7/2/02

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDE PROGRAMS
ENVIRONMENTAL CHEMISTRY LABORATORY
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July 02, 2002

MEMORANDUM   DP Barcode: D279315

SUBJECT: R-Metolachlor Lab Method Validation
             Report No. ECM0200W2

             Aubry E. Dupuy, Jr.

FROM: Aubry E. Dupuy, Jr., Chief
             BEAD/Environmental Chemistry Lab

TO: Mark Corbin
             EFED/Environmental Risk Branch 1 (7507-C)

        As requested ECL has completed an Environmental Chemistry Lab validation for
R-metolachlor in water, MRID #454996-12, using a method submitted by Syngenta Crop
Protection, Inc., entitled "Analytical Method for the Determination of R-Metolachlor in Water
by Enzyme Immunoassay Including Validation Data".

        This method was evaluated with reagent water fortified at three levels, 0.10 ppb,
0.20 ppb, and 2.00 ppb, and with quadruplicate analyses at each level.

        The attached method lab evaluation report includes three parts:

Part I: Summary and Conclusions

                 In this section any problems encountered with the method and
                 how they were handled are discussed. ECL's opinion of how well the
                 method performed is also presented.

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Part II: Analytical Results

In this section the individual results of each sample at each spiking level are listed. The average percent recovery and relative standard deviation (RSD) for each spiking level is also presented here.

Part III: Experimental Details

In this section any modifications that were made, instrument parameters, representative sample calculations and standard curve are listed and/or discussed.

If you have any questions concerning this report, please contact Henry Shoemaker at (228) 688-1222 or Aubry Dupuy at   (228) 688-3212.

Attachments

cc: Christian Byrne, QA Officer
        BEAD/Environmental Chemistry Lab

        Henry Shoemaker, Chemist
        BEAD/Environmental Chemistry Lab

ENVIRONMENTAL CHEMISTRY METHOD LAB EVALUATION REPORT
NUMBER ECM 0200W2

R-Metolachlor in Water

ENVIRONMENTAL CHEMISTRY LABORATORY

BIOLOGICAL AND ECONOMIC ANALYSIS DIVISION

Prepared by: _____   Date: 6/15/02
          Henry Shoemaker, ECL Chemist

Reviewed by: _____   Date: 06/25/02
          Christian Byrne, ECL QA Officer

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PART I

SUMMARY AND CONCLUSIONS

ECL has completed an Environmental Chemistry Method Laboratory Evaluation of R-metolachlor in water. This method, MRID# 454996-12, submitted by Syngenta Crop Protection, Inc., is titled "Analytical Method for the Determination of R-Metolachlor in Water by Enzyme Immunoassay Including Validation Data".

In order to evaluate this method a water matrix was fortified with R-metolachlor at 0.10, 0.20, and 2.0 ppb. All samples were done in replicates of four at each level. The registrant's method limit of detection (LOD) of 0.10 ppb and the method limit of quantitation (LOQ) of 0.20 ppb of R-metolachlor in water were confirmed by this data. ECL found the precision to be within the target limits of ≤20% relative standard deviation (RSD) at or above the (LOQ). For example, the (RSD) is 8.8% at the (LOQ) level and 7.2% at the (10 x LOQ) level. The mean recoveries of 80.0% for R-metolachlor at the (LOQ) level and 89.1% at the (10 x LOQ) level are well within the target range of 70% to 120%.

ECL encountered no problems with the method and feel that it could be used as described by the registrant for low-cost monitoring of water for R-metolachlor. However, since there can be some potential for cross-reactivity from other compounds, ECL emphasizes the necessity of confirmatory analysis by a conventional technique.

PART II

ANALYTICAL RESULTS FOR R-METOLACHLOR

EPA RECOVERIES IN WATER

Sample	Added (ppb)	Found (ppb)	Recovery (%)	Statistics
RM 01	0	0		
RM 02	0	0		
RM 03	0.10	0.09		
RM 04	0.10	0.08		
RM 05	0.10	0.10		
RM 06	0.10	0.08		
RM 07	0.20	0.15	75.0	mean(Rec) = 80.0%
RM 08	0.20	0.16	80.0	SD = 7.07
RM 09	0.20	0.15	75.0	RSD = 8.8%
RM 10	0.20	0.18	90.0	
RM 11	2.0	1.81	90.5	mean(Rec) = 89.1%
RM 12	2.0	1.79	89.5	SD = 6.42
RM 13	2.0	1.61	80.5	RSD = 7.2%
RM 14	2.0	1.92	96.0	

PART III

EXPERIMENTAL SUMMARY

(a)   Principle of Method

A 0.50 mL aliquot of a water sample is added to a polystyrene culture tube coated with R-metolachlor antibody. The assay is carried out by sequential addition of enzyme conjugate, wash water, and color reagent. The reaction is terminated by acidification. Quantification is performed spectrophotometrically at 450 nm.

(b)   Source of Analytical Reference Standard

Analytical standard of racemic metolachlor, lot# S98-2315, was obtained from the EPA National Pesticide Standard Repository, Fort Meade, Maryland, with certified purity of 98%. Refer to Appendix I for the chemical name and structure of R-metolachlor.

(c)   Source of Sample Matrix

Matrix used was reagent grade water, Fisher Optima, lot 991185.

(d)   Instrumentation for Quantitation

Photometer, fixed wavelength spectrophotometer RPA-1 RaPID Analyzer, Strategic Diagnostics Inc.

(e)   Modification of Method

The RPA-1 spectrophotometer was set up to use a ln/logit B data transformation calculation, while the registrant used a log/linear regression function. A comparison of the two methods yields almost identical results.

(f)   Time for Experiment

The time for preparing and analyzing a set of fourteen samples, including standards and controls, is one day for one person.

Samples Data :

```
Spl#     Abs      Conc
-----------------------------
  1     1.580    0.01nd
ID: RM 01      Blank
```

```
  2     1.578    0.01nd
ID: RM 02      Blank
```

```
  3     1.178    0.09
ID: RM 03
```

```
  4     1.205    0.08
ID: RM 04
```

```
  5     1.147    0.10
ID: RM 05
```

```
  6     1.219    0.08
ID: RM 06
```

```
  7     1.001    0.15
ID: RM 07
```

```
  8     0.954    0.16
ID: RM 08
```

```
  9     0.994    0.15
ID: RM 09
```

```
 10     0.919    0.18
ID: RM 10
```

```
 11     0.157    1.81
ID: RM 11
```

```
 12     0.159    1.79
ID: RM 12
```

```
 13     0.176    1.61
ID: RM 13
```

```
 14     0.148    1.92
ID: RM 14
```

END OF RUN
05-13-02 12:50:49

(g)   <u>Calculations</u>

1. Standard Curve

The RPA-1 RaPID® Analyzer contains preprogrammed data reduction
capabilities which calculates a standard curve for each analytical set using the
absorbances of the calibration standards; which had concentrations of 0.10 ppb,
0.30 ppb, 0.80 ppb, and 2.20 ppb of R-metolachlor. The calibration curve is
constructed using linear regression after performing a ln(conc.) and Logit(B/B_0)
data transformation of the concentration and absorbance values respectively.

The calibration standard curve equation is:

Logit (B/B_0) = slope (ln of conc.) + Y intercept

Where:
Logit (B/B_0) = ln $\{(B/B_0) / [1-(B/B_0)]\}$

B = the absorbance for a specific sample.

B_0 = the absorbance measured for the zero standard.

Conc.= the concentration of R-metolachlor (ppb).

2. Calculation of Analyte in Samples

Standards were processed and analyzed in duplicate. The RPA-1 Analyzer
measured the absorbances of each sample and calculated the concentrations (ppb)
of R-metolachlor using the regression equation generated from calibration
standards in the sample set.

3. Example of manual calculation

    Sample no. RM 12 for R-metolachlor

    To manually calculate the concentration (ppb), use the following equation:

    Conc. $= e^a$, where:

    $a = [\text{Logit}(B/B_0) - (Y \text{ intercept})] / \text{slope}$

Mean absorbance of sample RM 12 = 0.159
Slope of calibration curve = - 1.081
Y intercept of calibration curve = - 1.585
Mean absorbance of zero standard = 1.613

$B/B_0 = 0.728 / 2.460 = 0.0986$

$\text{Logit}(B/B_0) = \ln[0.0986/(1 - 0.0986)] = -2.213$

$\text{Conc} = e^{(-2.213 + 1.585)/(-1.081)}$

$= e^{(0.5809)}$

$= 1.79 \text{ ppb}$

(h)   Graphs and Data

        Pages (8) and (9) contain a print-out of the calibration curve and data generated by the R-metolachlor standards and samples.

```
******** OHMICRON ********
```

PROTOCOL :   METOLACH

TECH ID : *Henry Shoemaker*
LOT #   : *21012*
EXP DATE: *nov/02*

Data Reduct :Lin.Regression
Xformation: Ln/LgtB
Read Mode :   Absorbance
Wavelength :   450 nm
Units   :   PPB

EQUATION OF LINE :
```
-----------------
```
Slope   =   -1.081
Intercept =   -1.585
Corr (r) =   0.9992

Transformed Data :
```
-----------------
```

Conc	Abs
-2.30	0.877
-1.20	-0.210
-0.22	-1.404
0.79	-2.423

Calibrator Data:
```
-----------------
```

Conc	Abs Diff	%CV	Predic %Diff
0.00	1.619		
	1.608		
Mean	1.613	0.5	
0.10	1.141		0.10
	0.002		2.1
	1.138		0.10
	0.003		2.8
Mean	1.139	0.1	0.10
	0.003		2.5
0.30	0.731		0.27
	-0.025		-9.2
	0.713		0.29
	-0.014		-4.8
Mean	0.722	1.7	0.28
	-0.020		-7.0
0.80	0.341		0.78
	-0.020		-2.5
	0.295		0.92
	0.122		13.2
Mean	0.318	10.2	0.85
	0.046		5.5
2.20	0.131		2.17
	-0.030		-1.4
	0.131		2.17
	-0.030		-1.4
Mean	0.131	0.0	2.17
	-0.030		-1.4

```
    ---------------------------

Cal. Curve : ↕
------------

0.9x|
    |
    |
    |
0.1+|
    |              x
Abs |
-0.8+|
    |
    |
    |                   x
-1.6+|
    |
    |
    |
    |
-2.4+----+----+----+----x
  -2.30              0.79
              Conc
◄
```
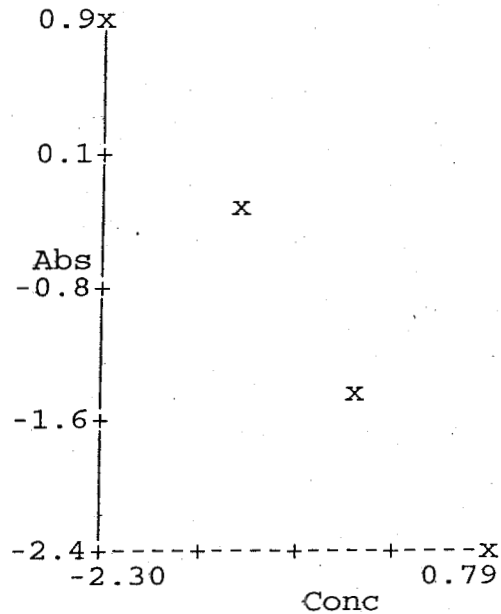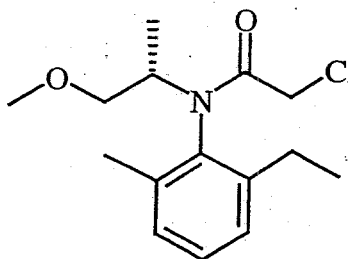
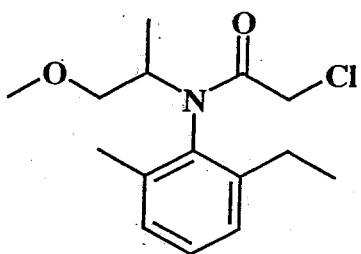Acetamide, 2-chloro-*N*-(2-ethyl-
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methoxy-1-methylethyl]-
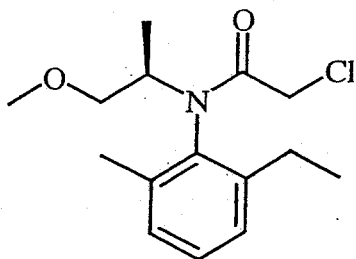
Acetamide, 2-chloro-*N*-(2-ethyl-
6-methylphenyl)-*N*-[(1*R*)-2-
methoxy-1-methylethyl]-

S-Metolachlor
(CGA-77102)

R-Metolachlor
(CGA-77101)

Metolachlor

Acetamide, 2-chloro-*N*-(2-ethyl-
6-methylphenyl)-*N*-[-2-methoxy-
1-methylethyl]-