ENVIRONMENTAL CHEMISTRY METHOD EVALUATION REPORT

NUMBER: ECM 0033S1

AN ELISA IMMUNOASSAY METHOD FOR THE DETERMINATION OF RESIDUES OF METHOMYL IN SOIL.

ENVIRONMENTAL CHEMISTRY SECTION (ECS)

ANALYTICAL CHEMISTRY BRANCH

BIOLOGICAL AND ECONOMIC ANALYSIS DIVISION

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PART I

SUMMARY AND CONCLUSION

We have completed an Environmental Chemistry Method Evaluation on methomyl in soil. This method, Dupont number AMR-2396-92, is an enzyme-linked immunoassay (ELISA) for measurement of methomyl over a range of 0.05 to 5.0 parts per billion (ppb).

Following the suggestion of EFGWB, we fortified a soil matrix with methomyl at 5.0, 10, and 50 ppb. In order to evaluate the method at a level near the limit of detection, we also fortified soil at 2.5 ppb. All samples were done in replicates of four at each level. The Dupont method limit of quantitation (LOQ) of 10 ppb was validated by our data. Recoveries for methomyl at the (LOQ) ranged from 116% to 120% with relative standard deviation (RSD) of 1.6%. Although this method involves a simple extraction and no clean-up, we found the recoveries and precision to be good at or above the Limit of Quantitation. The unfortified soil sample analyses showed some methomyl response. This response could be from methomyl already in the soil, or from cross reactivity or matrix effect. Since the amount found in the unfortified soil is less than ten percent of the (LOQ) it is of little consequence.

We feel that the method could be used for low-cost monitoring of soil for methomyl. However, since there can be some cross-reactivity from other compounds, we emphasize the necessity of confirmatory analysis.

We encountered no problems with the method. However, the pre-coated microplates and some reagents were obtained from the registrant since they are not readily available from commercial sources. EFED should make sure that Dupont understands that they are responsible for making the precoated microplates commercially available or for licensing their technology to one of the kit manufacturers. They are responsible for making the test kits commercially available to all potential users.

PART II

ANALYTICAL RESULTS FOR METHOMYL

EPA RECOVERIES IN SOIL

Sample no.	Added(ppb)	Found(ppb)	Recovery Data
01	0	1.00	mean(ppb)= 0.75
02	0	0.62	sd = .226
03	0	0.50	rsd = 30.2%
04	0	0.86	
26	2.50	3.54	mean(ppb)= 3.65
27	2.50	3.32	sd = .395
28	2.50	3.50	rsd = 10.8%
29	2.50	4.22	mean recovery= 146%
05	5.00	6.18	mean(ppb)= 6.52
06	5.00	6.60	sd = .252
07	5.00	6.78	rsd = 3.9%
08	5.00	6.50	mean recovery = 130%
09	10.0	11.8	mean(ppb)= 11.7
10	10.0	11.6	sd = .192
11	10.0	12.0	rsd = 1.6%
12	10.0	11.6	mean recovery = 117%
13	50.0	49.4	mean(ppb)= 49.0
14	50.0	45.8	sd = 2.63
15	50.0	48.6	rsd = 5.4%
16	50.0	52.2	mean recovery = 98%

PART III

EXPERIMENTAL SUMMARY

(a) Principle of Method

Polyclonal anti-methomyl antibodies(Ab) and buffer are added to a sample containing an unknown amount of methomyl and incubated. The antibodies bind to methomyl molecules present in the sample (if any).

Aliquots of the solution are added to wells on a 96-well microplate, which have been coated with a methomyl derivative-Ovalbumin conjugate. Any excess (Ab) not bound to methomyl in the sample will bind to methomyl immobilized on the microwell plate. The plate is then washed to remove any (Ab) not bound to the plate. The amount of (Ab) bound to the microwell is an inverse measure of the amount of methomyl in the sample.

To detect the (Ab) bound to each microwell, an anti-rabbit antibody conjugated to an enzyme,alkaline phosphatase(Ab-E), is added to each well and incubated. This (Ab-E) will bind to any anti-methomyl antibodies bound to the microwell. The microwell plate is then washed to remove any unbound (Ab-E).

The alkaline phosphatase substrate, para-nitrophenyl-phosphate,is added to the microwells. The enzyme-substrate reaction produces a yellow color which is inversely proportional to the concentration of methomyl in the sample. The Microplate Reader quantitates absorbance in each well at 405 nm. Computer software is used to construct a standard curve from standards run on the plate, and to calculate the methomyl concentrations of each unknown on the plate.

(b) Source of Analytical Reference Standard

Methomyl standard was supplied and certified at 99.77% by E.I.Dupont de Nemours & company, Wilmington, Del.

(c) Source of Sample Matrix

Soil was obtained from Dupont from a supply of soils used in their recovery trials. The soil was characterized as silt loam with 2.3% organic matter and pH of 6.6.

(d) Instrumentation for Quantitation

1. Vmax Kinetic Microplate Reader with 405 nm optical filter, Molecular Devices Corporation.

2. ULTRAWASH PLUS, automatic microplate washer/aspirator, Dynatech Laboratories, Inc.

(e) Modification of Method

We used microplates supplied by Dupont and already coated with Coating Antigen Reagent. The method includes a procedure for coating blank microplates.

(f) Sample Extraction

A 10 gram soil sample is extracted with 20 ml of acetone/phosphate buffered saline solution (PBS). The supernatant is filtered and a 1.0 ml aliquot is brought to near dryness under nitrogen and reconstituted with 10 ml of phosphate buffered saline solution (PBS).

(g) Calculations

The absorbance of each of the 96 wells of the microwell plate is read on a microplate reader equipped with a 405 nm filter and processed by a computer program which generates a standard curve based upon a four-parameter logit function. The standard four-parameter logit curve generated is sigmoidal in shape with optical density (OD) on the Y-axis and the log of the concentration of methomyl on the X-axis. Each calibration standard and sample is pipetted into three wells of the microplate and analyzed in triplicate. The average of the optical density (OD) readings for these three wells is then used to interact with the standard curve to calculate the concentration of methomyl in each sample. For comparison, a semi-log curve fit would provide reasonably good data and can be used to check the calculations.

(h) Graphs and Data

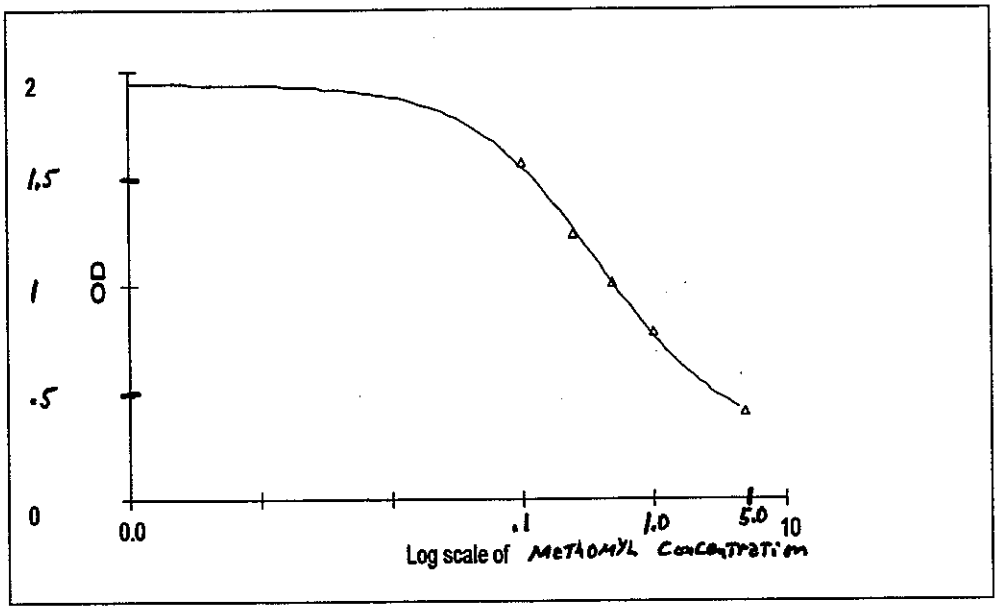
The following pages contain a print-out of the standard curve and data generated by the methomyl calibration standards and selected samples.

Curve Fit: 4-Parameter

Corr. Coeff: 1.00

$y = (A-D)/(1 + (x/C)^B) + D$

A= 1.95 B= 0.890 C= 0.391 D= 0.265



STANDARD	Std. Value	Well	OD	Mean	Std Dev	CV	Calc. Value	Sample ID
STD01	0	B2	1.964	1.943	0.045	2.332	<<<<<	
		C2	1.891				0.009	
		D2	1.974				<<<<<	
STD02	0.1	B3	1.588	1.577	0.014	0.911	0.090	
		C3	1.561				0.100	
		D3	1.583				0.092	
STD03	0.25	B4	1.295	1.247	0.047	3.769	0.234	
		C4	1.247				0.267	
		D4	1.201				0.303	
STD04	0.5	B5	1.050	1.017	0.029	2.832	0.454	
		C5	0.997				0.523	
		D5	1.004				0.514	
STD05	1	B6	0.808	0.784	0.021	2.728	0.898	
		C6	0.767				1.020	
		D6	0.777				0.989	
STD06	5	B7	0.428	0.418	0.017	4.074	4.796	
		C7	0.399				6.106	
		D7	0.429				4.760	

DATA FILE: 11169405
DESCRIPTION:
PROTOCOL: methomyl
DESCRIPTION:
MODE: Endpoint AUTOMIX: On
WAVELENGTH: 405
CALIBRATION: On

PLATE BLANK	Mean OD	Std Dev	CV	Well	OD	Sample ID
BL	0.108	0.002	1.580	A2	0.108	SUBSTRATE BLANKS
				A3	0.107	
				A4	0.106	
				A5	0.108	
				A6	0.109	
				A7	0.109	
				A8	0.108	
				A9	0.110	
				A10	0.112	
				A11	0.107	

STANDARDS	Mean OD	Std Dev	CV	Well	OD	Sample ID
STD01	1.943	0.045	2.332	B2	1.964	0.0 ng/ml
				C2	1.891	
				D2	1.974	
STD02	1.577	0.014	0.911	B3	1.588	0.1 ng/ml
				C3	1.561	
				D3	1.583	
STD03	1.247	0.047	3.769	B4	1.295	0.25 ng/ml
				C4	1.247	
				D4	1.201	
STD04	1.017	0.029	2.832	B5	1.050	0.50 ng/ml
				C5	0.997	
				D5	1.004	
STD05	0.784	0.021	2.728	B6	0.808	1.0 ng/ml
				C6	0.767	
				D6	0.777	
STD06	0.418	0.017	4.074	B7	0.428	5.0 ng/ml
				C7	0.399	
				D7	0.429	

UNKNOWNS	Mean OD	Std Dev	CV	Well	OD	Factor	PPb	Sample ID
UNK01	1.719	0.080	4.672	B8	1.803	20.00	0.995	Unfortified Soil
				C8	1.711			
				D8	1.643			
UNK02	1.794	0.100	5.579	B9	1.874	20.00	0.619	"
				C9	1.827			
				D9	1.682			
UNK03	1.816	0.061	3.336	B10	1.879	20.00	0.493	"
				C10	1.813			
				D10	1.758			
UNK04	1.740	0.022	1.249	B11	1.749	20.00	0.855	"
				C11	1.757			
				D11	1.716			

MOLECULAR DEVICES

UNKNOWNS	Mean OD	Std Dev	CV	Well	OD	Factor	ppb	Sample ID
UNK09	0.954	0.010	1.091	E6	0.966	20.00	11.77	Fortified at 10 ppb
				F6	0.946			
				G6	0.951			
UNK10	0.959	0.034	3.558	E7	0.981	20.00	11.65	"
				F7	0.920			
				G7	0.977			
UNK11	0.947	0.014	1.510	E8	0.963	20.00	12.00	"
				F8	0.935			
				G8	0.944			
UNK12	0.961	0.017	1.770	E9	0.968	20.00	11.55	"
				F9	0.942			
				G9	0.974			
UNK13	0.538	0.010	1.881	E10	0.550	20.00	49.37	Fortified at 50 ppb
				F10	0.533			
				G10	0.532			
UNK14	0.554	0.019	3.340	E11	0.564	20.00	45.85	"
				F11	0.566			
				G11	0.533			
UNK15	0.541	0.006	1.174	H2	0.545	20.00	48.61	"
				H3	0.545			
				H4	0.534			
UNK16	0.528	0.024	4.618	H5	0.519	20.00	52.23	"
				H6	0.556			
				H7	0.510			