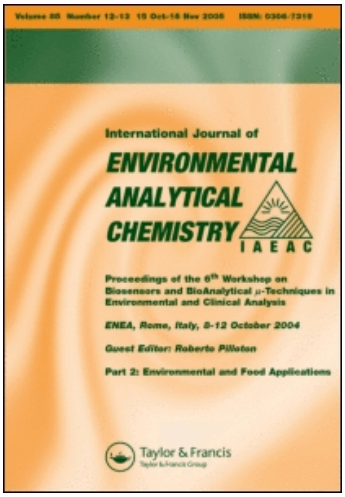

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RAPID ANALYSIS OF PCBs IN SOIL BY ENZYME IMMUNOASSAY

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A commercially available kit has been applied successfully to screening for polychlorinated biphenyls (PCB's) in soil samples. The kit uses a competitive inhibition Enzyme ImmunoAssay (EIA) for recognition of the PCB structure. Test specificity is restricted to PCB's, primarily Aroclors 1016, 1242, 1248, 1254, and 1260. Soil sample preparation and analysis can be performed in the field or lab using disposable kit components. Screening of unidentified Aroclors at 4 levels from 1 to 50 μg/g in soil is possible using the calibrators in the kit. This screening use has been reviewed by the USEPA Office of Solid Waste and will be proposed for inclusion in the 4000 series of screening methods in the next SW846 update. Conventional analysis (Soxhlet/GC-ECD) and the EIA kit were compared by Manitoba Hydro using 112 field samples over one year. EIA technology was found to be an effective screening tool for determining PCB concentration at contaminated sites. Routine use of these kits in conjunction with conventional Soxhlet extraction procedures has increased the lab's testing capability and reduced the amount of samples requiring conventional testing, providing substantial savings to the corporation.

KEY WORDS: PCB, soil, immunoassay, EIA, screening.

INTRODUCTION

Antibodies are specific binding proteins produced by all mammals for the purpose of self-protection. Their value to both animal and analyst resides in the enormous variety of different specificities which are made, typically thought to be 10 million or more in the mouse and similar in other mammals. In the late 1950's Yalow and Berson[1] showed how to produce and use specific antibodies for analysis of chosen target molecules such as proteins. This method was soon modified to allow the detection of specific small molecules[2,3]. Immunoassays have been used for over three decades in the medical field with excellent reliability. The original research use of immunoassays has evolved into a multibillion dollar per year clinical market including a wide array of tests for everything from disease organisms to drugs of abuse, therapeutic drugs, and hormones. Many of these tests use enzymes coupled in various ways to the specific antibody for generation of a colored endpoint and are therefore called enzyme immunoassays (EIA's). The technical success of environmental immunoassays over approximately the last 15 years

has been substantial and has been reviewed succinctly by Van Emon and Lopez-Avila[4] and more definitively by Sherry[5]. However, commercial success has come only in the last few years, as indicated in the historical progression summarized in Table 1. Numerous kits are now commercially available for analysis of a variety of pesticides and industrial wastes. Their utility is based on the attributes summarized in Table 2. We will describe in this paper how one such kit was evaluated in an attempt to integrate kit based screening into the analytical process.

MATERIALS AND METHODS

Soil samples for EIA analysis were selected randomly from Manitoba Hydro's routine analytical load. Because the ability to obtain a representative sample is critical, composite sampling techniques and splitting devices such as a rifle splitter were used where appropriate. Manitoba Hydro's standard method for soil analysis is Soxhlet extraction of a 20–25 g sample and GC-ECD, performed according to ASTM 3304. The EIA kit used in this study was the EnviroGard™ PCB Kit (Millipore Corp., Bedford MA). For EIA analysis a 5 g soil sample is extracted by shaking for 2 minutes with 5 ml of methanol, filtered using a device provided in the kit, and analyzed directly. A schematic representation of the kit protocol is shown in Figure 1 and the incubation periods for the three steps are 15, 5, and 5 minutes, respectively. The key performance characteristics of the kit are listed in Table 3. Kits were used in the screening mode accepted by the US Environmental Protection Agency[8] and described in the kit insert.

Table 1 Evolution of use of competitive EIA.

Area of use	*Analytes*	*First use*
Medical research	Hormones and Drugs	1959[1,2,3]
Environmental research	Pesticides	1975[6]
	Industrial Wastes	1979[7]
Environmental monitoring	Pesticides	1987*
	Industrial Wastes	1990**

* This date refers to the first commercial availability of immunoassay kits which are now routinely used for pesticide monitoring (first sold by ImmunoSystems, Inc.)

** This date refers to the first commercial availability of immunoassay kits which are now routinely used for industrial waste monitoring (first sold by EnSys, Inc.)

Table 2 Possible justifications for using EIA kits for screening.

Reduced overall cost
More samples can be analyzed at the same cost
Higher resolution site mapping can be done at same cost
Cost per negative result is reduced
Rapid analytical results
Decisions can be made in the field
Fewer samples sent for GC analysis
Less demand for rush GC analysis, reducing burden on lab

Table 3 Key performance characteristics of EnviroGard™ PCB kit.

Primary target analytes are Aroclors 1016, 1242, 1248, 1254, 1260
Minimal interference from metals or non-PCB organics
Response is always "total PCB"; cannot distinguish among Aroclors
Semi-quantitative screening for total PCB in soil across 1–50 ppm range
Screen at > 50 ppm levels with dilution
Quantitative analysis possible in defined single Aroclor situations
Designed for maximum false negative rate of 1%; slight false positive bias
Routine QA is still required (field duplicates, matrix blanks and spikes, reference samples, SRM's)
Flexibility to incorporate most user chosen QA methods
Run time for extraction and analysis 30–60 minutes per batch
Batch sizes up to 16 samples
Entire extraction and analysis can be performed in field with hand portable equipment

This screening procedure is based on the comparison of the sample to a calibrator at a chosen PCB level, run in the same assay as the sample. The optical density (OD) of each EIA tube was measured with a spectrophotometer to provide an objective record of results. A sample having greater color than a calibrator contains less PCB than the calibrator concentration, while a sample having less color than a calibrator *may* contain more PCB than the calibrator concentration. The actual concentrations of the calibrator solutions are less than the nominal concentrations in order to guarantee detection of the less strongly detected Aroclors such as 1242, and to limit the frequency of false negative results. This strategy allows 99% confidence detection of positive samples at any calibrator level. The target false positive rate is less than 10% at any calibrator level, but will depend on the distribution of individual sample concentrations.

RESULTS AND DISCUSSION

Results were interpreted relative to 3 different calibrators at 1, 10, and 50 μg/g nominal values (Table 4) to give a more detailed classification of samples than possible using a single calibrator. The actual concentrations of these calibrators were 0.5, 5, and 22 μg/g Aroclor 1248, respectively, because of the strategy described in the Materials and Methods section above. One set of field duplicates was run per batch of 16 samples; no discrepancies were seen. Matrix blanks were not used because of the many GC-ECD confirmed negative samples. Neither matrix spikes nor standard reference materials (SRM's) were used in this study.

The data of Table 4 illustrate the ability of the PCB EIA to effectively screen out negative samples. Sample 8, which is a false negative if the test results are strictly interpreted, was actually 0.5 μg/g by GC, which is technically at the detection limit for the EIA protocol used in this study. Only one other false negative result was observed, 10 μg/g for sample 71 by GC-ECD, which is at the boundary between the two concentration classifications. At first inspection, Table 4 may seem to indicate a high false positive rate in two areas, samples 18 to 70 and samples 79 to 92. However, the EIA results for all of samples 18 to 70 were all very close to the 1 μg/g calibrator, based on interpolation of the sample OD readings between the 1 and 10 μg/g calibrators. The 36 samples showing GC-EIA disagreement in the group from 18 to 70 are all apparent false positives. The mean GC-ECD value for this group was 0.33 ± 0.27 μg/g, which is very close to the actual 0.5 μg/g concentration of the 1.0 μg/g calibrator. Several samples, marked in Table 4 by darker shading, have GC-ECD values between the actual

Table 4 Comparison of results obtained by PCB EIA and GC-ECD. EIA results are relative to the calibrators included with the kit, at the nominal concentrations indicated. GC-ECD results are indicated by an X and PCB EIA results are indicated by shading. Results marked by darker shading indicate GC-ECD values between the actual calibrator concentration and the nominal calibrator concentration. See the Results and Discussion section for further explanation.

Sample	Semiquantitative results			
	< 1	< 10	< 50	≥ 50
1	X			
2	X			
3	X			
4	X			
5	X			
6	X			
7	X			
8		X		
9	X			
10	X			
11	X			
11	X			
12	X			
13	X			
14	X			
15	X			
16	X			
17	X			
18	X			
19		X		
20	X			
21	X			
22	X			
23		X		
24	X			
25	X			
26	X			
27	X			
28		X		
29	X			
30	X			
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32	X			
33	X			
34	X			
35	X			
36	X			
37	X			
38	X			
39		X		
40	X			
41		X		
42	X			
43	X			
44	X			
45		X		
46		X		
47		X		
48		X		
49		X		
50	X			
51		X		
52	X			
53		X		
54		X		
55		X		
56	X			
57	X			
58	X			
59	X			
60	X			

Sample	Semiquantitative results			
	< 1	< 10	< 50	≥ 50
61		X		
62		X		
63	X			
64		X		
65	X			
66	X			
67	X			
68	X			
69	X			
70	X			
71			X	
72		X		
73		X		
74		X		
75		X		
76		X		
77		X		
78		X		
79		X		
80		X		
81			X	
82		X		
83		X		
84			X	
85			X	
86			X	
87		X		
88		X		
89		X		
90		X		
91		X		
92		X		
93			X	
94			X	
95		X		
96			X	
97			X	
98			X	
99		X		
100		X		
101			X	
102			X	
103				X
104				X
105				X
106				X
107				X
108		X		
109				X
110			X	
111				X
112			X	

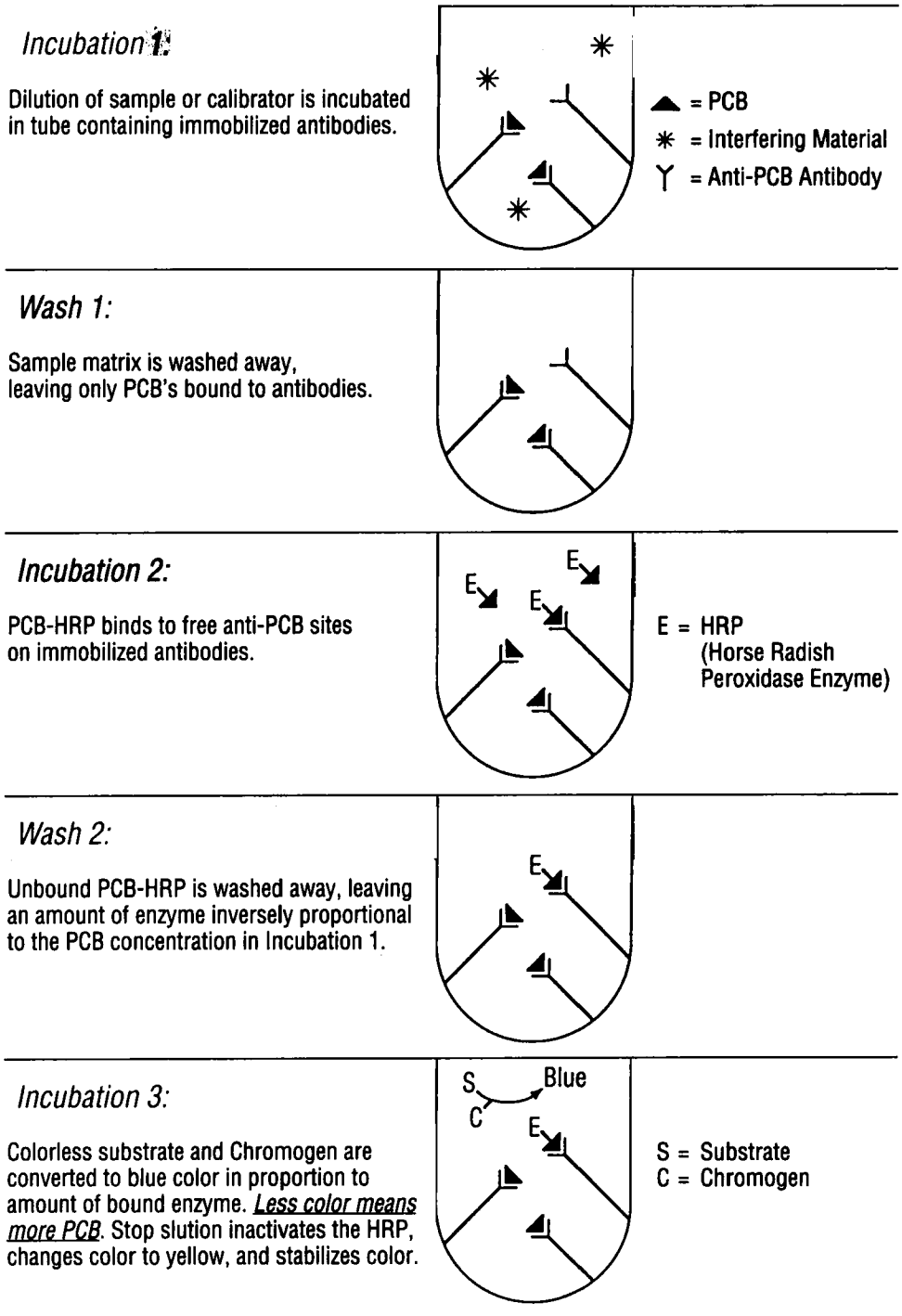

Figure 1 Schematic representation of the EnviroGard™ competitive EIA for PCB.

(0.5 μg/g) and nominal (1.0 μg/g) concentrations of the calibrator. These 9 samples therefore would be expected to appear as false positive results if the test were functioning perfectly. The fact that the samples in this study contained predominantly Aroclor 1260, which is recognized better than Aroclor 1248 in the test, probably accounts for many of the other apparent false positive results.

It is possible to reduce the false positive rate in such situations by using matching Aroclor calibrators. In this case, the Aroclor 1260 contaminated soils would be compared to calibrators of Aroclor 1260 having an actual concentration closer to the nominal concentration. This improvement is possible because part of the false positive bias built into the test is to compensate for the differences in test sensitivity among Aroclors. When the calibrator Aroclor matches the sample Aroclor, this portion of the bias can be removed by adjusting the actual concentration of the calibrator upward, closer to the nominal value. Additionally, it is possible to obtain quantitative data by comparing samples to a standard curve of the matching Aroclor. However, when using this method the analyst must assume a larger QA burden, including more rigorous QA procedures such as recovery of matrix spikes and analysis of SRM's, to maintain overall quality. An early example of this application for quantitative analysis was presented by Engle *et al.*[9]

Similar observations to those above can be made for samples 79 to 92. The 10 apparent false positive samples in this group have a mean GC-ECD value of 3.3 ± 2.3 μg/g, which parallels the above situation, but at a tenfold higher concentration. GC-ECD values for 2 of the 10 fall between the actual (5 μg/g) and the nominal (10 μg/g) calibrator values, and would be expected to give false positive results as described for samples 18 to 70 and marked in Table 4 by darker shading. It is important to note that the false positive rate of a semiquantitative test, which depends in part upon the sample distribution, will always rise when sample concentrations are clustered near the calibrator concentration, as is the case for the data of Table 4. Analysis of samples that are more broadly distributed typically yields false positive rates below 10%. The seemingly high false positive rate seen in this study is in fact the most desirable result for this screening program. These samples are precisely the ones most in need of confirmatory analysis to ensure accurate results, because they are close to the action level. The regulatory, fiscal, and public relations consequences of a high false negative rate demand that priority in test design be given to high confidence in negative results, as was done for this kit.

A summary of the specific benefits realized during this evaluation project is given in Table 5. The results of this study led to the development of the following confirmatory analysis strategy for QA during routine screening by EIA kit. All of the samples having

Table 5 Comparison of soil analysis by PCB EIA and GC-ECD for this evaluation.

	PCB EIA	*GC-ECD*
Sample size	5 g	20–25 g
Sample preparation	2 minute methanol shake	Soxhlet extraction
Cost per sample	$14 (overhead not included)	$100 (overhead included)
Sample throughput	20 per hour	≤ 5 per day
Typical turnaround time	1–2 hours	1–3 days
Start-up capital required	$1500 – 2500	$40,000
Analyst training required	≤ 5 hours	2 months

a concentration greater than the 1 μg/g calibrator were confirmed by GC-ECD, while a randomly chosen 20% of the samples having a concentration less than the 1 ppm calibrator were confirmed by GC-ECD.

CONCLUSIONS

The evaluation described above demonstrated that the PCB EIA kit is an effective screening tool for analysis of PCB's at contaminated sites. Use of the kit for sample screening reduced the number of samples requiring conventional testing, which in turn increased the testing capability of the laboratory. Manitoba Hydro then was able to devote expensive analytical resources to other projects and streamline its process of remediation and resale of PCB contaminated land. The overall productivity improvement ultimately provided substantial savings to the corporation.

Disclaimer

Any reference in this report to any specific commercial product, process, or service by tradename, trademark, manufacturer or otherwise does not constitute nor imply its endorsement or recommendation by Manitoba Hydro.

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