ENVIRONMENTAL CHEMISTRY METHOD EVALUATION REPORT

NUMBER: ECM 0033W1

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

ENVIRONMENTAL CHEMISTRY SECTION (ECS)

ANALYTICAL CHEMISTRY BRANCH

BIOLOGICAL AND ECONOMIC ANALYSIS DIVISION

02/06/95

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PART I

SUMMARY AND CONCLUSION

We have completed an Environmental Chemistry Method Evaluation on methomyl in pond water. 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 water matrix with methomyl at 0.5, 1.0, and 5.0 ppb. In order to evaluate the method at a level near the limit of detection, we also fortified water at 0.125 and 0.25 ppb. All samples were done in replicates of four or more at each level. The Dupont method limit of detection (LOD) of 0.1 ppb and limit of quantitation (LOQ) of 0.2 ppb were validated by our data. The recoveries of methomyl in eighteen samples, at or above the (LOQ), ranged from 76% to 112% with relative standard deviation (RSD) of 10.6%. Although this method involves no extraction or clean-up, we found the recoveries and precision to be good at or above the Limit of Quantitation.

We feel that the method could be used for low-cost monitoring of water 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 POND WATER

Sample no.	Added(ppb)	Found(ppb)	Recovery Data
01	0	0	
02	0	0	
03	0	0	
04	0	0	
17	0.125	0.09	mean(ppb)=.105
18	0.125	0.10	sd =.0152
19	0.125	0.11	rsd = 14.4%
20	0.125	0.09	mean recovery= 84%
21	0.125	0.11	
22	0.125	0.13	
23	0.25	0.28	mean(ppb)= .235
24	0.25	0.20	sd = .029
25	0.25	0.21	rsd = 12.3%
26	0.25	0.25	mean recovery = 94%
27	0.25	0.24	
28	0.25	0.23	
05	0.5	0.46	mean(ppb) = 0.50
06	0.5	0.49	sd = .029
07	0.5	0.52	rsd = 5.7%
08	0.5	0.52	mean recovery = 100%
09	1.0	0.99	mean(ppb)= 1.02
10	1.0	1.01	sd = .048
11	1.0	0.99	rsd = 4.7%
12	1.0	1.09	mean recovery = 102%
13	5.0	4.20	mean(ppb)= 4.25
14	5.0	3.82	sd = .417
15	5.0	4.15	rsd = 9.8%
16	5.0	4.82	mean recovery = 85%

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

Pond water was obtained locally from a pond at Stennis Space Center. The water was collected in pre-cleaned one gallon dark glass bottles. The water was kept refrigerated and brought to ambient temperature before aliquots were taken for fortification and analysis.

(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) 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.

(g) Graphs and Data

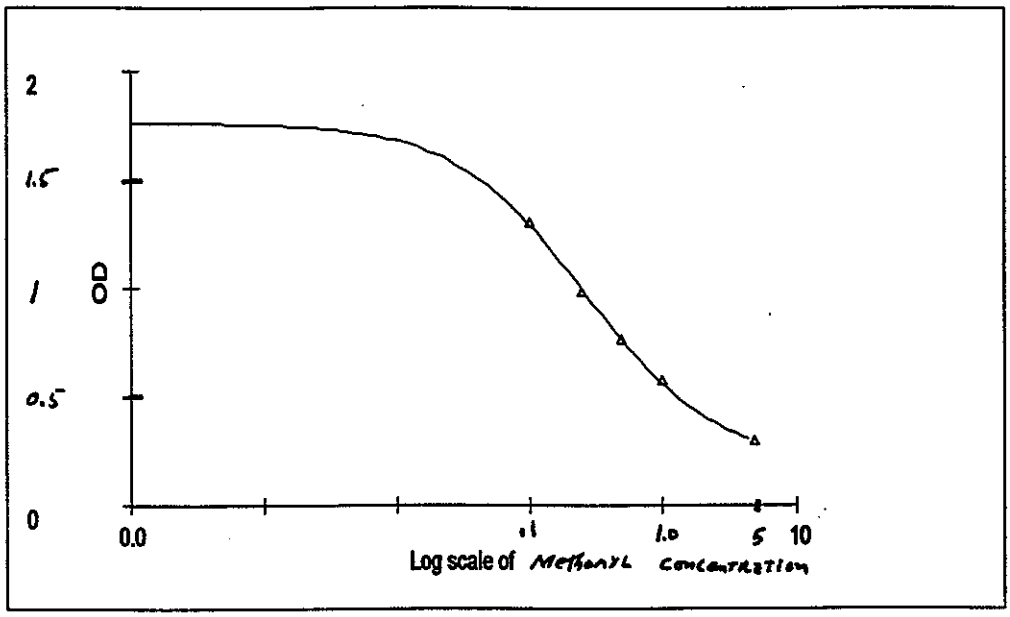
The following pages contain a print-out of the standard curve and data generated by the methomyl calibration standards and selected samples.

DATA FILE: 11089405
DESCRIPTION:
PROTOCOL: methomyl
DESCRIPTION:
MODE: Endpoint AUTOMIX: On
WAVELENGTH: 405
CALIBRATION: On

Curve Fit: 4-Parameter Corr. Coeff: 1.00

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

A= 1.77 B= 0.886 C= 0.268 D= 0.191



STANDARD	Std. Value	Well	OD	Mean	Std Dev	CV	Calc. Value	Sample ID
STD01	0	B2	1.851	1.767	0.082	4.649	<<<<<	
		C2	1.761				5.68e-4	
		D2	1.687				0.010	
STD02	0.1	B3	1.370	1.312	0.050	3.827	0.079	
		C3	1.283				0.107	
		D3	1.283				0.107	
STD03	0.25	B4	1.039	0.990	0.043	4.346	0.226	
		C4	0.964				0.280	
		D4	0.965				0.279	
STD04	0.5	B5	0.791	0.768	0.024	3.128	0.464	
		C5	0.769				0.496	
		D5	0.743				0.538	
STD05	1	B6	0.579	0.574	0.005	0.877	0.947	
		C6	0.569				0.984	
		D6	0.573				0.969	
STD06	5	B7	0.309	0.297	0.015	5.088	4.567	
		C7	0.280				6.418	
		D7	0.302				4.920	

DATA FILE: 11089405
DESCRIPTION:
PROTOCOL: methomyl
DESCRIPTION:
MODE: Endpoint AUTOMIX: On
WAVELENGTH: 405
CALIBRATION: On

PLATE BLANK	Mean OD	Std Dev	CV	Well	OD	Sample ID
BL	0.113	0.002	1.578	A2	0.114	SUBSTRATE BLANKS
				A3	0.111	
				A4	0.113	
				A5	0.113	
				A6	0.111	
				A7	0.115	
				A8	0.109	
				A9	0.113	
				A10	0.113	
				A11	0.114	

STANDARDS	Mean OD	Std Dev	CV	Well	OD	Sample ID
STD01	1.767	0.082	4.649	B2	1.851	0.0 ng/ml
				C2	1.761	
				D2	1.687	
STD02	1.312	0.050	3.827	B3	1.370	0.1 ng/ml
				C3	1.283	
				D3	1.283	
STD03	0.990	0.043	4.346	B4	1.039	0.25 ng/ml
				C4	0.964	
				D4	0.965	
STD04	0.768	0.024	3.128	B5	0.791	0.50 ng/ml
				C5	0.769	
				D5	0.743	
STD05	0.574	0.005	0.877	B6	0.579	1.0 ng/ml
				C6	0.569	
				D6	0.573	
STD06	0.297	0.015	5.088	B7	0.309	5.0 ng/ml
				C7	0.280	
				D7	0.302	

UNKNOWNS	Mean OD	Std Dev	CV	Well	OD	Sample ID
UNK01	1.802	0.079	4.372	B8	1.876	unfortified water
				C8	1.809	
				D8	1.719	
UNK02	1.794	0.092	5.145	B9	1.876	"
				C9	1.812	
				D9	1.694	
UNK03	1.754	0.055	3.108	B10	1.798	"
				C10	1.771	
				D10	1.693	
UNK04	1.851	0.065	3.531	B11	1.920	
				C11	1.843	
				D11	1.790	"

MOLECULAR DEVICES

UNKNOWNS	Mean OD	Std Dev	CV	Well	OD	Dil.Factor	ppb	Sample ID
UNK05	1.037	0.019	1.821	E2	1.032	2.000	0.455	Fortified at 0.5 ppb
				F2	1.020			
				G2	1.057			
UNK06	1.016	0.043	4.243	E3	1.054	2.000	0.485	"
				F3	1.024			
				G3	0.969			
UNK07	0.988	0.037	3.715	E4	1.026	2.000	0.525	"
				F4	0.953			
				G4	0.983			
UNK08	0.993	0.011	1.086	E5	0.985	2.000	0.516	"
				F5	1.005			
				G5	0.988			
UNK13	0.411	0.019	4.541	E10	0.408	2.000	4.196	Fortified at 5.0 ppb
				F10	0.394			
				G10	0.431			
UNK14	0.427	0.005	1.057	E11	0.426	2.000	3.815	"
				F11	0.422			
				G11	0.431			
UNK15	0.413	0.020	4.759	H2	0.416	2.000	4.150	"
				H3	0.392			
				H4	0.431			
UNK16	0.390	0.021	5.335	H5	0.374	2.000	4.816	"
				H6	0.413			
				H7	0.381			