Comparative Solid Phase Extraction, Solid Phase Microextraction, and Immunoassay Analyses of Metolachlor in Surface Runoff and Tile Drainage

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New analytical technologies are being developed as an aid to identify pollutants in the environment. An interlaboratory collaborative study was initiated to compare solid phase extraction−gas chromatography (SPE−GC), solid phase microextraction−gas chromatography (SPME−GC), and two enzyme-linked immunosorbent assay (ELISA) techniques for analysis of metolachlor in water samples from a field experiment. Concentration in the water samples ranged from <0.1 to 50 μg/L. Metolachlor concentration in water was highly correlated among methods (R^2 range 0.80−0.97). Slope of regression lines and intercept values between SPE−GC, immunoassay (IA) i, and IA ii techniques did not differ from unity or zero compared to SPME−GC analysis. The two immunoassay methods agreed well with each other (R^2 ranged from 0.94 to 0.97), and differences in concentration were mostly attributed to matrix effects. SPME and ELISA have great potential as tools to detect metolachlor in natural waters.

Keywords: *Solid phase microextraction; SPME; solid phase extraction; SPE; immunoassay; ELISA; EIA*

INTRODUCTION

Herbicides are an integral part of crop production systems. In Ontario, herbicides accounted for 70% of the 7200 tonnes of pesticides used in 1988 (Moxley 1989). Metolachlor is the most widely used herbicide with 1709 tonnes being applied to 499 000 ha in 1988 for annual grass control in soybean (*Glycine max*), field corn (*Zea mays*), and field bean (*Phaseolus* spp.). Most of this use occurred in southern Ontario in close proximity to the Great Lakes. Thus, it is inevitable that some metolachlor will enter the Great Lakes from agricultural runoff.

Metolachlor has been detected in surface runoff, tile drainage, streams, rivers, lakes, and groundwater (Gaynor *et al.*, 1995; Frank *et al.*, 1990; Squillace and Thurman, 1992; Thurman *et al.*, 1991; Chesters *et al.*, 1989). In most cases concentrations are below the Canadian water quality guideline (CWQG) of 50 μg/L for drinking water but near the CWQG of 8 μg/L for freshwater aquatic life (Kent *et al.*, 1991). Monitoring for metolachlor in Canadian waters requires specialized instrumentation such as a gas chromatograph with specific detectors and highly trained personnel.

Several new technologies have been developed for detection of metolachlor in water and other substrates. Enzyme-linked immunosorbent assay (ELISA) and solid phase microextraction (SPME) are two such technologies for metolachlor and other pesticides in water.

Several commercial firms now produce ELISA kits with specificity for metolachlor. ELISA methods involve production of an antibody which is attached to a protein molecule coated in microwells of an analytical plate (*e.g.*, IDTEK, Sunnyvale, CA) or attached to a paramagnetic particle (*e.g.*, Ohmicron, Newtown, PA). An enzyme conjugate is formed from a derivative of the analyte and an enzyme. The analyte and enzyme conjugate compete for binding sites with the antibody forming the basis of the analysis. A chromogenic agent is added which reacts with the enzyme conjugate to produce a colored product, and the resulting color intensity is compared to a set of standards for analyte concentration. Color intensity is inversely proportional to concentration of analyte. Analyte concentrations greater than the highest standard require dilution as concentration should not be extrapolated outside the analytical range of the test. When used as a screen of environmental samples, dilution is not generally done since concentrations greater than the range of the test would require further testing and confirmation by another technique. ELISA is cost effective and requires little technical skill to perform the test.

Ceramic fibers coated with liquid polymeric sorbent such as poly(dimethylsiloxane) form the basis for SPME (Arthur *et al.*, 1990, 1992a,c). The coated fibers are exposed to the test solution or to the headspace over the solution to adsorb a portion of the analyte largely determined by its partition coefficient. After equilibration or a fixed sorbing period, the sorbed analyte is thermally desorbed in the injection port of a gas chromatograph and the analyte is analyzed by conventional capillary gas chromatography. SPME offers a rapid, solvent-free, simple, and inexpensive method for analysis of volatile gases and analytes in liquid matrices. The technique has been evaluated for analysis of industrial pollutants in the environment and natural compounds extracted from plants (Arthur *et al.*, 1992b; Potter and Pawliszyn, 1992; Yang and Peppard, 1994; Hawthorne

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et al., 1992; Zhang and Pawliszyn, 1993). No studies have been published on its use for herbicide analysis in environmental samples.

A collaborative study was undertaken by the authors to compare ELISA, SPME, and solid phase extraction (SPE) methodologies for analysis of metolachlor in field runoff samples. The primary objective was to evaluate the precision of the methodologies to detect metolachlor as suitable analytical techniques to quantify the analyte in environmental samples. Metolachlor stability in stored samples was also assessed.

MATERIALS AND METHODS

Field Plots and Herbicide Application. The water samples were collected during a natural rain event from 16 plots measuring 15 × 67 m established on a Brookston clay loam at Woodslee, Ontario, Canada. Details of the study on the effect of crop/tillage/drainage control management systems on water quality of surface runoff and tile drainage have been reported (Tan *et al*., 1993). Metolachlor at 1.68 kg/ha was applied preemergence on May 13, 1994, with a Chelsea sprayer through 8004 EVS flat fan (banded application, intercropped) or 8004 VS (broadcast application, no intercrop) nozzles (TeeJet) calibrated to deliver 265 L of water/ha at 210 kPa. Herbicide was applied in a 38 cm wide band over the seeded row in the ryegrass intercrop treatments and over the entire area in the non-intercropped treatments. Thus, the non-intercropped treatments received 2 times the amount of herbicide as the intercropped treatments, but the application rate was similar.

Sample Collection. A 4 L sample of water was collected manually in glass bottles at selected times during the first rain event after herbicide application which produced runoff (August 13 and 14, 1994). Thirty-nine samples in total were collected including 15 surface runoff and 24 drainage samples. These samples provided a wide range in metolachlor concentration and sufficient samples for method comparison. The samples were stored at 4 °C prior to analysis and transported to the collaborating laboratories. Samples were not filtered during storage or transport because previous studies on this soil had determined that herbicide adsorbed to sediment was not a major source of loss (Gaynor *et al*., 1992, 1995; Gaynor and van Wesenbeeck, 1995). Loss of metolachlor by adsorption on glass or sediment and degradation was assessed by reanalysis 222 days after collection on August 13, 1994.

SPE Analysis. A suitable aliquot (100−250 mL) of water was filtered through a 0.45 μm filter (Gelman GN-6). Metolachlor was extracted from the filtered water on a cyclohexyl Bakerbond SPE cartridge (Baker cat. no. 7212-03) after preconditioning with 3 mL of methanol and 3 mL of distilled, deionized water. The cartridge was dried after loading and metolachlor eluted with 1.5 mL of methanol. The volume was adjusted to an appropriate volume and analyzed on a Varian 3400 gas chromatograph (GC) using a thermionic sensitive detector operated in the nitrogen mode. The He carrier had a flow rate of 10 mL/min. Air and hydrogen flow rates to the detector were 175 and 4.5 mL/min, respectively. The cryogenic injector was cooled with CO_2, and aliquots of the test samples were injected on-column (injector programmed from 70 to 210 °C at 180 °C/min with a 12.5 min hold) to a 15 m DB-5 capillary column, temperature programmed from 150 to 240 °C at 20 °C/min with a 5.5 min hold. Retention time for metolachlor was 7.3 min. Recovery from test samples fortified with 1−500 μg of metolachlor/L averaged >90%. Measured concentrations in the water were not corrected for recovery.

SPME Analysis. An unfiltered, 30 mL aliquot was transferred to a 40 mL Teflon-lined septum screw cap vial. A 100 μm poly(dimethylsiloxane)-coated fiber (Supelco) was inserted into the sample which was stirred for 15 min with a 13 × 8 mm Teflon-coated magnetic bar at 60% of the maximum stir rate during sampling. The fiber was removed after sampling, inserted into the GC (Hewlett Packard 5890), and thermally desorbed in the injection port for 2 min at 200 °C. The DB-5 30 m × 0.25 mm fused silica gel column was temperature programmed at 10 °C/min from 100 to 250 °C with a final hold for 2 min. Metolachlor had a retention time of 14.2 min with a He flow rate of 60 mL/min and was detected on an electron capture detector (ECD). Each sample was run in triplicate. A standard curve was prepared from standards in 30 mL of high-performance liquid chromatography grade water at concentrations ranging from 2 ng/L to 20 mg/L.

Immunoassay Analysis. Two immunoassay (IA) technologies, (i) plastic microwells precoated with antibody which specifically binds metolachlor (IDTEK) or (ii) metolachlor specific antibody attached to paramagnetic particles (Ohmicron), were assessed. Procedures were followed according to manufacturers specifications supplied with the kits. Samples with metolachlor concentrations greater than the highest standard provided by each kit (>8 μg/L of test i or >5 μg/L of test ii) were diluted to within range and reanalyzed. In each case a 200 μL aliquot was assayed. Standards of known metolachlor concentration provided with the kits were analyzed with each batch of samples to ensure proper calibration of the instruments and adherence to the procedures.

Test i. Seven standards (0, 0.25, 0.5, 1, 2, 4, and 8 μg/L) containing no analyte or metolachlor and test solution were added to individual cells in the sensitized ELISA microwell module. Distilled water containing no metolachlor or 0.5 μg/L metolachlor were prepared in the laboratory and included with each batch of analysis to provide internal quality assurance. Enzyme conjugate solution (50 μL) was added to each well and incubated on an orbital plate shaker for 10 min. After incubation, the solutions were aspirated from the wells and wash solution was added. The wash/aspiration sequence was repeated four times. A 200 μL substrate solution was added to each well and incubated for 10 min on an orbital plate shaker. At the end of the incubation, 50 μL of stopping solution was added and mixed for 10 min. The absorbance reading of each well was determined on a microplate reader fitted with a 650 nm filter within 90 min of adding the stop solution.

Test ii. Four standards (0, 0.1, 1, and 5 μg/L) containing no analyte and metolachlor, a control standard, and test solution were added to individual tubes. Enzyme conjugate (250 μL) and metolachlor antibody coupled to magnetic particles (500 μL) were added and the contents mixed on a vortex mixer and incubated for 30 min. After incubation the tubes in the rack were combined with a magnetic base for 2 min to allow separation of the magnetic particles from solution. The solution was drained from the tubes and 1 mL of wash solution added, and the magnetic particles were allowed to separate for 2 min. The wash procedure was repeated. The samples and magnetic rack were separated, and 500 μL of color reagent was added and mixed for 1−2 s on a vortex mixer. The samples were incubated for 20 min, 500 μL of stopping solution was added, and the absorbance was determined within 15 min on a RPA-I photometric analyzer at 450 nm.

For each immunoassay test, a log−logit standard curve was prepared from absorbance readings of standards. Concentration of analyte was determined from the linear plots. Concentrations of blind standards included with the kits were within the acceptable range for each batch of determinations.

Concentrations of metolachlor in each sample determined by the two IA and SPE methods (dependent *y* variable) were compared by regression analysis with the SPME method (independent *x* variable). Loss of metolachlor in storage was assessed by reanalysis of selected samples using IA test ii after 222 days from collection. Significance of difference between the first and last determined concentration was assessed by regression analysis (SAS, 1989).

Linearity of immunoassay test ii to predict metolachlor concentration was assessed by diluting the analyte to selected concentrations in the range of the standard curve. Least-squares mean regression of concentration (*y*) on dilution factor (*x*) was used to detect departures from zero in the slope of the regression line (SAS, 1989). A zero slope would indicate no specific or nonspecific interferences for metolachlor with immunoassay test ii.

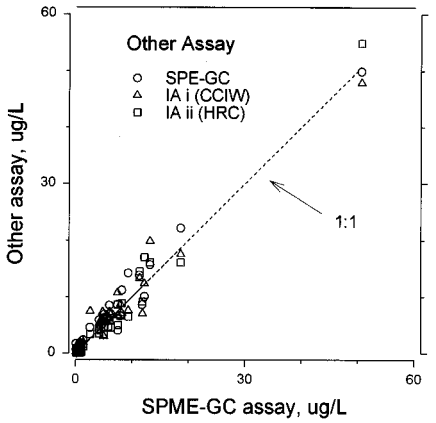

Figure 1. Comparison of SPE−GC, IA i, and IA ii analyses of metolachlor in water with that determined by SPME−GC analysis.

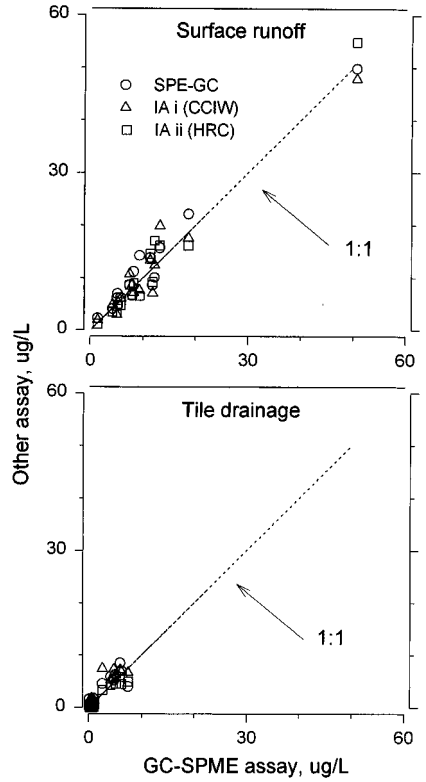

Figure 2. Comparison of SPE−GC, IA i, and IA ii analyses of metolachlor in surface runoff and tile drainage with that determined by SPME−GC analysis.

RESULTS AND DISCUSSION

Concentration of metolachlor in the water samples ranged from <0.1 to 54.9 μg/L (Figure 1). Larger metolachlor concentrations were found in surface runoff (1.4−54.9 μg/L) than in tile drainage (0.01−8.5 μg/L) (Figure 2). Metolachlor was surface applied; therefore, greater concentrations would be expected in surface runoff than in tile drainage (Gaynor *et al.*, 1992, 1995).

Metolachlor concentrations by SPE and the two IA techniques were compared with that determined by SPME (Figure 1). SPME was selected as the independent variable for comparison because samples were analyzed in triplicate and GC conditions were optimized providing greater confidence in the results in the absence of samples of known concentration or inclusion of field spikes. For the SPME method, coefficient of variation (CV) in metolachlor concentration within the three replicates ranged from 1% to 10% for the 39 samples with a mean of 5%. Coefficient of variation in metolachlor concentration by immunoassay test ii ranged from 1% to 12% ($n = 3$) with a mean of 5% for 17 samples. Metolachlor concentrations determined by each method correlated well (R^2 ranged from 0.95 to 0.97) with that from SPME (Table 1). Regression coefficients (slope of the regression) were near unity for each of the three methods, and intercept values were not significantly different from zero.

Table 1. Regression Coefficient (Slope), Intercept, and Correlation Coefficient (R^2) for Metolachlor Determination in Water by Four Analytical Techniques[a]

comparison, *x* vs *y*	source	slope	intercept	R^2
SPME, SPE	all	1.00 ± 0.03	0.56 ± 0.32	0.96
SPME, IA i	all	0.95 ± 0.03	0.62 ± 0.35	0.95
SPME, IA ii	all	1.07 ± 0.03	−0.52 ± 0.29	0.97
IA ii, IA i	all	0.88 ± 0.03	1.12 ± 0.29**	0.97
SPME, SPE	surface	0.97 ± 0.05	1.27 ± 0.83	0.96
SPME, IA i	surface	0.94 ± 0.06	0.77 ± 0.99	0.95
SPME, IA ii	surface	1.10 ± 0.05	−0.90 ± 0.86	0.97
IA ii, IA i	surface	0.85 ± 0.04	1.59 ± 0.75*	0.97
SPME, SPE	tile	1.00 ± 0.11	0.32 ± 0.31	0.80
SPME, IA i	tile	1.14 ± 0.10	0.27 ± 0.30	0.85
SPME, IA ii	tile	0.87 ± 0.06	−0.07 ± 0.16	0.92
IA ii, IA i	tile	1.32 ± 0.07	0.33 ± 0.18	0.94

[a] Asterisks denote significance at *$P = 0.05$ and **$P = 0.01$.

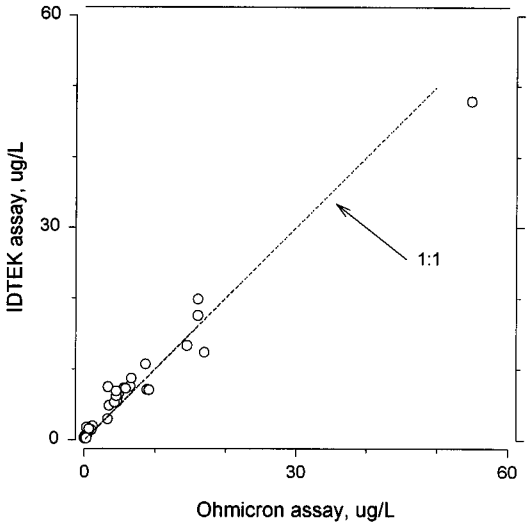

Figure 3. Comparison of the two immunoassay procedures for analysis of metolachlor in water.

Comparisons between the two immunoassay tests were highly correlated ($R^2 = 0.97$), but test i tended to overestimate metolachlor concentration at <20 μg/L and underestimated the largest concentration (>48 μg/L) compared to test ii (Figure 3). The intercept was offset by 1.12 μg/L ($P < 0.01$). This may reflect differences in selectivity of the antibody to substances in the matrix or other factors related to variations associated with dilution or analysis (Lawruk *et al.*, 1993). However, this does not reduce the suitability of the test as a field screen since samples outside the range of the test (>5 or 8 μg/L for tests ii and i, respectively) would be identified as requiring confirmation by GC. No data have been published on comparison among ELISA test kits from different sources. Comparisons between ELISA and GC analyses suggest that metolachlor concentrations appear generally larger by ELISA (Fleeker and Cook, 1991; Hall *et al.*, 1993; Thurman *et al.*, 1991; Lawruk *et al.*, 1993).

Table 2. Regression Parameters for the Estimate of Metolachlor Concentration Determined by Immunoassay Test ii at Different Dilutions in the Range 0.1−5.0 μg/L

source	sample ID	no. of determinations	metolachlor[a] (μg/L)	slope	intercept (μg/L)	R^2
surface	6	13	3.3 ± 0.1	−0.04 ± 0.07	3.4 ± 0.2	0.03
	10	16	8.4 ± 0.4	−0.15 ± 0.14	9.0 ± 0.7	0.08
	26	6	54.9 ± 2.6	1.14 ± 0.34*	34.5 ± 6.3	0.91
	29	19	17.0 ± 0.7	−0.03 ± 0.12	17.2 ± 1.2	0.01
	35	13	4.7 ± 0.1	0.04 ± 0.05	4.6 ± 0.2	0.04
	36	13	4.6 ± 0.1	0.09 ± 0.07	4.4 ± 0.2	0.14
tile	9	13	4.9 ± 0.1	0.04 ± 0.07	4.8 ± 0.2	0.03
	20	13	4.3 ± 0.2	−0.13 ± 0.09	4.6 ± 0.3	0.15
	21	17	5.8 ± 0.3	−0.01 ± 0.18	5.9 ± 0.5	0.01
	22	15	3.3 ± 0.1	−0.04 ± 0.08	3.5 ± 0.2	0.03
	32	13	4.6 ± 0.3	0.31 ± 0.11*	3.7 ± 0.4	0.40

[a] Average concentration for the number of determinations indicated. *Slope significantly different from zero at $P < 0.05$.

Comparisons in Surface Runoff. Values determined for metolachlor concentration in surface runoff did not differ among the four methods of analysis (Table 1). Regression coefficients were not significantly different from unity, and intercept values did not differ from zero ($P > 0.05$). This indicates that each method was suitable for determining metolachlor concentration in this substrate over a wide range of concentrations.

The two immunoassay technologies were highly correlated ($R^2 = 0.97$), but a slope less than one (0.85 ± 0.05) suggests differences in response to metolachlor. The intercept was greater than zero (1.59 ± 0.75, $P = 0.05$) (Table 1). The coefficient of regression of less than one (slope = 0.85 ± 0.04) indicates that immunoassay test i predicted smaller metolachlor concentrations in surface runoff than immunoassay test ii (Table 1). However, the regression line is highly influenced by the largest concentration measured (>48 μg/L) which was underestimated by test i (Figure 3). This is evident from the positive offset of the intercept (1.59 ± 0.75 μg/L) (Table 1). Other studies, comparing analysis by ELISA with GC, found deviations in regression coefficients from unity (Goh *et al.*, 1991, 1992). Some of the complexities of interactions which affect ELISA and GC results have been discussed by others (Goh *et al.*, 1991; Brady *et al.*, 1995). Bias in results can be affected by substrate matrix, dilution of samples, nature of antibody used in the immunoassay (polyclonal vs monoclonal antibody), and presence of cross-reacting products as well as other undefined factors.

Comparisons in Tile Drainage. The correlation coefficients were not as high among methods for tile drainage (0.80−0.94) as for surface runoff, but slopes did not differ from one, and intercepts were zero (Table 1). SPE and IA test i returned metolachlor concentrations similar to those from SPME (slopes and intercepts did not differ from unity or zero, respectively). Immunoassay test ii had the best R^2, but the slope value of 0.87 ± 0.06 (Table 1) indicates this method yielded smaller metolachlor concentration values than SPME.

Immunoassay test i returned greater metolachlor concentration values than immunoassay test ii (slope = 1.32 ± 0.07), consistent with a concentration of <20 μg/L in surface runoff. The intercept did not differ from zero (0.33 ± 0.18 μg/L, $p = 0.07$) but was indicative of the bias toward larger concentrations for those samples with metolachlor concentrations of <20 μg/L. Immunoassay test ii was conducted 111 days later than test i. Metolachlor concentrations determined at 222 days were compared with those at 111 days after collection using immunoassay test ii. Larger metolachlor concentrations at 222 days are indicated from the regression data by a slope > 1.0 and an intercept greater than zero

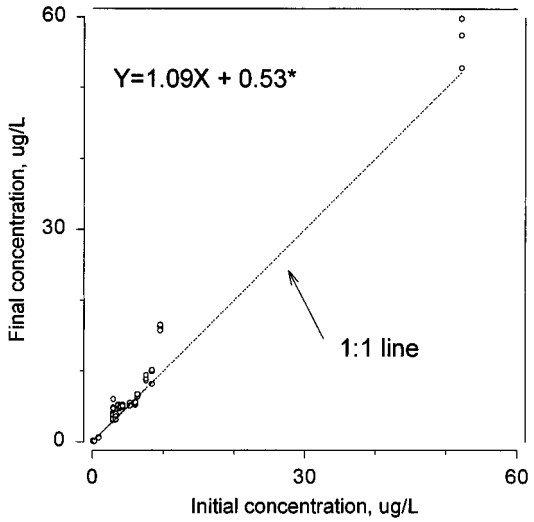

Figure 4. Metolachlor concentration in water determined by immunoassay test ii 111 (initial) and 222 (final) days after collection. Three determinations of 17 samples. Asterisk denotes significance of intercept at $p = 0.05$.

(Figure 4). No metolachlor was found in samples which previously had initial metolachlor concentrations near 0.1 μg/L, the smallest concentration of the standard curve. Metolachlor is rapidly depleted from solution by low-density plastic materials (Topp and Smith, 1992), but no data relating to its adsorption to glass or degradation in water at low temperature were found.

Metolachlor is rapidly degraded by microorganisms in soil (LeBaron *et al.*, 1988; Chesters *et al.*, 1989). Few studies are reported for fate in aquatic environments. LeBaron *et al.* (1988), in their review, calculated a half-life of greater than 200 days for metolachlor hydrolysis in water for the range in pH from 1 to 9, assuming first-order kinetics. Photolysis was not considered to be a significant pathway for dissipation in our study since samples were stored in the dark except when handled for shipping or analysis.

Linearity of Immunoassay Test ii upon Dilution. The linearity of test ii was assessed from departures in metolachlor concentration at different dilutions within the range of standards (0.1−5.0 μg/L). Departures from zero of the regression coefficient (slope) and differences between intercept and average concentration of metolachlor determined by selected dilutions would suggest either specific or nonspecific interferences in the assay with test ii. Regression analysis of measured metolachlor concentration at different dilutions detected no change in slope from zero or in intercept values (Table 2) except for two samples (no. 26 and 32). Greater

metolachlor concentration was predicted from the larger (20× and 6×, respectively) than smaller dilution as reflected by the smaller intercept compared to the average metolachlor concentration for these two samples. Metolachlor and alachlor, a related chloroacetamide, displayed no departure from expected concentration between diluted and nondiluted samples containing <4 μg/L herbicide (Lawruk *et al.*, 1992, 1993).

Our studies indicate good agreement between SPE, SPME, and IA techniques for analysis of metolachlor in field-derived water samples. The advantages of immunoassay analysis for initial screening of environmental samples have been discussed (Brady *et al.*, 1995; Hall *et al.*, 1990, 1992, 1993; Bushway *et al.*, 1991, 1992; Feng *et al.*, 1990). SPME also shows great promise as a tool in environmental monitoring (Arthur *et al.*, 1992b; Potter and Pawliszyn, 1992; Zhang and Pawliszyn, 1993; Sarna *et al.*, 1994). Development of a wider range of ceramic fiber-coating materials other than poly(dimethylsiloxane) will increase the application of SPME to a wider group of chemicals permitting multiple analyte sampling and analysis. In addition, a wide range of environmental matrices may be sampled directly or by headspace SPME enabling analyses to be performed less expensively and promoting more extensive and statistically defensible monitoring than has hitherto been possible. Further, solventless analytical techniques allow economic savings with regard to both purchase and disposal of the solvents currently required for residue analyses.

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