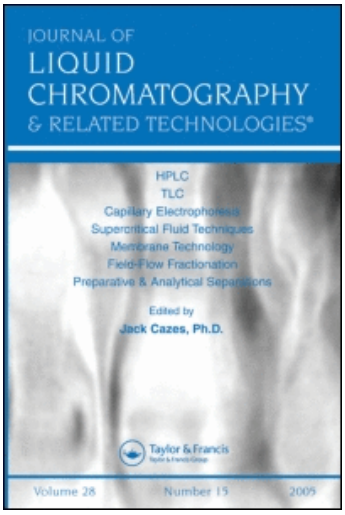

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High Performance Liquid Chromatographic Determination of Alachlor in Alginate-Based Formulations

J. W. Kuan[a]; A. B. Pepperman[a]
[a] Southern Regional Research Center, New Orleans, Louisiana

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF ALACHLOR IN ALGINATE-BASED FORMULATIONS

J.W. Kuan, A.B. Pepperman

Southern Regional Research Center
1100 Robert E. Lee Boulevard
New Orleans, Louisiana 70124

ABSTRACT

A reverse phase HPLC method for the quantitative determinations of alachlor [2-chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl)acetamide] and its degradation products in alginate-based alachlor controlled release formulations has been developed. Sonication and the addition of sodium ethylenediaminetetraacetate as a chelating agent were employed to disintegrate the formulation matrix. The disintegrated formulations were extracted with acetonitrile. The acetonitrile extracts were analyzed for alachlor and its degradation products by HPLC. A C_{18} column with a mobile phase of 65% acetonitrile and 35% water was used for separation. A UV detector set at 215 nm was selected for quantitation, and a photodiode array detector was used for confirmation. The developed method was used for the determinations of the percent active ingredient in freshly prepared and aged alachlor formulations made with and without oil (linseed, soybean, or corn oil). The method was also applied to monitor the rate of release and the fate of alachlor in controlled-release studies of alachlor formulations in water.

Alachlor degradation products, N-demethoxymethylalachlor and 2,6-diethylaniline (trace), were found in the formulations with oil but not in formulations without oil. The degradation of alachlor continued slowly as the formulations aged.

INTRODUCTION

Alachlor [2-chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl)acetamide] is a preemergence herbicide used for weed control in agronomic corps such as corn, soybean, peanut, rice, and potato. It acts as a herbicide by inhibiting protein synthesis and root elongation in susceptible plants.[1] Alachlor is one of the most widely used herbicides in the United States, with an annual application rate of about 85 million pounds.[2] Through its regular use, improper disposal, and accidental spills, alachlor was one of the pesticide contaminants most often found in the groundwater and wells.[3-5]

Alachlor is available commercially as an emulsifiable concentrate, clay granules, and microcapsules. The alachlor in commercial formulations is commonly assayed by organic solvent extraction followed by gas chromatography (GC). Typically, solvent or solid phase extraction is used to extract alachlor in water and soil, and the alachlor is quantitatively determined by GC or high performance liquid chromatography (HPLC).[6,7] GC/MS or LC/MS were usually employed for the quantitation, identification, and confirmation of alachlor and its metabolites.[8-10] Recently, due in part to the commercial availability of several alachlor enzyme-linked immunosorbent assay (ELISA) kits, this technique has been accepted and used for the analysis of alachlor in environmental water and food samples.[11,12] Despite drawbacks such as occasional false positives[13] and low cross-reactivity with metabolites, ELISA is still a good tool for the screening of environmental samples because it is rapid, simple to operate, inexpensive, and portable.

Herbicide controlled release formulations (CRFs) have potential benefits of enhanced weed control and corp protection, improved safety of handling, reduced losses to volatilization and leaching.[14] Connick et al.,[15] and Pepperman and Kuan[16] have demonstrated that incorporated clay and oil into herbicide-alginate formulations retards the release of herbicides. We have incorporated clay and oil into alginate-based alachlor controlled release formulations in our continuing search for a CRF which exhibits the best efficacy and has the least adverse impact on the environment.

After curing, our alginate-based formulations that incorporated oil and other adjuvants such as clay formed rather rigid beads which were not readily dissociated. Traditional methods involving simple organic solvent extraction did not recover all of the alachlor in the formulations. In a previous report, an HPLC method for the determination of percent active ingredient (% a.i.) in metribuzin-alginate formulations was described.[16] In this method, the % a.i. was obtained indirectly by the analysis of the formulation filtrates. The method was satisfactory for the determination of % a.i. in freshly prepared formulations, but could not be employed for the residual analysis of herbicide formulations that had been applied to the soil or partially extracted with water. The objective of the present study was to develop a direct method for the analysis of alachlor and its degradation products in the alginate-based formulations to monitor the fate of alachlor in the formulations at various stages of usage and release.

MATERIALS AND METHODS

Chemicals

Alachlor [technical grade, 94% pure] and N-demethoxymethylalachlor [2-chloro-N-(2,6-diethylphenyl)acetamide, 99.7% pure] were supplied by Monsanto Company, St. Louis, MO.[17] Technical grade alachlor was recrystallized from an ethyl acetate-hexanes mixture at 4 °C to a purity of about 99% with a mp of 40.5-41.5 °C. 2,6-Diethylaniline [99.5+% pure] was purchased from Aldrich Chemical Co., St. Louis, MO. Sodium alginate, Kelgin MV, was provided by Kelco, Division of Merck and Company, San Diego, CA, and kaolin clay was supplied by Thiele Kaolin Company, Wren, GA. Tween 20 [polyoxyethylenesorbitan monolaurate] and tetrasodium ethylenediaminetetraacetate dihydrate (sodium EDTA) were purchased from Sigma Chemical Co., St. Louis, MO. Raw linseed oil was purchased from a local hardware store, and soybean oil and corn oil were obtained from a local supermarket. Water purified with a NANOpure Ultrapure Water System (Barnstead/Thermolyne Corp, Dubuque, Iowa) through a 0.2 um final filter was used throughout the study. All other chemicals were either HPLC grade or reagent grade.

Apparatus

All HPLC analyses were performed on a Waters HPLC system (Waters Chromatographic Division, Millipore Corp., Milford, MA.). The system

consisted of a model 712 WISP auto-sampler, a model 600E Powerline multisolvent delivery system, a data system with model 991 Photodiode Array Detector V.6.22A Powerline software, a model 5200 printer/plotter, and two detectors: a model 486 IEEE tunable absorbance detector with analytical flow cell and a model 991 photodiode array detector (PDA). The detectors were installed parallel to each other and an automated switching valve was used to switch the direction of the flow to the detector. The HPLC was fitted with a Waters Nova-Pak C_{18} stainless steel column, 300 mm long x 3.9 mm i.d., 4 micron particle size. The mobile phase was 65% acetonitrile and 35% water at a flow rate of 0.7 mL/min. The solvents were sparged with helium at the flow rate of 30 mL/min. For quantitation, the model 486 detector was selected and was set at 215 nm, 1 AU full scale. Injection volume was 20 ul, each sample was injected twice, and the run time was 14 min. The PDA detector was used to acquire UV spectra and for confirmation of alachlor and the degradation products

Sonication was performed in an ultrasonic cleaner with a tank capacity of 3 L. and the dimensions of 3.75" H x 9.5" L x 5.5" W, model # SC-100H (Ultrasonic Industries, Clearwater, FL).

METHODS

Preparation of Alachlor Formulations

A typical alachlor formulation was prepared by first dissolving alachlor (1%) in methanol (5%). (All percentages herein are by weight, w/w). The oil (0-10%) and Tween 20 (0.5%) were added and the mixture was mixed with an overhead stirrer. While stirring the mixture at 200-250 rpm, the water (72.5-82.5%) was added very slowly to the mixture. Caution was taken not to add water too rapidly at the beginning to prevent precipitation and aggregation of alachlor. The clay (10%) was added, and the mixture was stirred at 350 rpm for 10 min. The sodium alginate (1%) was then added, and the mixture was stirred at 450 rpm for 1 hr. or until a homogeneous slurry was obtained. The slurry was added dropwise through Pasteur pipets into 0.25 M calcium chloride (twice the weight of the slurry) to form calcium alginate gel beads. The beads were weighed and allowed to harden for about 5 min. The liquid was removed by vacuum filtration through a coarse-frit Buchner funnel. The beads were rinsed with water and drained. The wet beads were spread on aluminum foil to air-dry at room temperature. Although the beads were essentially dry in 24 hours, they were dried for two weeks to allow for formation of a polymeric film

on the surface of the beads.[16] The formulation filtrate and its rinsate were combined and saved for the HPLC determination of % a.i. The % a.i. obtained from the analysis of the formulation filtrate was considered an "indirect method" for the determination of % a.i. in the formulation.

The color of the dried beads was off-white for formulations without oil and beige to light yellow-brown for oil-containing formulations. They were all spherically shaped with a diameter ranging from 1.5 mm (0% oil) to 1.7 mm (10% oil).

Preparation of Standards

Stock standards of alachlor, N-demethoxymethylalachlor, and 2,6-diethylaniline, 1.00 mg/mL, were prepared in HPLC grade acetonitrile. Working standard solutions of these compounds, 1-100 ppm, were prepared by diluting the stock standards with mobile phase (65:35 = acetonitrile:water). Working standard solutions were used for the construction of calibration curves. Standard alachlor solution was run routinely as a control.

Sample Preparation for HPLC Analysis

A formulation sample containing less than 10 mg of alachlor, ca. 0.1-0.2 g, was accurately weighed in an 8-dram (25x95 mm) vial with a Teflon-lined screw cap. Four mL of 0.05 g/mL (0.12 M) sodium EDTA was added to the sample. The vial was capped and mixed with a vortex mixer for 30 sec before it was placed in an ultrasonic bath. The sample mixture was sonicated for 10 min, followed by 30-60 sec of mixing with a vortex mixer. The total sonication time was 20-30 min or until the formulation was completely disintegrated. The sample was extracted with 8 mL of acetonitrile, and was mixed with a vortex mixer for 1-2 min. The extract was allowed to stand 10-20 min or until a clearly-defined phase separation occurred and the precipitate settled. The acetonitrile (upper) layer was then transferred to a clean vial and diluted to 15 mL with acetonitrile. A 1.0 mL aliquot of the diluted extract was further diluted with 3 mL of acetonitrile to precipitate more alginate. It was mixed with a vortex mixer for 30 sec then filtered through a 0.22 um Millex-GV filter (Millipore Corp., Bedford, MA). Before injecting into the HPLC, 2 mL of filtrate was mixed with 1.0 mL of water.

The combined formulation filtrate-rinsate was diluted to a known volume

with water. For HPLC analysis, 1.05 mL of diluted filtrate was mixed with 1.95 mL of acetonitrile. The mixture was filtered through a Millex-GV filter before injecting into the HPLC. The extract from the controlled release studies of alachlor formulations was prepared in the same manner as the filtrate.

RESULTS AND DISCUSSION

Factors Affecting the Analysis

1) Disintegration of alginate beads with EDTA

Water soluble sodium alginate was used to form a slurry with the herbicide and the other ingredients. The mixture when dropped into calcium chloride solution formed water insoluble calcium alginate beads. Calcium alginate can be rendered soluble by the addition of a ligand which will displace alginate and produce a water soluble calcium chelate. Sodium salts of citric acid, phosphoric acids such as tripolyphosphate and hexametaphosphate, and EDTA have been the most commonly used chelating agents to sequester the calcium in alginates. The stability constants (formation constants), log K, of these calcium chelates are: citrate 3.5; tripolyphosphate 5.2; hexametaphosphate 6.0; EDTA 10.7.[18] Because alachlor is not very stable in acidic solutions and EDTA has the strongest chelating power (highest formation constant), EDTA was selected as the chelating agent for the disintegation of alachlor-alginate beads. At pH 10-11, almost all the EDTA is non-protonated providing free EDTA ions for chelation with calcium. Sodium EDTA solutions, 0.05 g/mL (0.12 M) and 0.1 g/mL (0.24 M) which had pH of 10.7 and 10.9, respectively, were tested on an alachlor-oil-alginate formulation. The % a.i. obtained from using 0.1 g/mL was about 6% lower than the one with 0.05 g/mL. An EDTA concentration lower than 0.05 g/mL can be used for disintegration but it may require a longer sonication time. If an EDTA concentration higher than 0.1 g/mL (pH>11) is used, sodium alginate, specifically Kelgin MV, can depolymerize due to hydrolysis. The depolymerization produces many water soluble small molecules which may interfere with the alachlor analysis.

2) Sonication time

Sonication was employed to disintegrate the beads. The time required for complete disintegration of the beads depended on the oil content and the age of the beads. Formulations with no oil took less time than formulations with oil.

Also, aged formulations required a longer sonication time than fresh ones. It took about 20 min for the non-oil formulation and 30-80 min for oil formulations, depending on the percent oil used in the formulation and age of formulation, to disintegrate the beads completely. It is recommended that a brief pause be taken after each 10 min of sonication. The samples are removed from the sonication bath and swirled at high speed for 30-60 sec with a vortex mixer. Interrupted sonication prevents sample overheating which results in alachlor decomposition. Swirling of the samples aids in speeding up the disintegration of the beads. Crushing the beads prior to the sonication shortened the sonication time for the complete disintegration of non-oil formulations, but was not effective for the oil-containing formulations. Oil-containing beads, especially those with 8-10% oil, tended to flatten and stack together rather than crumble to small particles when crushed. Although it has been reported[19] that alachlor in water decomposed after lengthy sonication, no significant decomposition was found in our samples due to sonication.

3) Solvent extraction

Acetonitrile and ethyl acetate were evaluated as potential solvents for the extraction of alachlor from the EDTA-formulation mixture. Alachlor is very soluble and readily extractable in either solvent. However, with the use of ethyl acetate, multiple extractions and evaporation of the solvent from the extract prior to analysis were necessary. Since acetonitrile was used in the mobile phase, no drying of the extract was required when acetonitrile was used for sample extraction. Three different acetonitrile extraction procedures were tested on the same formulation: (1) once with 8 mL, (2) twice with 4 mL each, (3) first with 6 mL, second with 4 mL. The acetonitrile extracts were removed, combined (in 2 & 3), and diluted to 15 mL with acetonitrile. Alachlor was determined as described above. The % a.i. obtained for all three extraction procedures were the same. Hence, acetonitrile was chosen as the extraction solvent and each sample was extracted only once with 8 mL of acetonitrile.

Several problems were noted when acetonitrile was investigated as the extraction solvent. When the percentage of acetonitrile in the extraction mixture was lower than 50%, the extract was cloudy and it took a long time for the precipitates to settle. When the percentage was higher than 75%, the extract was very cloudy, and the precipitate became pasty and adhered to the wall of the vial when mixing, therefore, the phase separation was hard to attain and observe. Optimum extraction conditions were obtained when the percentage of acetonitrile in the extraction mixture ranged from 60 to 70%, e.g. acetonitrile:water = 3:2 to 2:1.

4) Absorption of alachlor on Millex-GV filters

During the development of the HPLC method for the analysis of alachlor, some absorption of alachlor on the Millex-GV filters was observed. To quantify the absorption, two experiments were conducted. In the first experiment, two sets of identical alachlor standards, 3 mL each, concentration: 10, 25, 100 ppm, were prepared in the purified water. One set of the standards was filtered through 0.22um Millex-GV filters, and the other set of standards was not filtered before the injections. The alachlor found in the filtered standards was only 77-79% of that found in the non-filtered ones. The percent absorption was similar regardless of the alachlor concentrations for the range tested (10-100 ppm). In the second experiment, standards were prepared directly in the mobile phase (acetonitrile:water = 65:35). No significant absorption of alachlor by the filters was observed.

Recovery Study

To verify the accuracy of the developed HPLC method for the determination of % a.i. in the alachlor formulations, a recovery study was conducted on an alachlor-linseed oil-alginate-clay formulation. The samples, in duplicate, were spiked with 0, 1, 3, 5 mg of alachlor. The average percent recovery of alachlor ranged from 96.5 to 101.3%.

Direct vs. Indirect Method for the Determination of % a.i. in the Formulations

Before developing the current method, designated as "direct method", the % a.i. in the alachlor formulation was determined by the HPLC analysis of alachlor in the formulation filtrate. The calculation of % a.i. in the formulation was based on the assumption that whatever was not found in the filtrate should be in the dried formulation. The % a.i. obtained by this method was considered an "indirect method". It was calculated as follows:

$$\% \text{ a.i.} = (A*W/T - F)*100/D$$

where: A= wt. of alachlor used in the preparation of formulation
W= wt. of wet beads (droppings in calcium chloride)
T= wt. of total ingredients
F= wt. of alachlor found in the filtrate
D= wt. of the dried formulation

Table 1

Direct vs. Indirect Method for the Determination of % Active Ingredient (a.i.) in the Formulations

Formulation	Direct, % a.i.		Indirect % a.i.**	Percent of direct/indirect	
#: oil type	ALC only	ALC+DMA		ALC only	ALC+DMA
A: no oil	6.63	6.63	7.01	94.6	94.6
B: linseed oil	4.67	4.84	5.27	88.6	91.8
C: soybean oil	4.63	4.83	5.22	88.7	92.5
D: corn oil	4.60	4.78	5.26	87.5	90.9

Formulations A,B,C,D all contained 1% alachlor, 10% clay, 1% alginate.
Formulation A= no oil.
Formulations B,C,D= 4% oil.
ALC, alachlor; DMA, N-demethoxymethylalachlor.
*Average of eight determinations.
**Average of four determinations.

A comparison study of these two methods was conducted on four formulations by analyzing both their filtrates (indirect method) and the dried formulations (direct method). These four formulations all had 1% alachlor, 10% clay, and 1% alginate, but A contained no oil and B, C, D contained 4% linseed oil, soybean oil, and corn oil, respectively. The average % a.i. obtained from four determinations by the indirect method and eight determinations by the direct method are shown in Table 1. The % a.i. determined by the direct method was consistently lower than by the indirect method. No significant alachlor decomposition products were found in either the filtrates or in extracts of the non-oil formulation. However, two alachlor degradation products, identified as N-demethoxymethylalachlor (DMA) and 2,6-diethylaniline (DEA) were found in oil-containing formulation extracts.

For the non-oil formulation (A), the % a.i. obtained by the direct method was about 95% of the value obtained by the indirect method, while the ratio of the two methods, direct/indirect, was 87-89% for the formulations containing oil (B,C,D). The relatively lower values of % a.i. by the direct method for formulations containing oil were partially due to the decomposition of alachlor

which was not accounted for. If the concentrations of DMA found in the formulations were included in the calculation, the ratio of direct/indirect was about 91-93%. DEA was not included in the calculations because only trace amounts of it were found. In the indirect method, small losses of alachlor on aluminum foil and to volatilization during the drying/curing stage (usually two weeks to allow linseed oil to cure) could not be accounted for in the calculation of % a.i. Judging from the recovery study and due to the possible losses of a.i. which can occur in the indirect method, the % a.i. obtained from the direct method should more accurately reflect the actual amount of the herbicide present in the beads.

Degradation of Alachlor in the Formulations

The presence of DMA and trace amounts of DEA in the formulations were identified and confirmed by comparison with the authentic compounds run under the same HPLC conditions. The retention time (RT) and response factor (RF) of DMA, DEA, and alachlor (ALC) were determined by the use of standards under the same conditions (see HPLC method). The RT and their relative RT were: DMA:ALC:DEA=5.39:9.40:10.00=0.5734:1.0000:1.0638. The relative response factors (RRFs) were DMA:ALC:DEA= 0.9689:1.0000:0.9575. The concentrations of DMA and DEA were calculated, based on their RRFs.

DMA is a major degradation product of alachlor in soil and water.[20,21] In the formulations we prepared and stored at room temperature, DMA was found only when oil was present and DMA concentration increased as the formulations aged. About 3% of alachlor in the formulations degraded to DMA two weeks after the preparation. The conversion continued slowly to about 5% in two months, and increased to 6-7% in 7 months. Formulations containing linseed oil had slightly slower degradation rates than formulations containing soybean oil or corn oil. A two-months-old formulation with linseed oil converted 4.4% alachlor to DMA, while soybean oil (5.5%) and corn oil (5.7%) formulations were higher in DMA. Since no significant alachlor degradation products were found in filtrates and non-oil formulations but were found in all formulations containing linseed, soybean, and corn oil, it is reasonable to believe that the decomposition of alachlor was caused by the interaction between alachlor and the oil in the formulations. The degradation of alachlor is affected by temperature, moisture, and surrounding environments. DMA was reported as a major degradation product from the hydrolysis of alachlor in acidic medium, and as one of the alachlor metabolites produced by soil

fungi.[22,23] DMA found in the oil-containing formulations probably resulted from the interaction of alachlor with free fatty acids in the oil or with products from the decomposition and/or oxidation of the oil. Free fatty acids have been reported as impurities in alginates from different sources.[24] Since there was no obvious degradation of alachlor in the non-oil formulation, it appeared that possible impurities in the alginate had no significant effect on the degradation. Alginates are hydrophilic polysaccharides which readily absorb moisture from the atmosphere. Linseed oil, being a drying oil, polymerized and formed a hard film on the surface of the beads.[16] The hard film on the beads may act as a barrier to protect the beads from oxidation and moisture, thus slowing the rate of alachlor decomposition. Soybean oil is a semi-drying, and corn oil a non-drying oil, hence, the alachlor decomposition rates were higher in formulations C and D than in B.

Determination of Alachlor and the Degradation Products in Partially Extracted Formulations

Controlled release studies were performed on some of the alachlor formulations in either static water and/or agitated (by shaking) water to determine the herbicide release properties. In the static water test (see ref. 25 for method), the release of alachlor from the formulations usually reached an equilibrium in a few days and no further increase of concentration was observed. To investigate the fate of the unreleased alachlor, an attempt was made to analyze the residual alachlor in those partially extracted formulations. Both original (non-extracted) and partially extracted formulations were analyzed by the developed method reported herein. Besides alachlor, DMA was the only other detectable compound found in the formulations. For partially extracted formulations, the total alachlor concentration was calculated by the addition of alachlor and DMA (converted to alachlor equivalent concentration) found in the water extracts and in the formulations.

The results of the controlled release study (CRS) in static water and the residue analysis of four representative alachlor formulations, in duplicate, are shown in Table 2. In comparison with the original formulations, the average overall percent recovery of alachlor from water extracts and partially extracted formulations were 96.7 to 99.3%. DMA was found in the extracts and the residues of the oil-containing formulations but was not found in non-oil formulation. Overall percent recovery increased slightly from 97.0-99.3% to 97.4-99.7% for oil-containing formulations if DMA was included in the calculations.

Table 2

Determination of Alachlor and Its Degradation Products in Partially Extracted Formulations

Formulation	Orig. Formulation		Amt. in Extract*		Resid. Formulation		Overall % Recovery	
#: Oil Type	Av. % ALC	Av. % ALC+DMA	ALC mg	ALC+DMA mg	% ALC	% ALC+DMA	ALC	ALC+DMA
E: no oil	6.92	6.92	67.66	67.66	4.87	4.87	94.5	94.5
			67.80	67.80	5.20	5.20	98.8	98.8
F: linseed oil	4.67	4.91	43.43	51.37	3.91	3.97	100.0	100.3
			43.94	51.94	3.82	3.88	98.6	99.0
G: soybean oil	4.67	4.97	41.97	52.14	3.86	3.93	97.1	97.6
			48.83	59.49	3.76	3.81	98.8	99.1
H: corn oil	4.70	5.01	46.97	57.94	3.71	3.76	96.4	96.8
			50.37	61.57	3.68	3.73	97.6	98.0

Formulations E,F,G,H all contained 1% alachlor, 10% clay, 1% alginate.
Formulation E= no oil.
Formulations F,G,H= 4% oil.
ALC, alachlor; DMA, N-demethoxymethylalachlor.
*Amount found in the extracts after formulations had been in static water for 96 hrs.

CONCLUSION

The developed reverse phase HPLC method allowed quantitation and identification of alachlor and its degradation products in alginate-based alachlor formulations. The method was also used to obtain the alachlor release profiles and the degradation rates in the controlled release studies of alachlor formulations in water. The major degradation product found in the alachlor-oil-alginate formulations was N-demethoxymethylalachlor. Either trace amounts or none of 2,6-diethylaniline were found in those formulations. The extent of alachlor degradation depended on the age and the oil type in the formulations (linseed< soybean < corn oil). The method as developed can be used to monitor the environmental fate of alginate-clay-oil containing alachlor formulations in the field or greenhouse. Some modification of the extraction method for soil samples may be necessary.

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17. This article reports the results of research only. Mention of a pesticide in this article does not constitute a recommendation for use by the U.S. Department of Agriculture nor does it imply registration under FIFRA as amended. The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

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