ATTACHMENT B

IMMUNOASSAY TEST INSTRUCTIONS

STRATEGIC DIAGNOSTICS INC.
EnviroGard® DDT in Soil Test Kit
73100

Intended Use
The EnviroGard DDT in Soil Test Kit is a qualitative or semi-quantitative field test for the detection of DDT and its metabolites DDD and DDE in soil. The EnviroGard DDT in Soil Test Kit allows rapid semi-quantitative screening for DDT at 0.2, 1.0, and 10.0 parts per million (ppm) in soils.

Test Principles
The EnviroGard DDT in Soil Test Kit is based on the use of polyclonal antibodies that bind either DDT or DDT-Enzyme Conjugate. These antibodies are immobilized to the walls of the test tubes. When DDT is present in the sample, it competes with the DDT-Enzyme Conjugate for a limited number of antibody binding sites.

Since there are the same number of antibody binding sites on every test tube and each test tube receives the same number of DDT-Enzyme Conjugate molecules, a sample that contains a low concentration of DDT allows the antibody to bind many DDT-Enzyme Conjugate molecules.

Therefore, a low concentration of DDT produces a dark blue solution. Conversely, a high concentration of DDT allows fewer DDT-Enzyme Conjugate molecules to be bound by the antibodies, resulting in a lighter blue solution.

NOTE: Color is inversely proportional to DDT concentration.

Darker color = Lower concentration
Lighter color = Higher concentration

Performance Characteristics
The EnviroGard DDT in Soil Test Kit will not differentiate between DDT, its metabolites, and other structurally similar compounds, but will detect their presence to differing degrees. The following table shows a number of compounds and the approximate concentration of each required to yield a positive result (Lower Limit of Detection or LLD), and the concentration required to inhibit one-half of the color developed by the Negative Control (IC50). Concentration is in parts per million (ppm) in soil.

Compound	LLD	IC50
p,p'-DDT (kit calibrator)	0.04	1.25
p,p'-DDD	0.01	0.3
p,p'-DDE	0.18	3.6
o,p'-DDT	4	93
o,p'-DDD	0.4	11
o,p'-DDE	3	93
DDA	0.002	0.04
Chloropropylate	0.007	0.08
Chlorobenzilate	0.03	0.35
Dicofol	0.14	2
Tetradifon	1.2	14
Thiobencarb	5	52
Tebuconazole	7	95
Neburon	17	284
Chloroxuron	24	216
Monolinuron	25	714
Diclofop	70	>1000

The following compounds have lower limits of detection > 100 ppm:

2,4-D	4-chlorophenoxyacetic acid
Chlorbromuron	Chlordane
Chlortoluron	Dicamba
Diflubenzuron	Diuron
Lindane	Linuron
MCPA acid	MCPB
Mecoprop	

Precautions
• Treat DDT, solutions that contain DDT and potentially contaminated soil samples as hazardous materials.
• Where appropriate, use gloves, proper protective clothing, and methods to contain and handle hazardous material.
• Store all test kit components at 4°C to 8°C (39°F to 46°F) when not in use.
• Do not freeze test kit components or expose them to temperatures greater than 37°C (99°F).
• Allow all reagents to reach ambient temperature (18°C to 27°C or 64°F to 81°F) before beginning the test.
• Do not use test kit components after the expiration date.
• Do not use reagents or test tubes from one test kit with reagents or test tubes from a different test kit.
• Use approved methodologies to confirm any positive results.
• Do not dilute or adulterate test reagents or use samples not called for in the test procedure; this may give inaccurate results.
• Tightly recap the DDT calibrator vials to prevent evaporative loss.
• Distribution of DDT in soils may be highly variable. The use of a composite sampling technique may be appropriate. Development of a sampling plan that assures adequate sample number and distribution is the responsibility of the analyst.
• DDT is light sensitive. Store soil extracts at 2°C to 7°C, shielded from direct light.

Materials Provided
EnviroGard DDT in Soil Test Kit
This test kit contains the following items:
20 Antibody-Coated Test Tubes
1 vial of Assay Diluent
1 vial of Negative Control (methanol)
1 vial of 0.2 ppm DDT Calibrator in methanol
1 vial of 1.0 ppm DDT Calibrator in methanol
1 vial of 10.0 ppm DDT Calibrator in methanol
1 vial of DDT-Enzyme Conjugate
1 vial of Substrate
1 vial of Stop Solution
1 20-place Test Tube Rack

22 Pipette Tips, yellow (for the Gilson M-25 Microman® Positive Displacement Pipettor)

Materials Required but Not Provided
You will also need several other items, some of which are included in the EnviroGard Soil Field Lab.

- Methanol-ACS reagent grade Methanol is required for soil extraction, but is not included in the EnviroGard Soil Extraction Kit. You must order it separately.
- EnviroGard Soil Extraction Bottle Kit

Use this kit for the extraction of DDT in soil samples. This kit contains enough devices to process 14 samples:
• 14, 30 mL LDPE Bottles with screw caps (each bottle contains stainless steel mixing beads)
• 14 filtration caps
• 14 Millex® HV_{13} filters
• 18 Wooden Spatulas
• 1 Syringe with coupler
• 1 Syringe coupler
• 14 Screw Top Glass Vials, 4.0 mL
• 14 Stoppers

• 18 Weigh Boats
• Gilson M-25 Microman Positive Displacement Pipettor
• Eppendorf™ Repeater® Pipettor and five Combitips® (3 x 12.5 mL, 1 x 5.0 mL, and 1 x 50 mL)
• Balance capable of accurately weighing 5 grams
• Differential Photometer or RPA-I Photometer
• Indelible marker for labeling test tubes
• Watch or timer
• Clean running water or a wash bottle containing tap or deionized water (500 mL)
• Calculator (optional)

Suggestions for Pipettor Use

• Practice using both pipettors (positive displacement and Repeater pipettor) with water and extra tips before you analyze your samples.
• Use a new tip each time you use the Repeater pipettor to avoid reagent cross-contamination. Label three 12.5 mL tips "Diluent", "Substrate" and "Stop," and one 5.0 mL tip "Conjugate".
• Draw the desired reagent volume into the Repeater pipettor and dispense one portion of the reagent back into the container to properly engage the ratchet mechanism. If you do not do this, the first volume delivered may be inaccurate.
• To add reagents using the Repeater pipettor, pipette down the side of the test tube just below the rim.
• To add samples and calibrators using the positive displacement pipettor, pipette down the side of the test tube just above the liquid level.
• The carryover volume of the positive displacement tips is minimal, but may affect results if you are going from a high to low DDT concentration. Use a new pipettor tip each time you pipette a new unknown.

Assay Procedure

Collect/Store the Sample

1. Collect soil in appropriately sized and labeled containers.
2. Take care to remove excess twigs, organic matter and rocks or pebbles from the sample. For best results, wet soils should be air-dried overnight and thoroughly mixed before testing.
3. Store soil samples at 4°C (39°F).

Prepare the Sample/Extract the Soil

1. Please follow the instructions from the EnviroGard Soil Extraction Bottle Kit to prepare the soil extract before the assay.
2. **5 ml** of **Methanol** will be used to extract DDT residue from a 5 gram soil sample. As per instructions, attach a **50 mL** Combitip to the Repeater pipettor and set the dial to **5**. Deliver once to add **5 mL** of **methanol** to the extraction vial, and cap tightly.

Perform the Test

NOTE: Allow all reagents and sample extracts to reach room temperature before you begin the test. Do not analyze more than 20 test tubes at a time.

1. The choice of calibrators to use in the test will depend on the selection of the analyst. The use of two calibrators may be appropriate if screening for a single level of DDT.

Remove the test tubes from the plastic bag and label them as follows*:

Tube Label	Tube Contents
NC	Negative Control
C1	0.2 ppm Calibrator
C2	1.0 ppm Calibrator
C3	10.0 ppm Calibrator
S1	sample 1
S2	sample 2
etc.	

You are not required to perform the assay in duplicate; however, doing so will increase the precision.
Place the test tubes in the test tube rack. Push down on each tube so that it is held firmly and does not fall out of the rack when shaken.
CAUTION: Do not "snap" the test tubes into the rack as this may result in a cracked tube.

2. Attach the **12.5 mL** Combitip labeled "Diluent" to the Repeater pipettor and adjust the dial to **2**. Add 500 microliters (µL) of Assay Diluent to each test tube.
3. Attach a clean pipette tip to the Microman pipettor and adjust the dial to "250". Add 25 µL of each calibrator (including Negative Control) to the corresponding test tube by placing the end of the pipette tip against the side of the tube (just above the level of the Assay Diluent) and dispensing the volume. Use a clean pipette tip each time.

CAUTION: Replace the caps on the calibrator vials immediately after use to minimize evaporation.
4. Using a clean tip for each sample, add 25 µL of each sample extract to the appropriately labeled test tube.
5. Attach the **5.0 mL** Combitip labeled "Conjugate" to the Repeater pipettor and adjust the dial to **1.** Add 100 µL of DDTEnzyme Conjugate to each test tube.
6. Shake the test tube rack to mix for 10 to 15 seconds. Leave the test tubes undisturbed for 15 minutes.
7. Vigorously shake out the test tube contents into a sink or suitable container. Fill the test tubes to **overflowing** with cool tap or distilled water, then decant and vigorously shake out the remaining water.
Repeat this wash step three more times, being certain to shake out as much water as possible on each wash. After the final wash, remove as much water as possible by tapping the inverted tubes on absorbent paper.
8. Attach the **12.5 mL** Combitip labeled "Substrate" to the Repeater pipettor and set the dial to **2**. Add 500 µL of Substrate to each test tube. Leave the test tubes undisturbed for 10 minutes.

NOTE: If a blue color does not develop in the Negative Control test tube within 10 minutes after adding the Substrate, the test is invalid and you must repeat it.

Interpret the Results

You can either interpret the results visually within 10 minutes after adding the Substrate to each test tube, or you can perform a more precise analysis with a photometer after you add the Stop Solution.

Visual Interpretation

After you add the Substrate, wait 10 minutes then mix the test tubes by shaking them for a few seconds until they are a uniform blue color. Compare the sample test tube to the calibrator test tubes against a white background. The test tube rack in the kit is well-suited for this purpose.

NOTE: The word DDT in the interpretation instructions below refers to "total DDT", i.e. the sum of p,p'-DDT, p,p'-DDD, and p,p'-DDE.

• If a sample test tube contains more color than the calibrator test tube, the sample contains DDT at a concentration lower than the calibrator.
• If a sample test tube contains less color than the calibrator test tube, the sample may contain DDT at a concentration greater than the calibrator.
• If the sample test tube contains color that is between the calibrator test tubes, the sample contains DDT at a concentration between the calibrator concentrations.
• If a sample test tube contains approximately the same amount of color as the calibrator test tube, the sample contains DDT at a concentration approximately equal to the calibrator.
• If the sample test tube contains less color than the 10 ppm Calibrator test tube, you may dilute a fraction of the soil extract in methanol (for example, 1:100) and perform the assay again. To determine the

concentration of the diluted extract multiply the result by the dilution factor. (Go to "Semi-Quantitative Interpretation" for further details.)

Photometric Interpretation

After you add the Substrate, wait 10 minutes then add the Stop Solution to each test tube.
WARNING: Stop solution is 1N Hydrochloric acid.

Attach the **12.5 mL** Combitip labeled "Stop" to the Repeater pipettor and set the dial to **2**. Add 500 µL of Stop Solution to each test tube. This converts the blue color in the test tubes to yellow.

NOTE: After you add Stop Solution to the test tubes, results should be read within 30 minutes.

Differential Photometer

1. Place a water blank test tube containing 1.5 mL of deionized water, or equivalent in the left (reference) well.
2. Place the Negative Control test tube into the right (sample) well. Record the optical density (OD) of the Negative Control.
3. Remove the Negative Control test tube and replace it with the 0.2 ppm Calibrator test tube to reactivate the photometer. Record the result. Repeat this step to determine the OD for each of the remaining calibrators and for each sample.

Semi-quantitative Interpretation

Compare the OD of each sample to the OD of each calibrator:
NOTE: The word DDT in the interpretation instructions below refers to "total DDT", i.e. the sum of p,p'-DDT, p,p'-DDD, and p,p'-DDE.
• If a sample OD is equal to the OD of a calibrator, the sample contains DDT at a concentration approximately equal to the calibrator.
• If a sample OD is greater than a calibrator OD, the sample contains less DDT than the calibrator.
• If a sample OD is lower than a calibrator OD, the sample may contain more DDT than that calibrator.
• If an assay result indicates that a soil sample contains greater than 10 ppm total DDT, but you need more specific information, the soil extract may be diluted 1:100 in neat methanol, and assayed again. You must then multiply the results of the re-assay by 100 to determine the approximate sample concentration.

NOTE: If you know in advance that the "action level" of interest is greater than 10 ppm total DDT in soil, the assay may be modified to pinpoint that particular concentration. For example:

If you wish to categorize samples as less than or greater than 250 ppm, you should dilute all sample extracts 1:250 in neat methanol (e.g. 20 µL extract plus 4.98 mL methanol) and compare the diluted extracts to the 1 ppm DDT kit calibrator. Due to the 250-fold dilution, the 1 ppm calibrator represents 250 ppm in the assay.

NOTE: If you are interested in action levels greater than 1000 ppm, please contact Technical Assistance for assistance.

Limitations of the Procedure

The EnviroGard DDT in Soil Test Kit is a qualitative/semi-quantitative screening test only. Actual quantitation of DDT by EnviroGard immunoassay is not possible due to the Test kit's cross-reactivity with DDT breakdown products and other similar compounds and to the variations in extraction efficiency inherent in the fast extraction protocol described in this product insert.

Soil sampling error may significantly affect testing reliability. The distribution of pesticides in different soils can be extremely heterogeneous. Soils should be dried and homogenized before analysis by any method. Split samples (i.e. for GC and immunoassay) should always derive from the same homogenate.

Ordering Information

Description	Catalog Number
EnviroGard DDT in Soil Test Kit	73100
EnviroGard Soil Extraction Bottle Kit	72010

Technical Assistance

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General Limited Warranty

Strategic Diagnostics Inc. warrants the products manufactured by it against defects in materials and workmanship when used in accordance with the applicable instructions for a period not to extend beyond a product's printed expiration date. **SDI MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE.**

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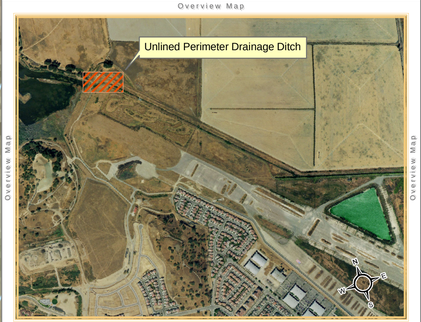
3099050 Rev 3 FEB 98

HAAF-UPDD-CSN-1241
HAAF-UPDD-CSB-1234
HAAF-UPDD-CSN-1230
HAAF-UPDD-CSB-1239
HAAF-UPDD-CSN-1237
HAAF-UPDD-CSS-1245
HAAF-UPDD-CSS-1204
HAAF-UPDD-CSB-1232
HAAF-UPDD-CSS-1224
HAAF-UPDD-CSS-1214
HAAF-UPDD-CSB-1212
HAAF-UPDD-CSN-1215

Overview Map



Overview Map

Total Excavation Volume = 536 cubic yards
Total Excavation Area = 10,719 sq. ft.

Legend

UPDD Excavation Recommendations

Analytical Results Vertical Extent Known	*Recommendation*
< 1 mg/Kg at 0 feet	Do Not Dig
< 1 mg/Kg at 0.5 feet	Dig to 0.5 feet
< 1 mg/Kg at 1.5 feet	Dig to 1.5 feet
< 1 mg/Kg at 2.0 feet	Dig to 2.0 feet

Excavation Areas >= 10 mg/Kg (Field Test Results, see note)

- @ Surface >= 10 mg/Kg
- @ 1.5 feet >= 10 mg/Kg

Other Data Layers

- Elevation Contours (feet)
- Channel Centerline
- Top of Bank Inflection
- Road Shoulder Inflection
- Dirt Road Centerline
- Confirmation Surface Soil Sample
- Confirmation Bottom Soil Sample

See section 3.5 of field sampling plan for explanation of sample identification numbers.

Feet
0 5 10 20 30 40 50
Scale 1:300 1" = 25 feet

US Army Corps of Engineers
Sacramento District
1325 J Street
Sacramento, CA 94805

Date: Aug 11, 2004

Prepared by: Michael O'Neill

N W E S

NAD27 CA State Plane Zone III

Notes:
About the Patterns: The patterns used on this map indicate areas where field tests registered results greater than or equal to 10 mg/Kg. The simple presence of any pattern indicates that concentration was found at some depth below grade. The type of pattern represents the depth at which that concentration was found. The lack of any pattern indicates that no field test results measured greater than 10 mg/Kg.

California

Hamilton Army Airfield

BRAC Remediation Project
Unlined Perimeter Drainage Ditch (UPDD)
Confirmation Sample Locations
Field Effort January, February, June 2004

Novato

Sheet Reference Number:

Figure 1

HAAF-SRW-CSN-1489
HAAF-SRW-CSW-1492
HAAF-SRW-CSB-1508
HAAF-SRW-CSN-1301
HAAF-SRW-CSW-1433
HAAF-SRW-CSS-1469
HAAF-SRW-CSB-1506
HAAF-SRW-CSS-1494
HAAF-SRW-CSB-1304
HAAF-SRW-CSN-1320
HAAF-SRW-CSB-1318
HAAF-SRW-CSN-1419
HAAF-SRW-CSW-1428
HAAF-SRW-CSB-1435
HAAF-SRW-CSB-1333
HAAF-SRW-CSB-1410
HAAF-SRW-CSN-1510
HAAF-SRW-CSE-1411
HAAF-SRW-CSN-1353
HAAF-SRW-CSB-1505
HAAF-SRW-CSB-1335
HAAF-SRW-CSW-1411
HAAF-SRW-CSS-1487
HAAF-SRW-CSB-1352
HAAF-SRW-CSB-1445
HAAF-SRW-CSS-1354
HAAF-SRW-CSE-1463
HAAF-SRW-CSE-1443
HAAF-SRW-CSE-1450
HAAF-SRW-CSE-1447

Legend

SRW Excavation Recommendations

Analytical Result Vertical Extent Known	*Recommendation*
< 1 mg/Kg at 0 feet	Do Not Dig
< 1 mg/Kg at 1 foot	Dig to 1 foot
< 1 mg/Kg at 2 feet	Dig to 2 foot
< 1 mg/Kg at 3 feet	Dig to 3 foot
< 1 mg/Kg at 4 feet	Dig to 4 foot

Excavation Areas >= 10 mg/Kg (Field Test Results, see note)

- @ Surface >= 10 mg/Kg
- @ 1 ft. >= 10 mg/Kg
- @ 3 ft. >= 10 mg/Kg

Other Data Layers

- Elevation Contours (feet)
- Runway Edge (Concrete or Asphault)
- Confirmation Surface Soil Sample
- Confirmation Bottom Soil Sample

See section 3.5 of field sampling plan for explanation of sample identification numbers.

Feet
0 5 10 20 30 40 50
Scale 1:360 1"=30'

Total Excavation Volume = 5605 cu. yds.
Total Excavation Area = 100,643 sq. ft.

Overview Map

Overview Map
Overview Map
Overview Map

US Army Corps of Engineers
Sacramento District
1325 J Street
Sacramento, CA 94805

Date: Aug 9, 2004

Prepared by: Michael O'Neill

N
E
W
S

NAD27 CA State Plane Zone III

Notes:
About the Patterns: The patterns used on this map indicate areas where field tests registered results greater than or equal to 10 mg/Kg. The simple presence of any pattern indicates that concentration was found at some depth below grade. The type of pattern represents the depth at which that concentration was found. The lack of any pattern indicates that no field test results measured greater than 10 mg/Kg.

California

Hamilton Army Airfield

BRAC Remediation Project
South of the Runway
Confirmation Sample Locations
Field Effort January, February, June 2004

Novato

Sheet Reference Number:

Figure 2