METHOD 4035

SOIL SCREENING FOR POLYNUCLEAR AROMATIC HYDROCARBONS BY IMMUNOASSAY

1.0 SCOPE AND APPLICATION

1.1 Method 4035 is a procedure for screening soils to determine when total polynuclear aromatic hydrocarbons (PAHs) are present at concentrations above 1 mg/kg. Method 4035 provides an estimate for the concentration of PAHs by comparison with a PAH standard.

1.2 Using the test kit from which this method was developed, $\geq$95% of samples confirmed to have concentrations of PAHs below detection limits will produce a negative result in the 1 ppm test configuration.

1.3 The sensitivity of the test is influenced by the binding of the target analyte to the antibodies used in the kit. The commercial PAH kit used for evaluation of this method is most sensitive to the three (i.e., phenanthrene, anthracene, fluorene) and four (i.e., benzo(a)anthracene, chrysene, fluoranthene, pyrene) ring PAH compounds listed in Method 8310, and also recognizes most of the five and six ring compounds listed.

1.4 The sensitivity of the test is influenced by the nature of the PAH contamination and any degradation processes operating at a site. Although the action level of the test may vary from site to site, the test should produce internally consistent results at any given site.

1.5 In cases where the exact concentration of PAHs are required, quantitative techniques (i.e., Methods 8310, 8270, or 8100) should be used).

1.6 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 An accurately weighed sample is first extracted and the extract filtered using a commercially available test kit. The sample extract and an enzyme conjugate reagent are added to immobilized antibody. The enzyme conjugate "competes" with the PAHs present in the sample for binding to the immobilized anti-PAH antibody. The test is interpreted by comparing the response produced by testing a sample to the response produced by testing standard(s) simultaneously.

2.2 A portion of all samples in each analytical batch should be confirmed using quantitative techniques.

3.0 INTERFERENCES

3.1 Chemically similar compounds and compounds which might be expected to be found in conjunction with PAH contamination were tested to determine the concentration required to produce a positive result. These data are shown in Tables 1 and 2.

3.2 The kit was optimized to respond to three and four ring PAHs. The sensitivity of the test to individual PAHs is highly variable. Naphthalene, dibenzo(a,h)anthracene, and

benzo(g,h,i)perylene have 0.5 percent or less than the reactivity of phenanthrene with the enzyme conjugate.

3.3 The alkyl-substituted PAHs, chlorinated aromatic compounds, and other aromatic hydrocarbons, such as dibenzofuran, have been demonstrated to be cross-reactive with the immobilized anti-PAH antibody. The presence of these compounds in the sample may contribute to false positives.

4.0 APPARATUS AND MATERIALS

PAH RISc™ Soil Test (EnSys, 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

7.1 Method 4035 is intended for field or laboratory use.

7.2 Follow the manufacturer's instructions for the test being used. Those test kits used must meet or exceed the performance indicated in Tables 3-7.

7.3 The action limit for each application must be within the operating range of the kit used.

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 kits.

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

9.0 METHOD PERFORMANCE

9.1 The extraction efficiency of a commercially available test kit was tested (PAH RISc™ Test, EnSys Inc.) by spiking phenanthrene, benzo(a)anthracene and benzo(a)pyrene into PAH negative soil matrices (PAH-116 and PAH-141 are field samples). The soils were spiked using detection limits established for each compound (see Table 1), extracted and determined by immunoassay. The results for these 3-, 4- and 5-ring PAHs (Table 4) demonstrated that they were extracted with good recovery and yielded the correct assay interpretation.

9.2 A single laboratory study was conducted with a commercially available test kit (PAH RISc™ Test, EnSys Inc.), using 25 contaminated soil samples. Four replicate determinations were made on each test sample and the data compared with values obtained using HPLC Method 8310. Several analysts performed the immunoassay analyses. The immunoassay data agreed in all cases with the external HPLC data obtained (Table 5).

9.3 An additional single laboratory validation study on 30 randomly selected, PAH-contaminated field samples from multiple sites was run by the USEPA Region X Laboratory. Results are reported in Table 6 on an as found basis, and reported in Table 7 normalized to phenanthrene, based on cross-reactivity data (from Table 1). The false positive rate at the 1 ppm action level was 13% for unnormalized results and 19% for normalized results based on 31 analyses. The false negative rate at 1 ppm was 0 in both cases. At the 10 ppm action level, the false positive rate was 19% unnormalized and 26% normalized. False negative rates at 10 ppm were 6% unnormalized and 3% normalized.

9.4 The probabilities of generating false positive and false negative results at an action level of 1 ppm are listed in Table 3.

10.0 REFERENCES

1. PAH-RISc™ Users Guide, EnSys Inc.

2. P. P. McDonald, R. E. Almond, J. P. Mapes, and S. B. Friedman, "PAH-RISc™ Soil Test - A Rapid, On-Site Screening Test for Polynuclear Aromatic Hydrocarbons in Soil", J. of AOAC International (accepted for publication document #92263)

3. R. P. Swift, J. R. Leavell, and C. W. Brandenburg, "Evaluation of the EnSys PAH-RISc™ Test Kit", Proceedings, USEPA Ninth Annual Waste Testing and Quality Assurance Symposium, 1993.

TABLE 1

Cross-reactivity of Method 8310 PAHs

Compound	Concentration Giving a Positive Result (ppm Soil Equivalent)	Percent Cross-Reactivity
2 Rings		
Naphthalene	200	0.5
3 Rings		
Acenaphthene	8.1	12
Acenaphthylene	7.5	13
Phenanthrene	**1.0**	**100**
Anthracene	0.81	123
Fluorene	1.5	67
4 Rings		
Benzo(a)anthracene	1.6	64
Chrysene	1.2	84
Fluoranthene	1.4	73
Pyrene	3.5	29
5 Rings		
Benzo(b)fluoranthene	4.6	22
Benzo(k)fluoranthene	9.4	11
Benzo(a)pyrene	8.3	12
Dibenzo(a,h)anthracene	>200	<0.5
6 Rings		
Indeno(1,2,3-c,d)pyrene	11	9.4
Benzo(g,h,i)perylene	>200	<0.5

TABLE 2

Cross Reactivity of Other PAHs and Related Compounds

Compound	Concentration Giving a Positive Result (ppm, Soil Equivalent)	Percent Cross-Reactivity
Other PAHs		
1-Methylnaphthylene	54	1.8
2-Methylnaphthylene	58	1.7
1-Chloronaphthylene	59	1.7
Halowax 1013	18	5.7
Halowax 1051	>200	<0.5
Dibenzofuran	14	7.2
Other Compounds		
Benzene	>200	<0.5
Toluene	>200	<0.5
CCA	>200	<0.5
Phenol	>200	<0.5
Creosote	5.4	18.5
2,4,6-Trichlorobenzene	>200	<0.5
2,3,5,6-Tetrachlorobenzene	>200	<0.5
Pentachlorobenzene	>200	<0.5
Pentachlorophenol	>200	<0.5
Bis(2-ethylhexyl) phthalate	>200	<0.5
Aroclor 1254	>200	<0.5
Aroclor 1260	>200	<0.5

TABLE 3

Probability of False Negative and False Positive Results
for PAHs at A 1 ppm Action Level

Spike Concentration Phenanthrene (ppm)	Probability of False Positive (Mean ± SD)	Probability of False Negative (Mean ± SD)
0	0% ± 0%	N/A
0.4	23% ± 17%	N/A
0.8	94% ± 13%	N/A
1.0	N/A	0% ± 0%

Results were obtained from spiking four different validation lots, using 3 operators, 12 matrices for a total of 201 determinations at each concentration of phenanthrene.

N/A = No false positive or negative possible above action limit.

TABLE 4

Spike Recovery of Phenanthrene, Benzo(a)anthracene and Benzo(a)pyrene

Compound	Spike (ppm)	Soil	PAH RISc™ Results
Blank	0	Wake	<1
Blank	0	PAH-116	<1
Phenanthrene	1	Wake	1-10
Phenanthrene	1	PAH-116	1-10
Phenanthrene	1	PAH-141	1-10
Phenanthrene	10	Wake	>10
Phenanthrene	10	PAH-116	>10
Phenanthrene	10	PAH-141	>10
Benzo(a)anthracene	1.6	Wake	1-10
Benzo(a)anthracene	1.6	PAH-116	1-10
Benzo(a)anthracene	16	Wake	>10
Benzo(a)anthracene	16	PAH-116	>10
Benzo(a)pyrene	8.3	Wake	1-10
Benzo(a)pyrene	8.3	PAH-116	1-10
Benzo(a)pyrene	83	PAH-116	>10

TABLE 5

Powerplant Field Samples (Soil) Evaluated by Immunoassay

Field Sample Number	EnSys Method Immunoassay (ppm)	Method 8310 HPLC (ppm)
PAH-137	>10	<21
PAH-141	<1	<21
PAH-118	1-10	<26
PAH-136	>10	26
PAH-139	>10	<28
PAH-126	1-10, >10	<32
PAH-127	>10	<33
PAH-122	>10	<33
PAH-138	>10	33
PAH-131	>10	<34
PAH-128	>10	<35
PAH-132	>10	<43
PAH-112	>10	<48
PAH-140	>10	50
PAH-130	>10	54
PAH-116	<1	<61
PAH-135	>10	71
PAH-133	>10	<91
PAH-119	>10	<100
PAH-120	>10	<161
PAH-124	>10	<167
PAH-134	>10	182
PAH-114	>10	<247
PAH-113	>10	<294
PAH-115	>10	<343

TABLE 6

Total PAH Content of Region X Field Samples Using EnSys
PAH RISc™ Immunoassay Test Kit

Sample ID	1 ppm Test		10 ppm Test		GC/MS Lab Result (ppm)[1]	False +/-	
	<1	>1	<10	>10		Eval @ 1 ppm	Eval @ 10 ppm
PAH-1		*		*	0.2	+	+
PAH-2				*	12.2		
PAH-3				*	16.0		
PAH-4	*				0.00		
PAH-5	*				0.5		
PAH-6		*		*	8.7		+
PAH-7				*	148		
PAH-8				*	182		
PAH-9		*		*	4.4		+
PAH-10		*		*	0.2	+	+
PAH-11	*				0.00		
PAH-12				*	85.4		
PAH-12Dup				*	85.4		
PAH-13				*	28.5		
PAH-14	*		*		0.3		
PAH-15		*			0.6	+	
PAH-16	*		*		0.00		
PAH-17		*		*	1.8		+
PAH-18		*	*		3.4		
PAH-19		*	*		6.7		
PAH-20	*		*		0.9		
PAH-21				*	43.2		

[1] Sum of all PAHs detected.

TABLE 6 (cont.)

Sample ID	1 ppm Test		10 ppm Test		GC/MS Lab Result (ppm)[1]	False +/-	
	<1	>1	<10	>10		Eval @ 1 ppm	Eval @ 10 ppm
PAH-22				*	72.8		
PAH-23		*		*	1.3		+
PAH-24		*	*		0.3	+	
PAH-25	*		*		0.4		
PAH-26			*		27.9		-
PAH-27	*		*		0.00		
PAH-28			*		16.4		-
PAH-29	*		*		0.4		
PAH-30		*	*		9.6		

TABLE 7

Total PAH Content of Region X Field Samples Using EnSys
PAH RISc™ Immunoassay Test Kit Normalized to Cross-reactivity

Sample ID	1 ppm Test		10 ppm Test		GC/MS Lab Result (ppm)[1]	False +/-	
	<1	>1	<10	>10		Eval @ 1 ppm	Eval @ 10 ppm
PAH-1		*		*	0.1	+	+
PAH-2				*	8.1		+
PAH-3				*	9.0		+
PAH-4	*				0.00		
PAH-5	*				0.2		
PAH-6		*		*	5.2		+
PAH-7				*	56.9		
PAH-8				*	73.2		

[1] Sum o f all PAHs detected.

TABLE 7 (cont.)

Sample ID	1 ppm Test		10 ppm Test		GC/MS Lab Result (ppm) [1]	False +/-	
	<1	>1	<10	>10		Eval @ 1 ppm	Eval @ 10 ppm
PAH-9		*		*	0.1	+	+
PAH-10		*		*	0.00	+	+
PAH-11	*				0.00		
PAH-12				*	47.3		
PAH-12Dup				*	47.3		
PAH-13				*	11.5		
PAH-14	*		*		0.2		
PAH-15		*			0.5	+	
PAH-16	*		*		0.00		
PAH-17		*		*	1.2		+
PAH-18		*	*		1.7		
PAH-19		*	*		3.6		
PAH-20	*		*		0.6		
PAH-21				*	27.5		
PAH-22				*	49.2		
PAH-23		*		*	0.8	+	+
PAH-24		*	*		0.1	+	
PAH-25	*		*		0.2		
PAH-26			*		13.5		-
PAH-27	*		*		0.00		
PAH-28			*		6.4		
PAH-29	*		*		0.2		
PAH-30		*	*		2.8		

[1] Sum of all PAHs detected.