Comparison of Enzyme-Linked Immunosorbent Assay and Gas Chromatography Procedures for the Detection of Cyanazine and Metolachlor in Surface Water Samples

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Enzyme-linked immunosorbent assay (ELISA) data from surface water reconnaissance were compared to data from samples analyzed by gas chromatography for the pesticide residues cyanazine (2-[[4-chloro-6-(ethylamino)-1,3,5-triazin-2-yl]amino]-2-methylpropanenitrile) and metolachlor (2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide). When ELISA analyses were duplicated, cyanazine and metolachlor detection was found to have highly reproducible results; adjusted R^2s were 0.97 and 0.94, respectively. When ELISA results for cyanazine were regressed against gas chromatography results, the models effectively predicted cyanazine concentrations from ELISA analyses (adjusted R^2s ranging from 0.76 to 0.81). The intercepts and slopes for these models were not different from 0 and 1, respectively. This indicates that cyanazine analysis by ELISA is expected to give the same results as analysis by gas chromatography. However, regressing ELISA analyses for metolachlor against gas chromatography data provided more variable results (adjusted R^2s ranged from 0.67 to 0.94). Regression models for metolachlor analyses had two of three intercepts that were not different from 0. Slopes for all metolachlor regression models were significantly different from 1. This indicates that as metolachlor concentrations increase, ELISA will over- or under-estimate metolachlor concentration, depending on the method of comparison. ELISA can be effectively used to detect cyanazine and metolachlor in surface water samples. However, when detections of metolachlor have significant consequences or implications it may be necessary to use other analytical methods.

Keywords: *Immunoassay validation; ELISA; gas chromatography; cyanazine; metolachlor*

INTRODUCTION

There is increasing interest in surface water monitoring for pesticide residues, with cyanazine (2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide) and metolachlor (2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide) being of particular interest. The U.S. Environmental Protection Agency (EPA) has classified cyanazine and metolachlor as group C carcinogens (U.S. EPA, 1996). Group C carcinogens are defined as those that have demonstrated limited evidence of carcinogenicity in animals but lack human data. However, a recent study (Roloff et al., 1992) showed that cyanazine is clastogenic and possibly carcinogenic in human cells. Increasing concern about cyanazine in drinking water is evident in the trends seen in EPA health advisory levels (HAL) for cyanazine. From 1989 to 1996, the HAL for cyanazine has decreased from 10 to 1 μg L^{-1} (U.S. EPA, 1989, 1996).

These concerns have fostered the need for more efficient and economical methods of detection. Current detection methods for cyanazine and metolachlor involve costly and time-consuming extraction procedures. Additionally, they require specialized instrumentation such as gas or liquid chromatography. The use of ELISA for the detection of pesticides emerged out of the need for more efficient, economical pesticide detection. ELISA is a viable alternative to gas and liquid chromatography. ELISA has been shown to be sensitive, reliable, cost-effective, and rapid (Van Emon and Lopez-Avila, 1992).

Surface water reconnaissance studies conducted by Mississippi State University in conjunction with the U.S. Geological Survey (308 South Airport Road, Pearl, MS 39208) involved collection of more than 500 surface water samples. All samples were analyzed for cyanazine and metolachlor using ELISA. Additionally, many of these samples were analyzed using traditional methods. Although the reliability of ELISA has already been demonstrated, that reliability needs to be assessed for a given sample type subjected to a particular preparation or extraction procedure (Huber and Ulvskov, 1993). The objective of this study was to determine the accuracy and utility of ELISA for detecting cyanazine and metolachlor in surface water samples by comparison with results from traditional methods.

MATERIALS AND METHODS

A total of 535 surface water samples from locations in Mississippi, Louisiana, Arkansas, and Tennessee was collected

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Table 1. Lower Limits of Detection (LLD) for Methods Used to Detect Cyanazine and Metolachlor in Surface Water Samples

method	LLD (μg L^{-1}) cyanazine	metolachlor
immunoassay		
millipore	0.140	0.070
ohmicron	0.035	0.050
gas chromatography		
GC/ECD[a]		0.700
GC/MS, CO[b]	0.004	0.002
GC/MS, KS[c]	0.050	0.050

[a] Cyanazine not detected by GC/ECD method. [b] Pesticide analysis was conducted by the U.S. Geological Survey laboratory in Arvada, CO (Plunkett et al., 1997, 1998). [c] Coupe et al., 1999.

Table 2. Summary of ELISA and Gas Chromatography Analyses of Water Samples Collected in 1996−1998

analytical method	number of samples analyzed	number of detections cyanazine	metolachlor
Millipore ELISA	85	70	16
Ohmicron ELISA	448	312	357
ELISA duplicates[a]	57	37	53
GC/ECD[b]	35		25
GC/MS, CO[c]	31	30	31
GC/MS, KS[d]	218	127	158

[a] Duplicates of Ohmicron ELISA analyses. [b] Cyanazine not detected by GC/ECD method. [c] Pesticide analysis was conducted by the U.S. Geological Survey laboratory in Arvada, CO (Plunkett et al., 1997, 1998). [d] Coupe et al., 1999.

in 1996, 1997, and 1998. At each site, samples were collected by strapping a 950-mL bottle into a metal cage attached to a length of rope. The bottle was lowered below the surface of the water 10−20 cm. Once filled, the bottles were capped and labeled with the date and sampling location. Samples were stored on ice for transportation. Samples were stored at 4 °C until analysis. Prior to analysis, samples were prefiltered to remove sediment. A peristaltic pump pushed samples through a 0.7-μm glass fiber filter (VWR Brand Glass Fiber Filter Grade 151 (28496−138), VWR Scientific Products, 1050 Satellite Blvd., Suwanee, GA 30024) held by a pressure-type filter holder. ELISA was used to determine the cyanazine (Cyanazine RaPID Assay, Ohmicron Corporation, 375 Pheasant Run, Newtown, PA 18940). Samples analyzed prior to June 1996 were analyzed using Cyanazine EnviroGard (Millipore Intertech, 397 Williams Street, Marlborough, MA 01752−9162) and Metolachlor RaPID Assay (Ohmicron Corporation, 375 Pheasant Run, Newtown, PA 18940). Samples analyzed prior to June 1996 were analyzed using Metolachlor EnviroGard (Millipore Intertech, 397 Williams Street, Marlborough, MA 01752−9162). Randomly selected duplicate samples were analyzed for 10% of surface water samples. The lower limits of detection (LLD) for cyanazine and metolachlor with the Millipore and Ohmicron ELISAs are listed in Table 1.

In addition to ELISA analysis, samples were analyzed by traditional methods to determine cyanazine and metolachlor concentrations. LLDs for these methods are listed in Table 1. Table 2 contains a summary of the samples analyzed by the different methods. Samples were analyzed by the Mississippi State University Weed Science Analytical Laboratory and U.S. Geological Survey laboratories in Arvanda, CO, and Lawrence, KS.

The Weed Science Analytical Laboratory prefiltered all samples. Samples were extracted via liquid−liquid extraction. Samples were cycled for 16 h. Rotary evaporation concentrated samples to near dryness. Samples were resuspended in hexane. Metolachlor content was determined by a gas chromatograph equipped with an electron capture detector (GC/ECD) (Hewlett-Packard 5890 Series II gas chromatograph, Hewlett-Packard Co., Little Falls Site 4300, 2850 Centerville Rd., Wilmington, DE 19808).

U.S. Geological Survey laboratories in Arvanda, CO, and Lawrence, KS, determined cyanazine and metolachlor contents. The U.S. Geological Survey supplied these data (Coupe et al., 1999). Samples were subjected to C_{18} solid-phase extraction followed by gas chromatography/mass spectrometry (GC/MS). Coupe et al. (1998) and Zuagg et al. (1995) described the procedures used by these laboratories. The difference in the procedures used by these two labs was the sample aliquot analyzed. The Colorado lab analyzed a 1-L sample aliquot, whereas the Kansas lab analyzed a 123-mL sample. Herbicide determination of the elutes was done on a gas chromatograph interfaced to a mass selective detector.

Herbicide detections were summarized to determine whether ELISA generated the same qualitative results as other analytical methods. False positives and false negatives were also determined. Herbicide concentrations were subjected to regression analyses (SAS Institute, Inc., 1988). Cyanazine and metolachlor concentrations obtained from ELISA analyses were regressed against duplicate ELISA analyses and gas chromatographic analyses. Tests were conducted on regression parameters to determine whether the slopes and intercepts were significantly different from 1 and 0, respectively. Regression analyses were conducted for data from Ohmicron ELISA only. Sufficient data for regression analyses were not available for Millipore ELISA. Significance during all statistical procedures was determined at $P < 0.05$.

RESULTS AND DISCUSSION

Data Summary. Although two different ELISAs were used in the course of this study, comparisons were not made between the Millipore and Ohmicron ELISAs. A change was made to use the Ohmicron ELISA to standardize laboratory procedures for cyanazine and metolachlor with analytical procedures for other pesticides. This change was made during the early stages of the study. Consequently, the amount of data collected using the Millipore ELISA was largely insufficient for making any comparison to the Ohmicron ELISA. Cyanazine was detected in 70 of 85 and 312 of 448 samples when analyses were conducted using the Millipore and Ohmicron ELISAs, respectively (Table 2). Millipore ELISA produced the same qualitative results for cy-

Table 3. Comparison of Cyanazine Analyses by ELISA and Traditional Methods for Water Samples Collected in 1996−1998

ELISA	analytical method	samples analyzed, no.[a]	same qualitative results, %[b]	detections, %[c]	false positives, %[d]	false negatives, %[e]
Millipore	GC/MS, CO[f]	2	50	50	0	50
	GC/MS, KS[g]	19	37	11	63	0
Ohmicron	ELISA duplicates	57	93	65	7	0
	GC/MS, CO[f]	29	86	86	4	10
	GC/MS, KS[g]	199	70	62	24	6

[a] Number of samples analyzed by ELISA and the traditional method or duplicated by ELISA. [b] Percent of samples for which both methods detected or failed to detect cyanazine. [c] Percent of samples for which both methods detected cyanazine. [d] Percent of samples for which ELISA and traditional methods detected and failed to detect cyanazine, respectively. [e] Percent of samples for which ELISA and traditional methods failed to detect and detected cyanazine, respectively. [f] Pesticide analysis was conducted by the U.S. Geological Survey laboratory in Arvada, CO (Plunkett et al., 1997, 1998). [g] Coupe et al., 1999.

Table 4. Comparison of Metolachlor Analyses by ELISA and Traditional Methods for Water Samples Collected in 1996−1998

ELISA	analytical method	samples analyzed, no.[a]	same qualitative results, %[b]	detections, %[c]	false positives, %[d]	false negatives, %[e]
Millipore	GC/MS, CO[f]	2	0	0	0	100
	GC/MS, KS[g]	19	63	5	5	32
Ohmicron	ELISA duplicates	57	88	81	0	12
	GC/ECD	35	80	71	20	0
	GC/MS, CO[f]	29	90	90	0	10
	GC/MS, KS[g]	199	82	73	16	2

[a] Number of samples analyzed by ELISA and the traditional method or duplicated by ELISA. [b] Percent of samples for which both methods detected or failed to detect metolachlor. [c] Percent of samples for which both methods detected metolachlor. [d] Percent of samples for which ELISA and traditional methods detected and failed to detect metolachlor, respectively. [e] Percent of samples for which ELISA and traditional methods failed to detect and detected metolachlor, respectively. [f] Pesticide analysis was conducted by the U.S. Geological Survey laboratory in Arvada, CO (Plunkett et al. 1997, 1998). [g] Coupe et al, 1999

anazine from 50 and 37% of samples when compared to data from U.S. Geological Survey (USGS) laboratories in CO and KS, respectively (Table 3). Ohmicron ELISA produced the same qualitative results from 86 and 70% of samples when compared to data from USGS laboratories in CO and KS, respectively (Table 3). Ohmicron ELISA results were highly reproducible, with duplicate analyses producing the same qualitative results from 93% of samples (Table 3). Millipore ELISA produced false positives for cyanazine detection for 0 and 63% of samples when compared to data from USGS laboratories in CO and KS, respectively (Table 3). The Ohmicron ELISA produced false positives from 4 and 24% of samples when compared to data from USGS laboratories in CO and KS, respectively (Table 3). False negatives for cyanazine detections were produced for 50 and 0% of samples when Millipore ELISA results were compared to data from USGS laboratories in CO and KS, respectively (Table 3). False negatives for cyanazine detections were produced for 10 and 6% of samples when Ohmicron ELISA results were compared to data from USGS laboratories in CO and KS, respectively (Table 3).

Metolachlor was detected in 16 of 85 and 357 of 448 samples when analyses were conducted using the Millipore and Ohmicron ELISAs, respectively (Table 2). Millipore ELISA produced the same qualitative results for metolachlor from 0 and 63% of samples when compared to data from USGS laboratories in CO and KS, respectively (Table 4). Ohmicron ELISA produced the same qualitative results from 80, 90, and 82% of samples when compared to data from Mississippi State University and USGS laboratories in CO and KS, respectively (Table 4). Ohmicron ELISA results were highly reproducible, with duplicate analyses producing the same qualitative results from 88% of samples (Table 4). Millipore ELISA produced false positives for metolachlor detection for 0 and 5% of samples when compared to data from USGS laboratories in CO and KS, respectively (Table 4). The Ohmicron ELISA produced false positives from 20, 0, and 16% of samples when compared to data from Mississippi State University and USGS laboratories in CO and KS, respectively (Table 4). False negatives for metolachlor detections were produced for 100 and 32% of samples when Millipore ELISA results were compared to data from USGS laboratories in CO and KS, respectively (Table 4). False negatives for metolachlor detections were produced for 0, 10 and 2% of samples when Ohmicron ELISA results were compared to data from Mississippi State University and USGS laboratories in CO and KS, respectively (Table 4).

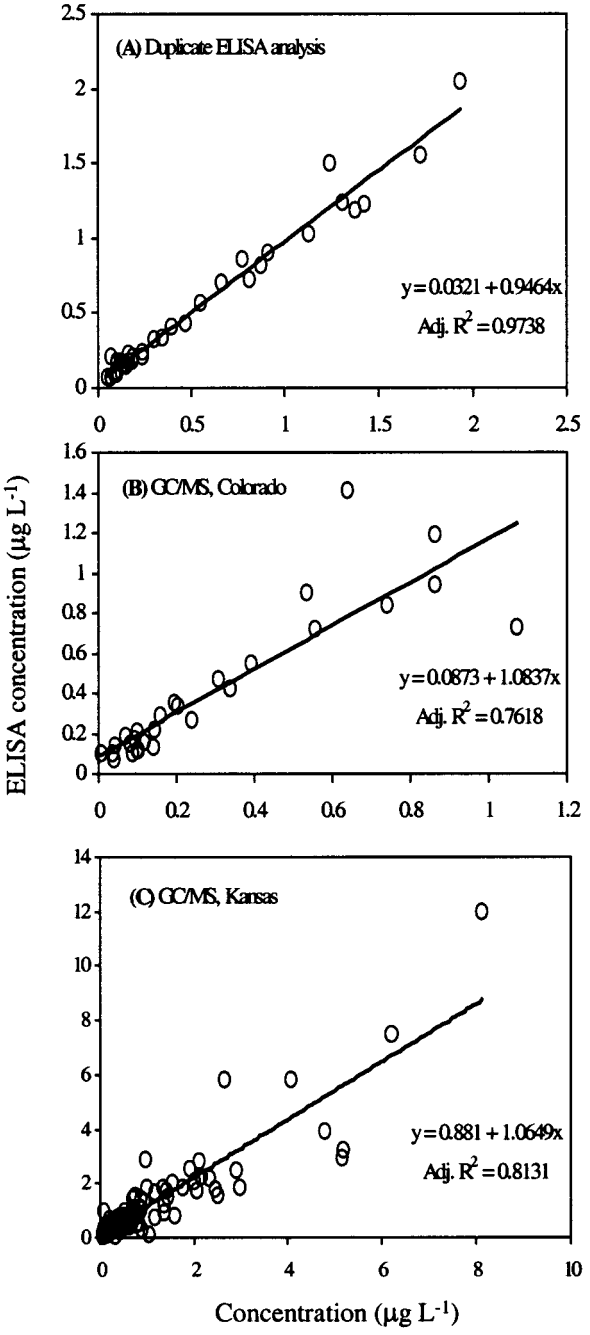

Figure 1. Cyanazine concentrations (μg L^{-1}) from ELISA analyses plotted against cyanazine concentration (μg L^{-1}) from duplicate ELISA analyses (A) and GC/MS data from U.S. Geological Survey laboratories in CO (B) and KS (C).

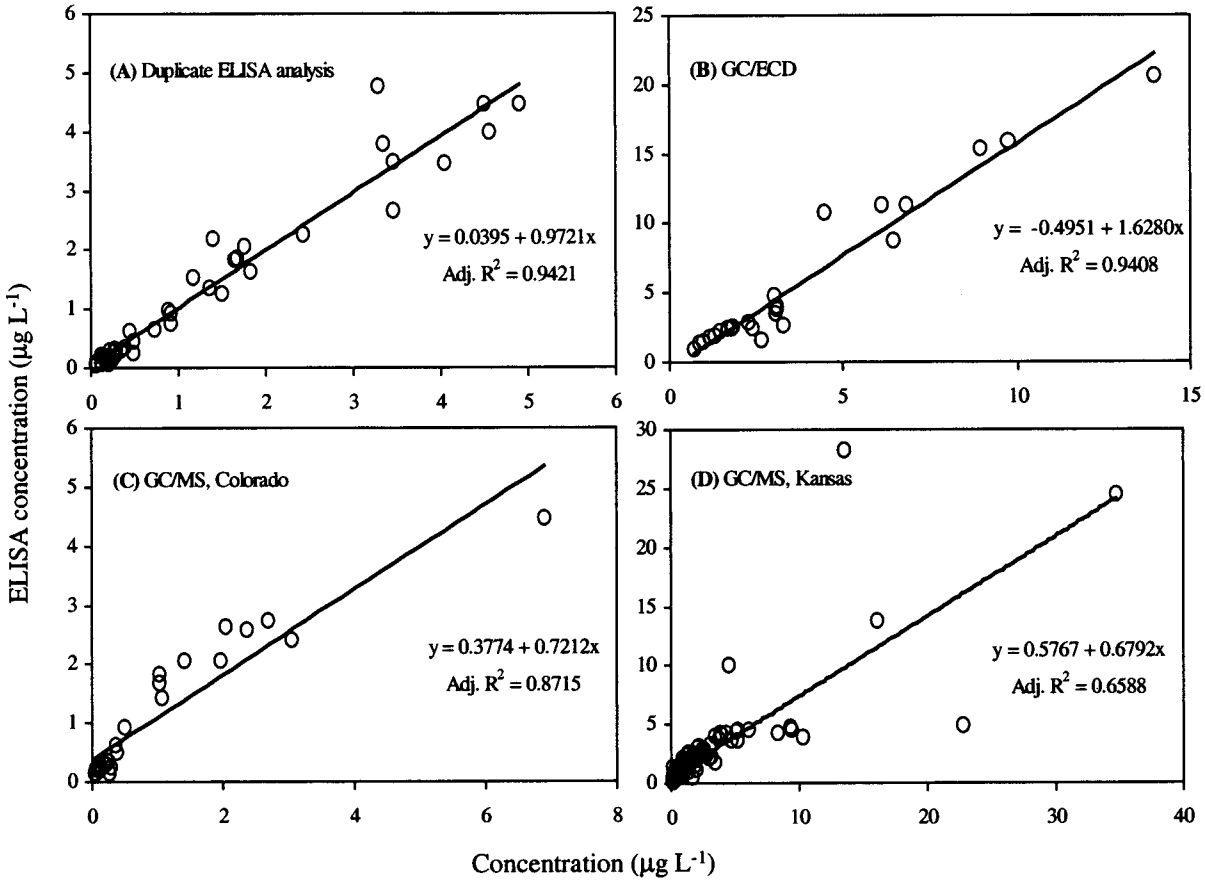

Figure 2. Metolachlor concentrations (μg L^{-1}) from ELISA analyses plotted against metolachlor concentration (μg L^{-1}) from duplicate ELISA analyses (A), GC/ECD data (B), and GC/MS data from U.S. Geological Survey laboratories in CO (C) and KS (D).

Table 5. Parameter Estimates and Analysis of Variance for Regression Models Where ELISA Results Were Dependent on the Results of Other Analytical Methods

herbicide	analytical method	n, number of samples	parameter estimates mg L^{-1}		adjusted R^2	*P*-value		
			y-intercept	slope		model[a]	intercept = 0[b]	slope = 1[c]
cyanazine	ELISA duplicates	37	0.0321	0.9464	0.9738	0.0001	0.0974	0.0455
	GC/MS, CO	27	0.0873	1.0837	0.7618	0.0001	0.0926	0.4850
	GC/MS, KS	124	0.0881	1.0649	0.8131	0.0001	0.2211	0.1605
metolachlor	ELISA duplicates	46	0.0395	0.9721	0.9421	0.0001	0.5548	0.4409
	GC/ECD	25	−0.4951	1.6280	0.9408	0.0001	0.2401	0.0001
	GC/MS, CO	26	0.3774	0.7212	0.8715	0.0001	0.0009	0.0001
	GC/MS, KS	146	0.5767	0.6792	0.6588	0.0001	0.0017	0.0001

[a] Probability that $F_{(0.05,\,1,\,n-2)}$ > F for the model $y = \beta_0 + \beta_1 x$; where *y* is the result of ELISA analysis and *x* is the results of another analytical method. If $P > 0.05$, the analytical method in column 2 is effective in predicting herbicide concentrations from ELISA results. If $P < 0.05$, the model was effective at predicting ELISA results. [b] Probability that $F_{(0.05,\,1,\,n-2)}$ > F for H_0: $\beta_0 = 0$. If $P > 0.05$, ELISA results did not over/underestimate herbicide concentration when the concentration detected by the method in column 2 wasis 0. However, if $P < 0.05$, ELISA results over/under-estimated herbicide concentrations in surface water samples when compared to the method in column 2 when the actual herbicide concentration = 0. [c] Probability that $F_{(0.05,\,1,\,n-2)}$ > F for H_0: $\beta_1 = 1$. If $P > 0.05$, ELISA results did not further over/under-estimate herbicide concentration as the concentration detected by the method in column 2 increased. However, if $P < 0.05$ and slope > 1 (or slope < 1), ELISA results overestimated (or underestimated) herbicide concentration as the detected herbicide concentration increased.

Regression Analysis. Regression analysis allowed the comparison of ELISA results to those from other procedures. These analyses generated regression models with slopes and intercepts for each comparison. Ideally, the regression model would have an intercept of 0 and a slope of 1. An intercept of 0 means that when herbicide concentrations were lowest neither ELISA nor a comparison method would over/under-estimate the sample concentration. A slope of 1 would indicate that, as the herbicide concentration detected by a comparison method increased, the concentration detected by ELISA would increase in the same manner. ELISA results for cyanazine and metolachlor are plotted against the results of other methods in Figures 1 and 2, respectively. The illustrations contain trendlines as well as regression equations. All models regressing ELISA results against other methods of detection were effective at predicting results of ELISA procedures at $P < 0.05$ (Table 5). When ELISA results were regressed against duplicate analyses the results of analyses for both cyanazine and metolachlor were highly reproducible. Cyanazine and metolachlor regression models had adjusted R^2 of 0.97

and 0.94, respectively (Table 5). Intercepts for both models were not different from 0 (Table 5). The slope of the regression model for cyanazine was less than 1 (Table 5). This would indicate that at higher concentrations ELISA results were not as reproducible as they were at lower concentrations. However, the slope for the metolachlor model was not different from 1 (Table 5). When the results of ELISA for cyanazine were regressed against GC/MS data from labs in CO and KS, the regression models were similar between laboratories (Figure 1, Table 5). Models of the data from the CO and KS labs had acceptable adjusted R^2 of 0.76 and 0.81, respectively (Table 5). Although the slopes were not significantly greater than one, they indicate that ELISA results were 6 to 8% higher than GC/MS results (Table 5). The higher ELISA results could be attributed to cyanazine losses during the extraction procedure for the GC/MS method (Lawruk et al., 1993b). The slopes were similar to that reported by Lawruk et al. (1993b). However, they reported negative intercepts, whereas both of the intercepts reported here are positive (Table 5). When ELISA results for metolachlor were regressed against GC/ECD data from Mississippi State University, the resulting model showed a high degree of correlation between the two methods; adjusted R^2 was 0.94 (Table 5). However, the slope (1.628) of this model was >1 (Table 5). As the metolachlor concentration detected by the GC/ECD method increased, the concentration detected by the ELISA increased at a higher rate. Lawruk et al. (1993a) stated that these higher metolachlor concentrations could be due to cross-reactivity with metabolites and other chloracetanilides, or could be due to the loss of analyte during the sample extraction necessary for the GC method. When ELISA results for metolachlor detection were regressed against GC/MS data from the labs in CO and KS the correlation between methods was somewhat lower, adjusted R^2 were 0.87 and 0.66, respectively (Table 5). Both models for the GC/MS data had intercepts > 0 (Table 5). When concentrations detected by GC/MS methods were close to 0, ELISA results over-estimated the metolachlor concentration by as much as 0.57 μg L^{-1} (Table 5). However, the slope for each model was < 1 (Table 5). As metolachlor concentrations detected by GC/MS methods increased, ELISA results increased at a slower rate. Thus, as concentrations detected by GC/MS methods increased, ELISA procedures underestimated metolachlor sample concentrations.

In making general comparisons of the two immunoassays, the Ohmicron ELISA produced the same qualitative results from a larger percentage of samples than did the Millipore ELISA. The Millipore ELISA had larger percentages of false positives and false negatives than did the Ohmicron ELISA. However, very few samples were analyzed using the Millipore ELISA compared to the number analyzed using the Ohmicron ELISA. Thus, the percentages of same qualitative results, false positives, and false negatives for the Millipore ELISA may be somewhat misleading. Even so, the nonagreement of Millipore ELISA with GC/MS data may be the result of variability of wells within microtiter plates or the desorption of antibody or other proteins that adversely affect precision and sensitivity (Engvall, 1980; Harrison et al., 1989; Howell et al., 1981; Lehtonen and Viljanaen, 1980). The data generated in this study should not be used to make judgment as to the effectiveness of the Millipore ELISA for detecting cyanazine and metolachlor.

Regression procedures which compared Ohmicron ELISA to other analytical methods showed that detection of cyanazine by ELISA more closely follows other analytical methods than did the detection of metolachlor by ELISA. When regressed against GC/MS data, models predicting ELISA results for cyanazine had intercepts and slopes that did not differ from 0 and 1, respectively. Models predicting ELISA results for metolachlor had intercepts and slopes that were significantly different from 0 and 1, respectively. The ELISA for cyanazine determination is statistically more reliable than the ELISA for metolachlor detection. These findings were suspected early-on during sample analysis. Establishing the standard curves for the metolachlor ELISA was more difficult than for the cyanazine ELISA because of high coefficients of variation for the metolachlor standards (data not shown). In cases where a particular standard's coefficient of variation exceeded a maximum value, that standard was not used in developing the calibration curve. Thus, the calibration curve was developed using a single absorbance value rather than an average of two samples at a particular concentration.

The ELISA in this study compared more favorably to GC/MS results when determining cyanazine content than when determining metolachlor content. However, ELISA is an efficient and cost-effective means for determining the cyanazine and metolachlor contents of water samples. Much less time is required for the ELISA procedure: hours compared to days to analyze the same number of samples by traditional methods. The economic benefits make ELISA a viable and attractive option when large numbers of samples require analysis. Standard procedures for the determination of cyanazine and metolachlor in surface water samples at Mississippi State University's Weed Science Analytical Laboratory cost approximately $36/sample, compared to less than $12/sample with ELISA (Boyette, 1999). When very accurate measurements are necessary, GC methods may be preferred over ELISA determination of metolachlor. However, if near real-time determination of cyanazine and metolachlor is necessary, ELISA is a useful option for analytical methodology.

ABBREVIATIONS USED

ELISA, enzyme linked immunosorbent assay; EPA, Environmental Protection Agency; HAL, health advisory level; LLD, lower limit of detection; GC/ECD, gas chromatography with electron capture detection; GC/MS, gas chromatography with mass selective detection.

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