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ATRAZINE CONTAMINATION OF WATER SUPPLIES FROM AGRICULTURAL USE IN NEBRASKA

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ATRAZINE CONTAMINATION OF WATER SUPPLIES FROM AGRICULTURAL USE IN NEBRASKA

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TITLE

Atrazine Contamination of Water Supplies

from Agricultural Use in Nebraska

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A Technique to Analyze Soil or Water For Multiple Herbicide Residues

Abstract A technique is described which uses soxhlet extraction with methanol for soil, and carbon tetrachloride extraction for water in the simultaneous analysis of atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine], simazine [2-chloro-4,6-bis(ethylamino)-s-triazine], cyanazine [2-((4-chloro-6-(ethylamino)-s-triazin-2-yl) amino)-2-methylpropionitrile], alachlor [2-chloro-2',6' -diethyl-N-(methoxymethyl)acetanilide], propachlor [2-chloro -N-isopropylacetanilide], and metolachlor [2-chloro-N -(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) -acetamide]. Herbicide residues were separated from coextractants by high pressure liquid chromatography (HPLC). Qualitative and quantitative determinations were accomplished using a gas-liquid chromatograph (GLC) equipped with a nitrogen-phosphorus specific thermionic detector. Due to the efficiency of HPLC clean-up and selectivity of the nitrogen-phosphorous detector, all six herbicides could be separated and accurately quantified at minimum concentrations of 10 ppb (soil) or 200 ppt (water).

Additional index words: high pressure liquid chromatography, gas-liquid chromatography, solvent extraction, soxhlet extraction.

INTRODUCTION

Recent discovery of atrazine as a groundwater contaminant under irrigated farmland in Nebraska has spurred the need for analytical techniques in which large numbers of soil or water samples can be rapidly analyzed by similiar procedures for trace levels of herbicide(s) residue. Existing techniques for extracting herbicides from water require that the water be allowed to percolate through a column packed with resins which retain organics dissolved in the water (3). Analysis for trace levels of herbicides (< 1 ppm) requires extraction from several liters in order to cumulate sufficient amounts for identification and quantification. Consequently extraction by resin filled columns is prohibitively slow. Direct solvent extraction should provide a more rapid means for extracting small amounts of herbicide from large volumes of water.

Separation of herbicides from coextractants (clean-up) has traditionally been accomplished by a separate chromatographic separation prior to final qualification and quantification by gas-liquid chromatography (4,6). Clean-up using high pressure liquid chromatogrphy should be adaptable to either soil or water extracts.

Soxhlet extraction, which allows for freshly distilled solvent to percolate repeatedly through the soil sample has

been shown to be an effective means of extracting herbicide(s) from soil (5).

The objective of this research was to (a) test the practicality of solvent extraction of water, (b) evaluate HPLC clean-up of extracts from either water or soil so as to produce a preparation suitable for final analysis by GLC, and (c) evaluate the aforementioned analytical technique for the simultaneous analysis of six herbicides.

MATERIALS AND METHODS

Extraction of water. A suitable extraction solvent must have a high solubility towards nonpolar organic molecules and be relatively immisicible in water. It is preferable that the solvent be heavier than water for ease of use. Carbon tetrachloride, which has a specific gravity of 1.63 g/ml and is practically immiscible in water (2), fits these criteria. Tap water (10 L) was spiked at 200 ppt with one of the herbicides (^{14}C-labeled). Labeled and nonlabeled herbicide were combined to yield an approximate specific activity of 40,000 dpm/ug. Water was subsequently extracted three times with 500 ml of glass-distilled carbon tetrachloride. Cumulative extract (1.5 L) was reduced in volume by rotary vacuum distillation (59 C) and taken to dryness in a scintillation vial with a gentle stream of N gas and water bath at 59 C. The maximum volume of solvent which could be conveniently handled in the vacuum

distillation apparatus was 1.5 L. Thus, 500 ml was the largest volume used in each extraction. Duplicate aliqouts of the spiking solution were placed directly into scintillation vials so as to determine the efficiency of recovery. Extraction was repeated five times for each herbicide and the recovery averaged. An additional five water samples were spiked with a composite of all six herbicides (200 ppt, nonlabeled) and the average recovery and standard deviation determined for the complete procedure (extraction, HPLC clean-up, and GLC analysis).

Extraction was performed in a 12 L separatory funnel mounted in a wooden frame with an electric mixer suspended overhead such that the blades of the mixer reached into the center of funnel. Preliminary trials with ^{14}C-labeled atrazine indicated that equilibrium between the amount of atrazine in the water and carbon tetrachloride phases was achieved in less than a minute of mixing. Five minutes of mixing were used for routine extractions to assure complete partitioning of all herbicides.

Extraction of soil. Methanol was used in the solvent extraction of soil. This solvent was chosen because of its efficiency (5), low cost, and limited health hazard. Herbicide extraction varies with the soil type, therefore two different soils were used in extraction and analysis: Dix sandy loam (Torriorthentic haplustoll) and Sharpsburg

silty clay loam (Typic Argiudoll) Textural analysis for the Dix sandy loam was sand 72%, silt 17%, clay 11%, with a pH of 5.8, and 1.7% organic matter. Textural analysis for the Sharpsburg silty clay loam was sand 11%, silt 58%, clay 31%, with a pH of 5.5, and 4.2% organic matter. The soil was air-dried and passed through a 10 mesh screen. Fifty grams of each soil were spiked to 1 ppb of each of the ^{14}C-labeled herbicides and placed in cellulose extraction thimbles. Soxhlet extractions were repeated five times for each soil and herbicide combination. Solvent was maintained at a 63 to 66 C (low boil), therefore recycling every 10 to 15 min. Consequently, each sample was leached 12 to 18 times during the 3 h extraction period. Extracts were reduced in volume, taken to dryness in scintillation vials, and counted using procedures identical to those described for water extraction. Duplicate aliqouts of the herbicide spiking solutions were added directly to scintillation vials to determine the efficiency of the extraction process.

Clean up of water or soil extract. A Hewlett-Packard 1084B liquid chromatograph equipped with a a C-8 reverse phase column was used for clean-up. The entire extract from either soil or water was dissolved in 200 ul of methanol and injected. The effluent was collected during the time interval during which the herbicides of interest would be elluting from the column. The ellution times were determined by injecting a standard solution containing all 6

herbicides. Previous trials with ^{14}C-labeled atrazine indicated that the position and width of the herbicide peak was not altered by contaminates in the sample. It was also found that collecting the effluent between 0.2 min prior to and 0.2 min after the end of the peak gave virtually complete recovery (97 ± 5%) of the herbicide of interest. However, in normal samples the amount of coextractants was so great as compared with the amount of herbicide that no individual herbicide peaks could be detected.

The methanol:water ratio was held at 52:48 during the first 10 min of the run; after which time methanol was increased to 56%. Under these conditions atrazine, cyanazine, simazine, and propachlor elluted between 3.2 and 7.8 min; alachlor and metoalchlor between 12.5 and 15.4 min (Fig. 1). Effluents were collected in a 50 ml separatory funnel, extracted three times with 5 ml of carbon tetrachloride, and the combined carbon tetrachloride extract was taken to dryness under a stream of N gas.

Quanitative and qualitative analysis. Residues were redissolved in 2 ml hexane for analysis with a Hewlett Packard 5840B gas-liquid chromatograph equipped with a 1.6 m by 4.0 mm glass column packed with 3% OV-25 on 100/120 mesh chromosorb WHP. Column oven temperature maintained at 210 C, injection port at 215 C, and the detector at 300 C. Velocity of the nitrogen carrier gas was 20 ml/min. Both a nitrogen-

phosphorus specific flame ionization thermionic detector and an electron capture detector were used. P-5 carrier gas (95% argon and 5% methane) was used with the electron capture detector. Peak area was determined by a dedicated micro-processor.

RESULTS AND DISCUSSION

Extraction of water. Solvent extraction of water resulted in recoveries greater than 50% for atrazine, simazine, cyanozine, and alachlor (Table 1). Recovery of propachlor and metoachlor were less than 50%, due to their higher water solubilites. Solvent extraction appears to be limited to compounds with a strong preferential solubility in organic solvents unless a much greater volume of extraction solvent were to be used. Water extracts appeared very clean, as no particulate matter was visible. Extract residues were redissolved in 0.2 ml of methanol prior to injection into the HPLC.

Extraction of soil. Soxhlet extraction of soil resulted in high recoveries of the herbicides tested (Table 2,3). With both soils, small amounts of particulate materials entered into the extract solvent. While this material did not affect herbicide recovery as indicated by the percent of radioactivity recovered, this material had to be removed before the preparation could be injected into the HPLC. The amount of this material was so great in the Sharpsburg silty

clay loam extract that centrifugation was required in order to obtain an acceptable preparation for injection into the HPLC. Extracts from the Dix sandy loam and the Sharpsburg silty clay loam (cleaned by centrifugation) left a deposit of fine, insoluble particulate matter on the walls of the test tube used for drying. Herbicide adherence to this fine material probably represents the main loss of recovery between the extract and final quantification by GlC.

Clean-up of water or soil extracts. HPLC proved to be a very effective and rapid means of cleanup evidenced by the almost complete lack of extraneous peaks in the final chromatogram (Figure 2) when the nitrogen-phosphorus detector was used, and by the precision of recovery for the herbicides tested (Table 2,3). The methanol:water ratio could be varied continually during HPLC clean-up, thereby changing the herbicide peak position. Delaying the first peak allows for more contaminates to be elluted from the column prior to collection of the herbicide containing fraction. Manipulation of the methanol:water ratio resulted in the clustering of the herbicide peaks which minimized effluent volume.

Quanitative and qualitative analysis. The electon-capture detector was overly sensitive and produced enough spurious peaks to prevent adequate resolution of the herbicides. The nitrogen-phosphorus detector was more selective and

satisfactory resolution was achieved (Figure. 2). Separation of atrazine and simazine was particularly difficult and separation remained incomplete with the column used.

Loss of recovery between the initial extract and the final GLC determination averaged about 3% for all herbicides in the water analysis. This probably reflects loss that occurred during transferral of the preparation between glassware and during extraction and clean-up. The comparable loss from the soil preparations was much greater, approximating 25 to 30%. This probably was due to herbicide adherence to the fine soil particles which had to be removed from the extract. Reversal of this herbicde bonding should greatly increase recovery.

Preliminary results (data not reported) indicated that recovery of individual herbicides was consistent with concentrations less than what was used in this experiment. Therefore the minimal level of detection is limited only by the detection and integration limits of the GLC system. The success of this technique for simultaneous analysis of six herbicides was largely due to the efficiency of HPLC clean-up and the extreme selectivity of the nitrogen-phosphorus detector.

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Table 1. Water solubility, extraction efficiency, and final recovery from water for six herbicides.

Herbicide	Water solubility[a]	Extraction efficiency[b]	Recovery and precision of the complete procedure[c]	
	(ppm)	------(%)-----		σ
Atrazine	33	67	64	5
Simazine	4	55	52	5
Cyanazine	160	58	54	5
Alachlor	242	54	51	5
Propachlor	580	45	41	5
Metolachlor	532	29	26	4

[a]Mullison, W. R. (Chairman) 1979. Herbicide handbook. Weed Sci. Soc. Am., Champaign, IL. 497 pp.

[b]Five replications were used for each herbicide. Each trial consisted of 10 L of water which had been spiked with ^{14}C-labeled herbicide (200 ppt) and extracted three times with 500 ml carbon tetrachloride. Recovery was determined by liquid scintillation counting of the extract.

[c]Five replications of 10 L of water which had been spiked with a composite of all six herbicides (200 ppt each, nonlabeled). Each trial consisted of extraction, HPLC clean-up, and GLC quantification.

Table 2. Efficiency of extraction from a Dix sandy loam and the entire analytical procedure for six herbicides.

Herbicide	Extraction efficiency[a]	Recovery and precision of the complete procedure[b]	
	(%)		6
Atrazine	86	61	7
Simazine	81	55	7
Cyanazine	89	62	6
Alachlor	76	53	7
Propachlor	88	64	7
Metolachlor	94	65	7

[a] Five replications were used for each herbicide. Each trial consisted of 50 g of soil which had been spiked with ^{14}C-labeled herbicide (50 ppb) and extracted 3 h with methanol. Recovery was determined by liquid scintillation counting of the extracted residue.

[b] Five replications of 50 g soil which had been spiked with a composite of six herbicides (50 ppb each, nonlabeled). Each trial consisted of soxhlet extraction, HPLC clean-up, and GLC analysis.

Table 3. Efficiency of extraction from a Sharpsburg silty clay loam and the entire analytical procedure for six herbicides.

Herbicide	Extraction efficiency[a]	Recovery and precision of the complete procedure[b]	
	-------(%)-------		6
Atrazine	78	63	6
Simazine	82	58	7
Cyanazine	87	60	7
Alachlor	71	55	7
Propachlor	69	66	7
Metolachlor	89	69	7

[a]Five replications were used for each herbicide. Each trial consisted of 50 g, spiked with ^{14}C-labeled herbicide (50 ppb) and soxhlet extracted 3 h with methanol. Particulate matter in the extract was removed by 3 centrifugations and resuspension in fresh methanol. Recovery was determined by liquid scintillation counting of the extracted residue.

[b]Five replications of 50 g of soil which had been spiked with a composite of six herbicides (50 ppb each, nonlabeled). Each trial consisted of extraction, separation of particulate matter by centrifugation, HPLC clean-up, and GLC quantification.

Figure 1. HPLC chromatograph of the elution pattern of six herbicides and the collection period required for recovery. A C-8 reverse phase column was used. The methanol:water ratio was held at 52:48 during the first 10 min of the run, after which time methanol was increased to 56%. Velocity of liquid phase was 2 ml/min and a 540 nm ultra-violet detector was used.

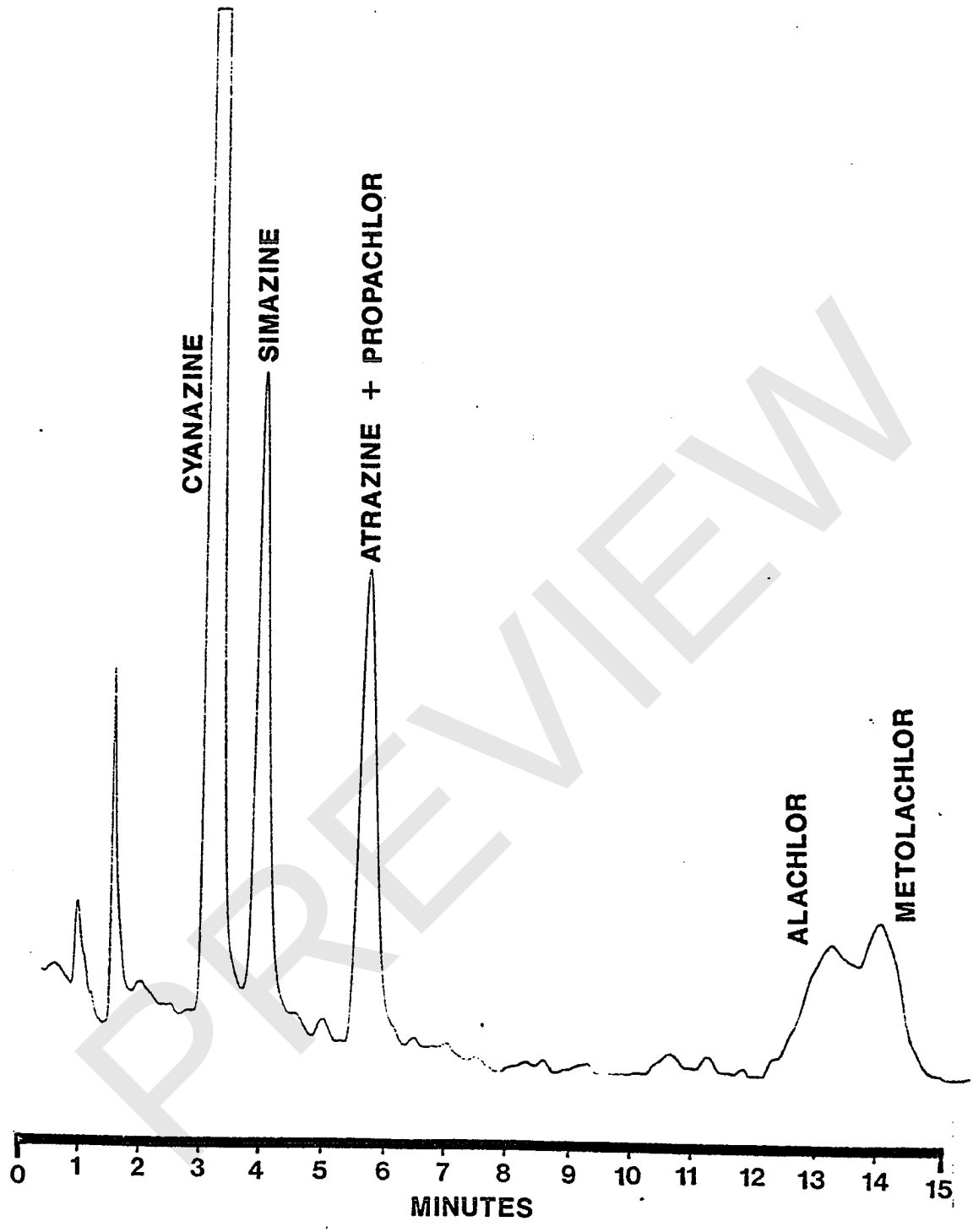

Figure 2. GLC chromatograph of the extract from the Dix sandy loam spiked with six herbicides. The glass column was 1.8 m X 2.0 mm (ID) packed with 3% OV-25 on 100/120 mesh chromosorb WHP. Velocity of the nitrogen carrier gas was 20 ml/min. Column oven temperature was held at 300 C, injection port at 215 C, and detector at 300 C. Nitrogen carrier gas flow was 20 ml/min. A nitrogen-phosphorus specific thermionic detector was used.