METHOD 4051

HEXAHYDRO-1,3,5-TRINITRO-1,3,5-TRIAZINE (RDX) IN SOIL BY IMMUNOASSAY

1.0 SCOPE AND APPLICATION

1.1 Method 4051 is a procedure for screening soils to determine when hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX, CAS No. 121-82-4) is present at concentrations above 0.5 mg/kg Method 4051 provides an estimate of the concentration of RDX by comparison with a reference.

1.2 Using the test kit from which this method was developed, 99+% of soil samples containing 0.25 ppm or less of RDX will produce a negative result and 99+% of soil samples containing 1.0 ppm will produce a positive result.

1.3 In cases where the exact concentration of RDX is required, quantitative techniques (i.e., Method 8330) 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

Test kits are commercially available for this method. The manufacturer's directions should be followed. In general, the method is performed using an extract of a soil sample. Samples and an enzyme conjugate reagent are added to immobilized RDX antibody. The enzyme-RDX conjugate "competes" with RDX present in the sample for binding to an immobilized RDX antibody. The enzyme-RDX conjugate bound to the 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

Chemically similar compounds and compounds which might be expected to be found in conjunction with RDX contamination were tested to determine the concentration required to produce a positive test result. Table 1 provides the concentrations of compounds tested with the D TECH™ test kit that are required to elicit a positive response at the MDL, as well as the concentration required to yield 50% inhibition compared to the standard curve.

4.0 APPARATUS AND MATERIALS

Immunoassay test kit: D TECH™ RDX (Strategic Diagnostics 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 HAULING

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 indicated in Tables 3-6.

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 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. Do not mix reagents from one kit lot with a different kit lot.

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

8.6 Method 4051 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 Table 1 provides data on the minimum concentrations of possible interferants and co-contaminants required to elicit a positive response in the test kits evaluated.

9.2 Twenty six soil samples, known to not be contaminated with RDX, were extracted and analyzed using the D TECH™ RDX kit to determine the extent of soil matrix effects on the performance of the test kit. The results are provided in Table 2, and show that false positive results are not attributable to soil components.

9.3 Thirty soil samples, known to not be contaminated with RDX, were each spiked with RDX at one-half and two times the MDL (0.25 and 1.0 ppm respectively). These samples were analyzed with the D TECH™ RDX test kit to determine the error rate of the assay. The results are presented in Table 3.

9.4 Ten different soil types, all known not to be contaminated with RDX, were spiked with RDX. The spiked soil samples were each analyzed six times with the D TECH™ kit to determine the extraction efficiency of the method. The data are presented in Table 4.

9.5 Table 5 presents the results of analysis of three soils spiked at approximately 0.4, 1 and 3 ppm RDX. Each sample was analyzed using Method 8330 and in triplicate using the D TECH™ kit.

9.6 Tables 6A through 6D present the results of four field trials. Freshly collected (Table 6A, 6B and 6D) and archived (6C) soil samples, were analyzed by commercial laboratories using Method 8330 and the D TECH™ test kit. The tables provide results for both analyses, and evaluate the agreement between the two.

10.0 REFERENCES

1. D TECH™ TNT Users Guide , SDI/Em Sciences.

2. Haas, R.J., and B.P. Simmons, "Measurement of Trinitrotoluene (TNT) and Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in Soil by Enzyme Immunoassay and High Performance Liquid Chromatography (EPA Method 8330)", California Environmental Protection Agency, Department of Toxic Substances Control, Hazardous Materials Laboratory, March, 1995.

TABLE 1

CROSS REACTANTS - D TECH™ RDX TEST KIT

SAMPLE	MDL[a] (ppb)	IC_{50}[b] (ppb)	% CROSS REACTIVITY[c]
RDX [d]	5	25	100
HMX [d]	150	800	3
TNT (trinitrotoluene)	> 500	> 500	< 1
Tetryl [d]	> 500	> 500	< 1
TNB (trinitrobenzene)	> 500	> 500	< 1
2-amino-4,6-dinitrotoluene	> 500	> 500	< 1
4-amino-2,6-dinitrotoluene	> 500	> 500	< 1
2,4-dinitrotoluene	> 500	> 500	< 1
2,6-dinitrotoluene	> 500	> 500	< 1
1,3-dinitrobenzene	> 500	> 500	< 1
nitrobenzene	> 500	> 500	< 1
2-nitrotoluene	> 500	> 500	< 1
3-nitrotoluene	> 500	> 500	< 1
4-nitrotoluene	> 500	> 500	< 1
nitroglycerine	> 500	> 500	< 1
pentaerythritoltetranitrate	> 500	> 500	< 1

The following compounds were not detected at or above 500 ppm (100x the method MDL for RDX):

Atrazine	Benzo(a)pyrene	Benzo(b)fluoranthene	Benzene
Aroclor 1254	Acenaphthene	Dibenz(ah)anthracene	Chrysene
Acetone	Acenaphthalene	Fluoranthene	Fluorene
Toluene	1,2-Benzanthracene	Benzo(k)fluoranthene	Pyrene
Ethylbenzene	Indeno(123-cd)pyrene	Benzo(ghi)perylene	Xylene
Naphthalene	Methanol	Phenanthrene	

[a] The Method Detection Limit (MDL) is defined as the lowest concentration of compound that yields a positive test result.

[b] The IC_{50} is defined as the concentration of compound required to produce a test response equivalent to 50% of the maximum response.

[c] % Cross Reactivity is determined by dividing the equivalent RDX concentration by the actual compound concentration at IC_{50}.

[d] RDX = hexahydro-1,3,5-trinitro-1,3,5-triazine
HMX = octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
Tetryl = methyl-2,4,6-trinitrophenylnitramine

TABLE 2

SOIL MATRIX EFFECTS

Soil ID #	Soil Type	State	D TECH™ Result (ppm)
1	Low OM Clay Loam	DE	< 0.5
2	Sassafras Sandy Loam	DE	< 0.5
3	Cecil Sandy Clay Loam	GA	< 0.5
4	Davidson Clay Loam	GA	< 0.5
5	Shontik-Casa Grande Clay Loam	AZ	< 0.5
6	Trix Sandy Clay Loam	AZ	< 0.5
7	Trix-Casa Grande Clay Loam	AZ	< 0.5
8	Yolo Loam	CA	< 0.5
9	Capay Silty Clay	CA	< 0.5
10	Sycamore Silt Loam	CA	< 0.5
11	Dennis Silt Loam	KA	< 0.5
12	Luray Silty Clay Loam	OH	< 0.5
13	Wooster Silt Loam	OH	< 0.5
14	Vienna Loam	SD	< 0.5
15	Opal Clay	SD	< 0.5
16	Raulb Silt Loam	IN	< 0.5
17	Rockfield Silt Loam	IN	< 0.5
18	Cisne Silt Loam	IL	< 0.5
19	Muscatine Silt Loam	IL	< 0.5
20	Avonburg	IL	< 0.5
21	Matapeake Silt Loam	DE	< 0.5
22	Evesboro Low OM Sand	DE	< 0.5
23	Selbyville High OM Sand	DE	< 0.5
24	Casa Grande Clay Loam	AZ	< 0.5
25	Grundy Silty Clay Loam	KA	< 0.5
26	Drummer Silty Clay	IL	< 0.5
27	Non-Soil Control	-	< 0.5

TABLE 3

FALSE NEGATIVE AND FALSE POSITIVE RATES, SOIL MATRIX[a]

Spike Concentration	False Positive Rate	False Negative Rate
0.25 ppm	0%	-
1.0 ppm	-	0%

[a] Thirty negative soils were spiked with RDX at one-half and two times the MDL (0.25 and 1.0 ppm, respectively). These samples were analyzed with the D TECH™ RDX test kit to determine the error rate of the assay.

TABLE 4

DETERMINATION OF EXTRACTION EFFICIENCY FROM SOIL SAMPLES[a]

Soil ID : Spike (ppm)	Mean RDX Concentration (ppm)	Standard Deviation	Coefficient of Variation (%)	Recovery (%)
101:1	0.53	0.19	35	53
106:1	0.88	0.13	15	88
108:1	0.86	0.23	26	86
109:1	0.66	0.22	34	66
110:1	0.70	0.14	19	70
116:1	0.96	0.12	13	96
117:1	0.92	0.42	46	92
123:1	1.00	0.45	45	100
126:1	1.03	0.25	24	103
128:1	1.02	0.18	18	102
Non-Soil Control:1	1.05	0.13	12	105
Average	0.86	0.23	27	86
101:6	4.92	0.54	11	82
106:6	6.15	0.84	14	103
108:6	5.69	1.09	19	95
109:6	6.11	0.93	15	102
110:6	6.12	0.46	8	102
116:6	6.26	1.21	19	104
117:6	5.71	0.72	13	95
123:6	6.05	0.8	13	101
126:6	6.82	0.33	5	114
128:6	6.02	0.62	10	100
Non-Soil Control:6	6.02	0.83	14	100
Average	5.98	0.75	13	100

TABLE 5

RECOVERY OF RDX SPIKED INTO REAL SOILS.

Soil ID	Spike Concentration (ppm)	Method 8330 (ppm)	D TECH™ (ppm)	AGREEMENT Y, FN, FP
106	0.4	0.32	< 0.5	Y
			< 0.5	Y
			< 0.5	Y
	1.0	0.83	0.5 - 1.5	Y
			0.5 - 1.5	Y
			0.5 - 1.5	Y
	3.0	1.79	> 2.0	FP
			> 2.0	FP
			≥ 2.0	FP
116	0.4	0.29	< 0.5	Y
			< 0.5	Y
			< 0.5	Y
	1.0	0.66	0.5 - 1.5	Y
			0.5 - 1.5	Y
			0.5 - 1.5	Y
	3.0	0.61	> 2.0	FP
			< 2.0	FP
			> 2.0	FP
128	0.4	0.31(0.25)	< 0.5	Y
			< 0.5	Y
			< 0.5	Y
	1.0	0.73(0.73)	< 0.5	FN
			0.5 - 1.5	Y
			0.5 - 1.5	Y
	3.0	0.75(2.27)	≥ 2.0	Y
			< 2.0	Y
			< 2.0	Y

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

TABLE 6A

COMPARISON OF D TECH™ SOIL RESULTS WITH METHOD 8330

Sample ID	Method 8330 (ppm)	D TECH™ (ppm)	AGREEMENT Y, FN, FP
S4	< 0.2	< 0.5	Y
S12	< 0.2	< 0.5	Y
S14	1.72	1.5 - 2.0	Y
S15	< 0.2	< 0.5	Y
S19	2.12	1.5 - 3.0	Y
S20	1.61	1.5 - 3.0	Y
S21	0.32	< 0.5	Y
T1-2	0.21	< 0.5	Y
T2-4	1.41	1.5 - 2.0	FP
T6-1	2.62	> 3.0	FP
T3-5	2.00	0.5 - 1.5	FN
T12-3	< 0.2	< 0.5	Y
T12-6	1.00	0.5 - 1.5	Y
T20-3	< 0.2	< 0.5	Y
T21-10	1.89	1.5 - 2.0	Y
T22-4	< 0.2	< 0.5	Y
T22-5	0.83	0.5 - 1.5	Y
T22-6	0.99	0.5 - 1.5	Y
T28-3	3.73	> 3.0	Y
T28-4	< 0.2	< 0.5	Y
T28-5	< 0.2	< 0.5	Y
T28-6	< 0.2	< 0.5	Y
T28-7	< 0.2	< 0.5	Y
T28-8	< 0.2	< 0.5	Y
T28-9	< 0.2	< 0.5	Y

TABLE 6A (cont.)

Sample ID	Method 8330 (ppm)	D TECH™ (ppm)	AGREEMENT Y, FN, FP
T28-10	0.28	< 0.5	Y
T28-11	1.51	1.5 - 3.0	Y
T28-12	1.3	1.5 - 3.0	FP
T28-13	0.6	0.5 - 1.5	Y
T31-4	1.22	1.5 - 2.0	FP
T12-5	0.26	< 0.5	Y

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

TABLE 6B

COMPARISON OF D TECH™ SOIL RESULTS WITH METHOD 8330

		Replicate 1		Replicate 2	
Sample ID	Method 8330 (ppm)	D TECH™ (ppm)	AGREEMENT Y, FN, FP	D TECH™ (ppm)	AGREEMENT Y, FN, FP
1	4.00	> 3.0	Y	> 3.0	Y
3	19.0	> 6.0	Y	> 6.0	Y
13	1.30	0.5 - 1.5	Y	0.5 - 1.5	Y
15	1.80	1.5 - 3.0	Y	1.5 - 3.0	Y
16	3.40	> 3.0	Y	> 3.0	Y
23	0.48	0.5 - 1.5	Y	0.5 - 1.5	Y
24	0.68	0.5 - 1.5	Y	< 0.5	FN
25	0.68	0.5 - 1.5	Y	0.5 - 1.5	Y
26	0.75	0.5 - 1.5	Y	0.5 - 1.5	Y
31	0.13	0.5 - 1.5	FP	0.5 - 1.5	Y
33	0.74	0.5 - 1.5	Y	0.5 - 1.5	Y
34	0.48	0.5 - 1.5	Y	0.5 - 1.5	Y
35	1.30	1.5 - 3.0	FP	1.5 - 3.0	Y
37	5.50	> 6.0	FP	> 3.0	Y
38	0.55	0.5 - 1.5	Y	0.5 - 1.5	Y
43	1.30	1.5 - 3.0	FP	1.5 - 3.0	FP
44	40.0	> 6.0	Y	> 6.0	Y
47	2.30	> 3.0	FP	> 3.0	FP
48	0.36	0.5 - 1.5	FP	< 0.5	Y
58	0.79	0.5 - 1.5	Y	0.5 - 1.5	Y
59	0.80	1.5 - 3.0	FP	1.5 - 3.0	FP
64	2.20	1.5 - 3.0	Y	1.5 - 3.0	Y
67	10.9	> 6.0	Y	> 6.0	Y
68	3.40	1.5 - 3.0	FN	1.5 - 3.0	Y
75	3.90	> 3.0	Y	> 3.0	Y
84	17.6	> 6.0	Y	> 6.0	Y
85	70.3	> 6.0	Y	> 6.0	Y
87	101	> 6.0	Y	> 6.0	Y
94	1.60	1.5 - 3.0	Y	1.5 - 3.0	Y
96	0.20	< 0.5	Y	< 0.5	Y

TABLE 6B (cont.)

		Replicate 1		Replicate 2	
Sample ID	Method 8330 (ppm)	D TECH™ (ppm)	AGREEMENT Y, FN, FP	D TECH™ (ppm)	AGREEMENT Y, FN, FP
97	5.40	> 3.0	Y	> 3.0	Y
98	< 0.05	< 0.5	Y	< 0.5	Y
99	< 0.05	< 0.5	Y	0.5 - 1.5	FP
105	130	> 60	Y	> 60	Y
111	< 1.0	> 3.0	FP	< 5.0	Y
113	< 1.0	< 5.0	Y	< 0.5	FN
115	3.00	< 5.0	Y	< 0.5	FN
119	36.0	> 30	Y	15 - 30	FN

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

TABLE 6C

COMPARISON OF D TECH™ SOIL RESULTS WITH METHOD 8330

Sample ID	METHOD 8330 (ppm)	D TECH™ (ppm)	AGREEMENT Y, FN, FP
1	17	15 - 30	Y
2	34	15 - 30	FN
3	48	> 30	Y
4	160	60 - 120	FN
5	650	150 - 300	FN
6	41	> 30	Y
7	360	50 - 150	FN
8	840	> 600	Y
9	69	> 60	Y
10	85	30 - 60	FN
19	17	> 6.0	Y
20	19	> 6.0	Y
11	4.3	> 3.0	Y
12	1.9	> 3.0	FP
13	4.9	> 3.0	Y
14	27	1.5 - 3.0	Y
15	1.2	1.5 - 3.0	FP
16	1.0	1.5 - 3.0	FP
17	0.82	0.5 - 1.5	Y
18	0.78	0.5 - 1.5	Y
21	0.67	< 0.5	FN
22	0.94	< 0.5	FN
23	< 0.4	< 0.5	Y
24	< 0.4	< 0.5	Y
25	< 0.4	< 0.5	Y
26	< 0.4	< 0.5	Y

TABLE 6C (cont.)

Sample ID	METHOD 8330 (ppm)	D TECH™ (ppm)	AGREEMENT Y, FN, FP
27	< 0.4	< 0.5	Y
28	< 0.4	< 0.5	Y
29	< 0.4	< 0.5	Y
30	< 0.4	< 0.5	Y

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

TABLE 6D

COMPARISON OF D TECH™ SOIL RESULTS WITH METHOD 8330

Sample	D TECH™ Dilution Factor	D TECH™ Results	8330 Results	AGREEMENT Y, FN, FP
1	1	<0.5	<0.17	Y
2	1	<0.5	<0.17	Y
3	1	<0.5	<0.17	Y
4	1	<0.5	<0.17	Y
5	1	0.5-1.5	<0.17	FP
6	1	<0.5	<0.17	Y
7	1	<0.5	<0.17	Y
8	1	0.5-1.5	<0.17	FP
9	1	<0.5	<0.17	Y
10	1	<0.5	<0.17	Y
11	1	0.5-1.5	<0.17	FP
12	1	0.5-1.5	<0.17	FP
13	1	<0.5	<0.17	Y
14	1	<0.5	<0.17	Y
15	1	<0.5	<0.17	Y
16	1	<0.5	<0.17	Y
17	1	<0.5	<0.17	Y
18	1	<0.5	<0.17	Y
19	1	<0.5	<0.17	Y
20	1	<0.5	<0.17	Y
21	1	<0.5	<0.17	Y
22	1	<0.5	<0.17	Y
23	1	<0.5	<0.17	Y
24	1	0.5-1.5	<0.17	FP

TABLE 6D (cont.)

Sample	D TECH™ Dilution Factor	D TECH™ Results	8330 Results	AGREEMENT Y, FN, FP
25	1	<0.5	<0.17	Y
26	1	<0.5	<0.17	Y
27	1	0.5-1.5	<0.17	FP
28	1	<0.5	<0.17	Y
29	1	<0.5	<0.17	Y
30	1	1.5-3.5	0.17-0.99	FP
31	1	<0.5	<0.17	Y
32	1	0.5-1.5	<0.17	FP
33	1	<0.5	<0.17	Y
34	1	0.5-1.5	0.17-0.99	Y
35	1	0.5-1.5	0.17-0.99	Y
36	1	<0.5	<0.17	Y
37	1	1.5-3.0	1.2	FP
38	1	<0.5	<0.17	Y
39	1	0.5-1.5	<0.17	FP
40	1	<0.5	<0.17	Y
41	1	<0.5	<0.17	Y
42	1	<0.5	3.8	FN
43	1	<0.5	<0.17	Y
44	1	<0.5	<0.17	Y
45	1	<0.5	<0.17	Y
46	1	1.5-3.0	<0.17	FP
47	1	<0.5	<0.17	Y
48	1	0.5-1.5	<0.17	FP
49	1	<0.5	<0.17	Y
50	1	0.5-1.5	<0.17	FP
51	1	<0.5	<0.17	Y

TABLE 6D (cont.)

Sample	D TECH™ Dilution Factor	D TECH™ Results	8330 Results	AGREEMENT Y, FN, FP
52	1	0.5-1.5	0.17-0.99	Y
53	100	50-150	100	Y
54	1	3.0-4.5	<0.17	FP
55	1	3.0-4.5	<0.17	FP
56	1	<0.5	<0.17	Y
57	1	0.5-1.5	<0.17	FP
58	1	<0.5	<0.17	Y
59	1	<0.5	<0.17	Y
60	1	1.5-3.0	1.1	FP
61	1	0.5-1.5	<0.17	FP
62	100	150-300	290	Y
63	10	15-30	46	FN
64	1	1.5-3.0	4.8	FN
65	1	1.5-3.0	0.17-0.99	FP
66	1	3.0-4.5	12	FN
67	100	50-150	150	Y
68	1	0.5-1.5	2.6	FN
69	100	50-150	140	Y
70	1	0.5-1.5	7.8	FN
71	1	1.5-3.0	3.2	FN
72	100	150-300	340	FN
73	10	45-60	55	Y
74	10	>60	67	Y
75	10	30-45	63	FN
76	1	1.5-3.0	2.4	Y
77	1	4.5-6.0	6.4	FP
78	10	>60	73	Y

TABLE 6D (cont.)

Sample	D TECH™ Dilution Factor	D TECH™ Results	8330 Results	AGREEMENT Y, FN, FP
79	10	15-30	14	FP
80	1	0.5-1.5	2.1	FN
81	1	3.0-4.5	2.4	FP
82	1	1.5-3.0	2	Y
83	10	>60	94	Y
84	1	>6	23	Y
85	100	50-150	150	Y
86	10	30-45	34	Y
87	100	50-150	150	Y
88	1	0.5-1.5	1.2	Y
89	1	<0.5	0.17-0.99	Y
90	1	<0.5	<15	Y
91	1	<0.5	<15	Y
92	1	<0.5	<2	Y
93	1	<0.5	<15	Y
94	1	<0.5	<5	Y
95	1	<0.5	<0.17	Y
96	1	<0.5	<15	Y
97	1	<0.5	<5	Y
98	1	<0.5	<0.17	Y
99	1	<0.5	<0.17	Y
100	1	<0.5	<0.17	Y

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