Determination of Metolachlor in Water and Soil by a Rapid Magnetic Particle-Based ELISA

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A competitive enzyme-linked immunosorbent assay (ELISA) for the quantitation of metolachlor in water and soil was developed using a magnetic particle-based solid phase. Metolachlor was covalently attached to bovine serum albumin carrier through the chloroacetamide moiety and the resulting herbicide–protein immunogen used in rabbits to produce polyclonal antibodies specific for metolachlor. The polyclonal antibodies are covalently attached to amine-terminated superparamagnetic particles and used as the solid phase in a rapid and sensitive immunoassay. This ELISA has a limit of detection of 0.05 ppb (parts per billion, nanograms per milliliter) in water and 3.0 ppb in soil. The ELISA compares favorably with GC measurements when water samples are analyzed ($r = 0.976$). Recoveries from fortified soils averaged 83% and 90% using 30-min and 24-h extractions with methanol/water (75:25 v/v), respectively.

INTRODUCTION

The interest in pesticide residue testing in water, soil, and food has increased dramatically over the past few years. Current testing methods involving gas (GC) and liquid (HPLC) chromatography are time-consuming and expensive and require specialized instrumentation. Growth in pesticide residue testing throughout the world has led to a need for faster, easier methods that permit the screening of large numbers of samples. The emergence of enzyme immunoassays, which are rapid, sensitive, accurate, and cost-effective, has provided the analytical chemist with an alternative to traditional methods (Van Emon and Lopez-Avila, 1992).

Metolachlor is a pre-emergence selective herbicide used for the control of annual grasses and some broad-leaved weeds in such crops as corn, soybeans, sorghum, peanuts, cotton, potato, and sugar cane. The active ingredient of Dual herbicide, metolachlor is one of the most widely used herbicides in the United States; an estimated 50 million pounds are applied per year (Gianessi and Puffer, 1991). As a result of its high usage, metolachlor residues have been detected in precipitation, wells, and surface and ground water through normal-use spraying, spills, and runoff (Frank et al., 1987; Goolsby et al., 1991; Thurman et al., 1992; Hall et al., 1993). Metolachlor has been classified in group C, a possible human carcinogen, by the EPA, which established a Health Advisory Limit in drinking water of 100 ppb (U.S. EPA, 1989). This category is for substances with limited evidence of carcinogenicity in animals and absence of human data. Health and Welfare Canada has set an interim maximum acceptable concentration for metolachlor in drinking water at 50 ppb (Health and Welfare Canada, 1989). The European Community has set a maximum admissible concentration for metolachlor, as well as other pesticides, in drinking water at 0.1 ppb and for the total of all pesticides at 0.5 ppb (EC Council, 1980). In soil, metolachlor is readily adsorbed to muck and clay constituents; however, the rate of dissipation is sufficiently rapid under field conditions to avoid any carryover to rotational crops the next season (LeBaron et al., 1988). If, however, soon after harvest of the treated crop, a sensitive crop such as cereal grains is planted, injury and yield reduction can occur, especially in cooler, drier climates (LeBaron et al., 1988).

The principles of enzyme-linked immunosorbent assay (ELISA) have been described (Hammock and Mumma, 1980) and applied to the detection of metolachlor in water and soil (Schlaeppi et al., 1991; Feng et al., 1992; Schmitt et al., 1992; Hall et al., 1992). These ELISAs utilized polystyrene wells or tubes on which antibody or hapten–protein conjugate is passively adsorbed. The desorption and leaching off of antibody or other proteins that are passively adsorbed to solid phases are major factors that adversely affect assay sensitivity and precision (Howell et al., 1981; Engvall, 1980; Lehtonen and Viljanen, 1980). Variability of wells within microtiter plates due to coating and desorption has been shown to be the greatest contributor to total assay imprecision (Harrison et al., 1989). Magnetic particle-based ELISAs have previously been described and applied to the detection of pesticide residues (Rubio et al., 1991; Itak et al., 1992, 1993; Lawruk et al., 1993), including the chloroacetanilide herbicide alachlor (Lawruk et al., 1992). The magnetic particle-based ELISA eliminates the imprecision problems of coated plates and tubes through the precise addition of antibody, which is covalently coupled to the magnetic solid phase, to each reaction tube. The uniform dispersion of particles throughout the reaction mixture provides rapid reaction kinetics. In the present work, we describe the development and evaluation of a competitive ELISA for the determination of metolachlor in environmental water and soil samples utilizing superparamagnetic particles as a solid support and means of separation.

MATERIALS AND METHODS

Reagents. Amine-terminated superparamagnetic particles of approximately 1-μm diameter were obtained from Advanced Magnetics, Inc. (Cambridge, MA). Horseradish peroxidase (HRP) and glutaraldehyde were purchased from Sigma Chemical Co. (St. Louis, MO). Metolachlor–HRP conjugate is available from Ohmicron Corp. (Newtown, PA). Hydrogen peroxide and 3,3′,5,5′-tetramethylbenzidine (TMB) were obtained from Kirke-

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0021-8561/93/1441-1426$04.00/0

gaard and Perry (Gaithersburg, MD). Metolachlor and related compounds as well as nonrelated cross-reactants were purchased from Riedel-de-Haen (Hanover, Germany). All other reagents were of reagent grade or chemically pure.

Apparatus. All spectrophotometric measurements were determined using the RPA-I Analyzer (Ohmicron) the detailed functions of which have previously been described by Rubio et al. (1991). A two-piece magnetic separation rack consisting of a test tube holder which fits over a magnetic base containing permanently positioned rare earth magnets is required. This two-piece design allows for a 60-tube immunoassay batch to be set up, incubated, and magnetically separated without removing the tubes from the holders (Itak et al., 1992). Gilson P-200 (Rainin, Woburn, MA) and Eppendorf repeating pipets (Eppendorf, Hamburg, Germany) were used to dispense liquids.

Antibody Generation. The metolachlor ligand, 4-[[(2-methoxy-1-methylethyl)(2-ethyl-6-methylphenyl)amino]-2-oxoethyl]thiobutanoic acid, was coupled to bovine serum albumin (BSA) according to the mixed anhydride procedure of Erlanger et al. (1957). The immunogen solution was brought to room temperature and dialyzed against 0.02 M sodium borate, pH 8.7, and deionized water (two changes) and lyophilized. The attachment of the BSA to the chloroacetamide moiety leaves the ring and side chains free, ensuring maximal sensitivity to these functional groups and minimal cross-reactivity with other chloroacetanilide herbicides. The metolachlor immunogen was dissolved in sterile saline solution to a concentration of 4 mg/mL. This solution was emulsified with an equal volume of Freund's complete adjuvant, and a total of 0.5 mL of the emulsion was injected in the hip muscle of three rabbits. After 20 and 45 days and at 30-day intervals thereafter, the rabbits were boosted with 0.5 mL of the emulsion using Freund's incomplete adjuvant. Whole blood (30–50 mL) was obtained 10 days after each boost, allowed to coagulate, and centrifuged to obtain the antiserum, which was stored at −70 °C.

Antibody Coupling Procedure. Rabbit anti-metolachlor coupled magnetic particles were prepared by glutaraldehyde activation of the magnetic solid phase as by Rubio et al. (1991).

Immunoassay Procedure. All water samples and diluted soil extracts were assayed by adding 200 μL of sample, 250 μL of metolachlor–HRP conjugate, and 500 μL of anti-metolachlor magnetic particles to a test tube and incubating for 30 min at room temperature. The magnetic rack was used to magnetically separate the reaction mixture. After separation, the magnetic particles were washed twice with 1.0 mL of deionized water to remove unbound conjugate and eliminate any potential interfering substances. The colored product was developed for 20 min at room temperature by the addition of 500 μL of hydrogen peroxide/TMB solution. The colored reaction was stopped and stabilized by the addition of 500 μL of 2 M sulfuric acid. The final concentrations of metolachlor for each sample were determined using the RPA-I analyzer by determining the absorbance at 450 nm. The observed sample results were compared to a linear regression line using a natural logarithm (ln) of the concentration vs logit B/B_0 standard curve (where B/B_0 is the absorbance at 450 nm observed for a sample or standard divided by the absorbance at the zero standard). The calibrators were prepared in the zero standard (0.025 M sodium acetate/0.15 M NaCl/0.1% gelatin preserved solution) and contained metolachlor at 0, 0.1, 1.0, and 5.0 ppb. Samples greater than 5.0 ppb were diluted in the zero standard for analysis, and sample concentrations were calculated by multiplying results by the appropriate dilution factor.

Water samples for method comparison were drinking, surface, and ground waters obtained from various locations throughout the United States and Canada and were analyzed as received. The gas chromatograph (GC) utilized a nitrogen–phosphorus detector and a 3% OV-101 column with a Chromosorb W-HP solid phase (100–120 mesh size). Helium was used as the carrier gas at a flow rate of 30 mL/min. The oven, injection, and detection temperatures were 190, 210, and 250 °C, respectively. The water samples were extracted according to the method of Hall et al. (1993), and solvent extracts were injected directly into the GC and the peak heights compared to external standards. The GC results were not corrected for procedural recoveries.

Table I. Composition of Soils Fortified with Metolachlor

soil	% sand	% silt	% clay	% humus	CEC[a]	pH
Sassafras sandy loam	60	29	11	2.02	6	7.0
silt loam	33	57	10	<1.0	NA[b]	6.0
Sharkey clay loam	38	22	40	3.0	24	6.1
muck sandy loam	72	21	7	46	78	4.5
Plano loam	38	48	26	4.5	12	6.1

[a] Cation-exchange capacity (mequiv/100 g of soil). [b] NA, analysis not available.

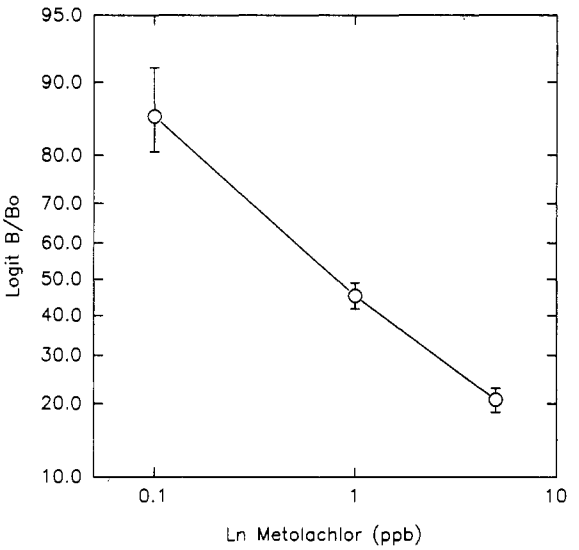


Figure 1. Dose response for metolachlor. Each point represents the mean of 68 determinations. Vertical bars indicate ±2 SD about the mean.

Water samples for the spike recovery study were prepared by adding 1.5 ppb of metolachlor to 326 drinking, surface, and ground waters collected from Florida, Idaho, Illinois, Kansas, Minnesota, New Jersey, Ohio, Pennsylvania, Rhode Island, Vermont, Washington, Wisconsin, and Canada. The recovery was determined by analyzing the samples before and after the addition of metolachlor and then subtracting the concentration of metolachlor present in the sample prior to spiking.

Soil Extraction and Analysis. Air-dried soils of known composition (Table I) were shaken for 2 h with water spiked with metolachlor to yield soil concentrations of metolachlor from 0.1 to 100 ppm. Soils were then air-dried for 3 days and ground with a mortar and pestle. Ten grams of soil was extracted for 30 min to 24 h by agitating in 30 mL of methanol/water (75:25 v/v). After settling for approximately 15 min, the extract supernatant was diluted at least 1:20 (50 μL in 950 μL) in the zero standard to eliminate solvent interferences. The diluted soil extract was assayed as described above, and the results were multiplied by the appropriate dilution factor to determine the soil metolachlor concentration (i.e., multiply by 60 for a 1:20 dilution to correct for the initial 1:3 dilution of soil with methanol/water).

RESULTS AND DISCUSSION

Dose Response Curve and Sensitivity. Figure 1 illustrates the mean standard curve for the metolachlor calibrators collected over 68 runs, linearly transformed using a ln/logit curve fit. The error bars, representing 2 SD, illustrate the reproducibility of the standard curve. The displacement at the 0.1 ppb level is significant, 86% B/B_0. The assay sensitivity was estimated to be 50 ppt (parts per trillion, picograms per milliliter) using the 90% B/B_0 concentration (Midgley et al., 1969). The sensitivity can also be defined as the mass equivalent of 2 or 3 times the standard deviation of the B_0 from its mean absorbance. The minimum detectable concentrations determined according to this method are approximately 33 and 55 ppt, respectively. This sensitivity exceeds the method

Table II. Precision of Metolachlor Measurement

	sample[a]			
	1	2	3	4
replicates	5	5	5	5
days	5	5	5	5
N	25	25	25	25
mean, ppb	0.34	0.74	1.90	3.37
%CV (within-assay)	6.5	5.2	7.3	5.4
%CV (between-assay)	9.2	5.7	4.6	2.6
%CV (total assay)	10.6	7.4	8.4	6.0

[a] Metolachlor-spiked ground water (sample 1 spiked at 0.3 ppb and sample 2 spiked at 0.75 ppb) and surface water (sample 3 spiked at 2.0 ppb and sample 4 spiked at 3.0 ppb) assayed in five singlicates each over 5 days.

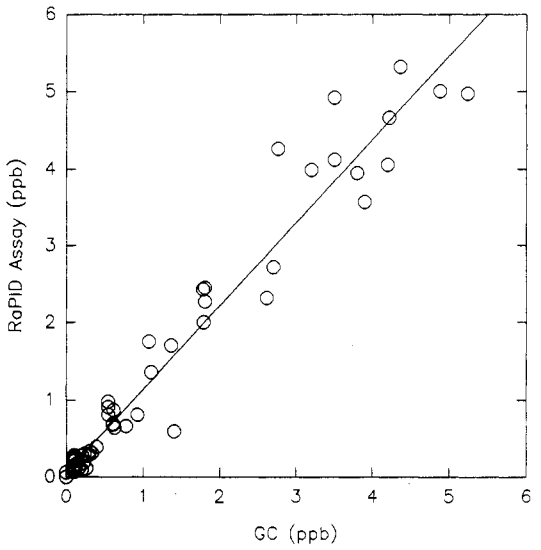


Figure 2. Correlation between metolachlor concentrations as determined by the magnetic particle-based ELISA and GC methods. $n = 58$, $r = 0.976$, $y = 1.08x + 0.063$.

detection limit reported for U.S. EPA Method 507 of 0.75 ppb using gas chromatography with a nitrogen–phosphorus detector (U.S. EPA, 1989b). Schlaeppi et al. (1991) have previously reported direct and indirect ELISAs using monoclonal antibodies with minimum detectable levels of 50 and 100 ppt, respectively, using a 2 SD from B_0 sensitivity definition. An indirect ELISA to detect metolachlor using polyclonal antibodies produced with a metalaxyl acid–BSA immunogen has been developed by Hall et al. (1992) with a lower detection limit of 2.0 ppb. Schmitt et al. (1992) have recently described an indirect ELISA using polyclonal antibodies with detection limits ranging from 0.2 to 1.5 ppb.

Precision. A precision study in which two surface and two ground water samples were spiked with metolachlor at 0.30, 0.75, 2.0, and 3.0 ppb and each was assayed five times in singlicate per assay on five different days is shown in Table II. The within- and between-day variation was determined by analysis of variance (ANOVA) (Bookbinder and Panosian, 1986) using SAS software (SAS Institute, 1988). Coefficients of variation (%CV) within- and between-day were less than 8% and 10%, respectively. The total %CV ($n = 25$) was less than 11% at all concentrations tested.

Method Comparison. Correlation of 58 environmental water samples obtained by the present ELISA method (y) and an established gas chromatography method (x) is illustrated in Figure 2. The regression analysis yields a correlation of 0.976 and a slope of 1.08 between methods. The apparent higher metolachlor sample concentrations could result from cross-reactivity of the antibody with metabolites and other chloroacetanilides or could be due to the loss of analyte during the sample extraction and concentration steps of the GC method (U.S. EPA, 1989a).

Table III. Accuracy of Metolachlor ELISA

amt of metolachlor added, ppb	metolachlor recovered[a]			
	mean, ppb	n	SD, ppb	% recovery
0.25	0.25	16	0.03	100
0.50	0.55	16	0.05	110
2.00	2.21	16	0.18	110
4.00	3.56	16	0.34	89
av				102

[a] Eight water samples each spiked at the described concentration and assayed in duplicate in the ELISA.

Table IV. Linearity upon Sample Dilution[a]

sample ID	undiluted	1:2	1:4	1:8
sample 1				
obtained, ppb	3.54	2.09	1.07	0.51
expected,[b] ppb	3.54	1.77	0.89	0.44
recovery, %		118	121	115
sample 2				
obtained, ppb	3.83	2.07	1.11	0.53
expected, ppb	3.83	1.92	0.96	0.48
recovery, %		108	116	111
sample 3				
obtained, ppb	2.10	1.08	0.48	0.25
expected, ppb	2.10	1.05	0.53	0.26
recovery, %		103	91	95

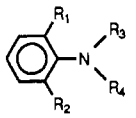
[a] Samples diluted with the zero standard. [b] Expected concentrations are derived from the metolachlor concentration obtained from the undiluted sample.

Accuracy. The accuracy of the ELISA was established by adding known amounts of metolachlor to eight water samples obtained locally. The water samples included two municipal drinking water sources, three small creeks, a reservoir, and two samples from the Delaware River approximately 70 mi apart. The accuracy was evaluated by analyzing the samples before and after the addition of metolachlor and subtracting the concentration of metolachlor before spiking. Table III summarizes the accuracy of the metolachlor ELISA. Added amounts of metolachlor were recovered quantitatively in all cases with an average assay recovery of 102%. An inaccurate recovery of the spiked metolachlor (either increased or decreased) would suggest the presence of an interference. The recovery of the spiked samples indicates that no sample matrix problems or interferences were present in the samples tested, and the accuracy of the ELISA is linear across the range of the assay.

Sample Dilution. Samples that apparently contain metolachlor can be diluted with the zero standard and reassayed to determine "parallel" dilution. If the positive result was due to either specific or nonspecific interefernces, the values of the diluted samples would not assay as expected, i.e., the standard curve should be parallel to the curve obtained by diluting a sample (Jung et al., 1989). If the ELISA were susceptible to interferences, the difference between expected and observed values would increase with increasing dilutions. Values obtained from three spiked ground water samples diluted in the zero standard showed agreement between measured and expected values (Table IV). The expected values are derived from the metolachlor concentration in the undiluted (neat) sample.

Specificity. The 50% inhibition concentration (I_{50}) was determined by estimating the amount of chloroacetanilide analogue necessary to displace 50% of the metolachlor–HRP conjugate. The least detectable dose (LDD) was determined as the concentration of analogue required to achieve 90% B/B_0, the limit of detection of

Table V. Specificity (Cross-Reactivity) of Chloroacetanilides and Unrelated Agrochemicals in the Metolachlor ELISA



compound	R_1	R_2	R_3	R_4	LDD,[a] ppb	I_{50},[b] ppb
metolachlor	CH_2CH_3	CH_2CH_3	$CH(CH_3)CH_2OCH_3$	$COCH_2Cl$	0.05	0.85
acetochlor	CH_3	CH_2CH_3	$CH_2OCH_2CH_3$	$COCH_2Cl$	0.06	6.55
metalaxyl	CH_3	CH_3	$CH(CH_3)CO_2CH_3$	$COCH_2OCH_3$	0.06	5.60
butachlor	CH_2CH_3	CH_2CH_3	$CH_2O(CH_2)_3CH_3$	$COCH_2Cl$	0.26	52.0
propachlor	H	H	$CH(CH_3)_2$	$COCH_2Cl$	1.00	2500
alachlor	CH_2CH_3	CH_2CH_3	CH_2OCH_3	$COCH_2Cl$	1.30	84.0

[a] Least detectable dose (90% B/B_0). [b] 50% inhibition concentration (50% B/B_0). The following pesticides were assayed at 10 000 ppb and found to have no reactivity in the assay: aldicarb, aldicarb sulfate, aldicarb sulfoxide, atrazine, benomyl, butylate, captan, carbaryl, carbendazim, carbofuran, cyanazine, 2,4-D, 1,3-dichloropropene, dinoseb, MCPA, metribuzin, pentachlorophenol, picloram, simazine, terbufos, thiophanat-methyl, thiabendazol.

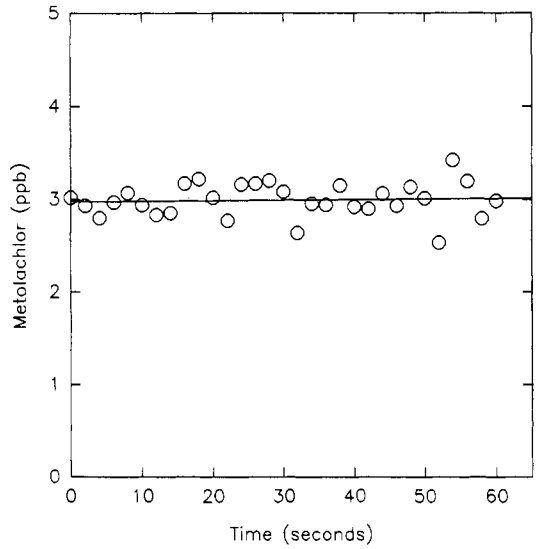


Figure 3. Assay drift: plot of 30 consecutive determinations of a single sample containing 3.0 ppb of metolachlor. Total time to add the magnetic particles was approximately 90 s (40 tubes total including the standards). Slope = 0.00069 ppb/s.

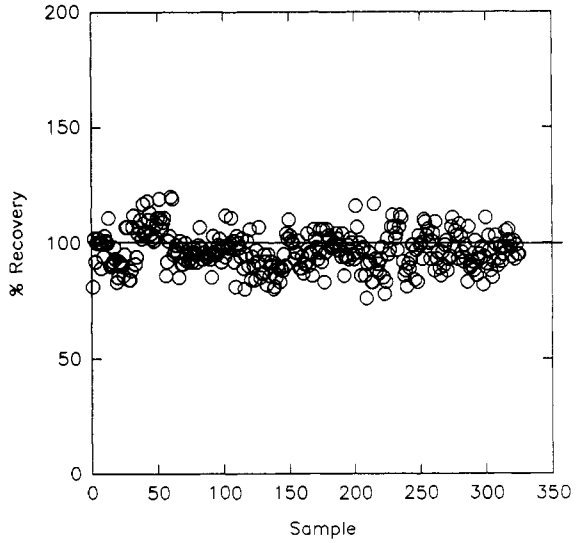


Figure 4. Sample spike recovery (percent) after fortification of 326 drinking, surface, and ground water samples with 1.5 ppb of metolachlor. Mean recovery was 96% (SD = 9%).

this ELISA. Table V summarizes the specificity data using a variety of chloroacetanilide analogues and their structures, as well as many nonstructurally related agricultural compounds. The differences in antibody binding to the chloroacetanilide compounds are pronounced given their structural similarities. Butachlor and alachlor differ only at the R_3 position from metolachlor but have substantially less reactivity in the ELISA (1.6% and 1.0%, respectively, at I_{50}). Acetochlor and metalaxyl, which have similar carbon/oxygen chain lengths at the R_3 position, have greater reactivity in the ELISA (13.0% and 15.2%, respectively, at I_{50}). These observations suggest that the antibody is most reactive to this alkyl side chain (R_3).

Drift. An optimized ELISA should exhibit little or no variation in sample values from the beginning to the end of a run due to timing. The time needed to complete sample and reagent additions depends upon the number of samples being assayed. The magnetic particle-based ELISA minimizes the drift effect because the immunological reaction is not initiated until the addition of the final reagent, the antibody-coupled magnetic particle, which can be added rapidly to all tubes with a repeating pipet. To evaluate drift, a water sample containing 3.0 ppb of metolachlor was assayed in 30 replicates or 40 tubes total including the standards and controls (approximately 2 s/tube). Figure 3 illustrates the insignificant drift of sample concentrations in this ELISA. The slope of the regression line (0.00069 ppb/s) suggests that for the 40-tube assay the analyte concentration difference from beginning to end would be minimal, 1.4% at the 3.0 ppb metolachlor level.

Sample Spike Recovery. Three hundred twenty-six drinking, surface, and ground water samples obtained from throughout the United States were fortified with a known concentration of metolachlor to evaluate sample matrix effects. The metolachlor concentration of the water samples ranged from less than 0.05 to 4.54 ppb before they were fortified. Figure 4 illustrates acceptable recovery when these samples were spiked with 1.5 ppb of metolachlor (range 76–120%), indicating that no sample matrix effects were present in this ELISA. The mean recovery of all samples was 96% (SD = 9%).

Interferences. The following compounds were added to blank and spiked metolachlor water samples at 250 ppm (parts per million, micrograms per milliliter) and evaluated for possible interference in the ELISA: nitrate, copper, nickel, thiosulfate, sulfite, sulfide, iron, calcium, and magnesium. Table VI summarizes that no interferences are present up to the tested levels of various common water components. In addition, sulfate to 10 000 ppm, NaCl to 1.0 M, silicates to 1000 ppm, and humic acid to 50 ppm exhibited no interferences. The concentrations of the compounds chosen are those that would most likely exceed levels found in environmental water samples (American Public Health Association, 1989).

Soil Fortification Study. Recovery of metolachlor in sandy loam soil spiked from 0.1 to 100 ppm was determined

Table VI. Effect of Possible Interfering Substances

compound	max concn of compound tested, ppm	0 ppb of metolachlor sample	1 ppb of metolachlor sample
nitrate	250	ND[a]	0.93
copper	250	ND	0.89
nickel	250	ND	0.98
thiosulfate	250	ND	0.93
sulfite	250	ND	1.01
sulfide	250	ND	1.02
sulfate	10 000	ND	0.87
iron	250	ND	0.89
magnesium	250	ND	0.85
calcium	250	ND	0.94
humic acid	50	ND	0.95
silicates	1000	ND	0.85
sodium chloride	1.0 M	ND	0.82

[a] ND, none detected (<0.05 ppb).

Table VII. Effect of Soil Extraction Time on Metolachlor Recovery[a]

metolachlor spike, ppm	% recovery at extraction time of				
	0.5 h	1.0 h	2.0 h	4.0 h	24.0 h
0.1	93.0	86.0	98.0	84.0	104.0
1.0	90.0	82.4	86.4	85.1	94.5
10.0	89.6	87.8	82.8	79.2	95.4
100.0	90.0	85.5	89.6	83.3	86.4
av	90.7	85.4	89.2	82.9	95.1

[a] Ten grams of metolachlor-spiked Sassafras sandy loam soil was extracted with 30 mL of methanol/water (75:25 v/v). Soil extracts were diluted in the zero standard and analyzed in duplicate in the ELISA. The unspiked soil assayed as less than the soil detection limit of 3 ppb.

Table VIII. Effect of Soil Type and Extraction Time on Recovery of Metolachlor[a]

soil texture	% recovery at extraction time of				
	0.5 h	1.0 h	2.0 h	4.0 h	24.0 h
sandy loam	90.0	87.8	82.8	79.2	94.5
silt loam	75.2	77.0	99.0	78.8	87.3
clay loam	82.4	78.8	86.9	81.5	80.1
muck	81.0	77.0	91.4	79.2	88.7
loam	87.3	93.6	99.5	96.3	97.7
av	83.2	92.8	91.9	83.0	90.0

[a] Percent recovery of soil spiked with 1000 ppb of metolachlor. Ten grams of each soil was extracted with 30 mL of methanol/water (75:25 v/v). Soil extracts were diluted 1:200 in the zero standard and analyzed in duplicate in the ELISA. All unspiked soil samples assayed as less than the soil detection limit of 3 ppb metolachlor.

Table IX. Assay Response with Metolachor Extracts Containing Methanol

methanol concn,[a] %	0 ppb of metolachlor sample	2.5 ppb of metolachor sample	% recovery[b]
0	ND[c]	2.29	92
0.2	ND	2.53	101
0.5	ND	2.58	103
1.0	ND	2.35	94
2.0	ND	2.44	98
5.0	ND	2.28	91
10.0	ND	1.97	79
25.0	ND	1.49	60
50.0	ND	1.05	42

[a] Methanol concentration v/v. [b] Acceptable range of metolachlor recovery was 100 ± 20%. [c] None detected (<0.05 ppb).

using extraction times from 30 min to 24 h (Table VII). The metolachlor recovery at the 24-h extraction was not substantially greater than that at the 30-min extraction (Tables VII and VIII), and the amount of metolachlor recovered was not dependent on the spike concentration. Recovery of metolachlor at 1000 ppb was also evaluated in various soils of known composition (Table VIII). The recoveries in the clay loam samples were lower than those in the sandy loam due to greater adsorption of the herbicide to the clay constituents (Somasundaram et al., 1991). Also, the higher cation-exchange capacity (CEC) of the clay loam increases the retention of metolachlor to soil, making the extraction less efficient (Bailey and White, 1964). The recovery of metolachlor is also affected by its breakdown in different soil types and binding to soil constituents. As with any analytical method, the extraction efficiency may vary with soil type and may extract substances that interfere with the analysis (Schneider and Hammock, 1992). The overall metolachlor soil spike recoveries using 30-min and 24-h extractions were 83% and 90%, respectively. All unspiked samples assayed as less than the soil detection limit of 3 ppb. Diluting the soil extracts in the zero standard eliminates the need for solvent evaporation and reduces any possible matrix or solvent interferences in the assayed sample, making it unnecessary to prepare standards containing methanol. At least a 1:20 dilution of the soil extract into the diluent is necessary to be below the 5% maximum methanol tolerance for this ELISA (Table IX). Using a 1:20 extract dilution provides a detection range of 3.0–300 ppb of metolachlor in soil. Samples containing greater than 300 ppb must be diluted further.

Conclusions. This work describes a magnetic particle-based ELISA for pesticide residues and its performance characteristics in the quantitation of metolachlor in water and soil. The assay results compare favorably to GC determinations, with no false negative values for the 58 samples analzyed using a cutoff of 0.1 ppb. The ELISA exhibits within- and between-assay precision of less than 10% and average accuracy of 102%, which provides consistent monitoring of environmental samples. The magnetic particle-based system is rapid and more sensitive than previously reported ELISAs (detection limits of 50 ppt to 2.0 ppb) and EPA Method 507 (detection limit of 0.75 ppb) for the determination of metolachlor. Detection of metolachlor in soil at the parts per billion level fulfills the sensitivity requirements for environmental monitoring. The specificity of the antibody employed allows for the detection of metolachlor in the presence of other pesticides and commonly found ground water and soil components. This magnetic particle-based ELISA for metolachlor provides results in less than 1 h without the problems of variability encountered with coated tubes, beads, and microtiter plates (e.g., coating variability and antibody leaching). The assay is also ideally suited for adaptation to on-site monitoring of metolachlor in water and soil.

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Received for review March 25, 1993. Accepted June 23, 1993.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts*, August 15, 1993.