TABLE 5 (cont.)

Test Interpretation at 1.0 mg/kg

Sample ID	Method 8081 (mg/kg)	Immunoassay (mg/kg)	Results Agree?
co-ss-2	3.6	POSITIVE	YES
co-ss-3	0.55	NEGATIVE	YES
co-ss-4	2.3	POSITIVE	YES
co-ss-5	<0.05	NEGATIVE	YES
co-ss-6	0.15	NEGATIVE	YES
co-ss-7	0.3	NEGATIVE	YES
co-ss-8	0.1	NEGATIVE	YES
co-ss-9	0.8	NEGATIVE	YES
co-ss-10	0.23	NEGATIVE	YES
co-ss-13	0.79	NEGATIVE	YES
co-ss-14	0.58	NEGATIVE	YES
co-ss-15	0.35	NEGATIVE	YES
co-ss-17	<0.05	NEGATIVE	YES
co-ss-20	0.18	NEGATIVE	YES
co-ss-21	0.06	NEGATIVE	YES
co-ss-22	<0.05	NEGATIVE	YES
co-ss-23	<0.05	NEGATIVE	YES
co-ss-24	1.2	POSITIVE	YES
co-ss-25	0.12	NEGATIVE	YES
co-ss-26	<0.05	NEGATIVE	YES
co-ss-27	<0.05	NEGATIVE	YES
co-ss-28	0.16	NEGATIVE	YES
co-ss-28-17D	0.18	NEGATIVE	YES
co-ss-29	0.69	NEGATIVE	YES
co-ss-30	0.73	POSITIVE	FALSE POSITIVE
co-ss-31	0.68	POSITIVE	FALSE POSITIVE
co-ss-32	<0.05	NEGATIVE	YES
co-ss-33	0.32	NEGATIVE	YES
co-ss-34	0.23	NEGATIVE	YES
co-ss-35	0.52	NEGATIVE	YES
co-ss-36	1.0	NEGATIVE	FALSE NEGATIVE
co-ss-41	<0.05	NEGATIVE	YES