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Final Report on Project

DEGRADATION OF ATRAZINE, ALACHLOR, METOLACHLOR IN SOILS AND AQUIFER MATERIALS

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SUMMARY AND CONCLUSIONS

The investigation was initiated to examine the fate of herbicides in the environment as it relates to their degradation and groundwater protection. We describe the simulated movement of herbicides through the soil in large columns and have shown that for two widely different soils--Plainfield sand and Plano silt loam--movement of herbicides to groundwater occurs in the order alachlor > metolachlor >> atrazine. Little or no volatilization of these compounds to the atmosphere was found.

Decomposition rates of herbicides were determined as half-life values (the time required to decompose 50% of the herbicide present) usually referred to as $t_{\frac{1}{2}}$-values, and as degradation rate constant (k). As $t_{\frac{1}{2}}$ increases, the longer the herbicide and its residues will stay in the environment. Since k-values are inversely related to $t_{\frac{1}{2}}$, k will increase as degradation rates speed up. The $t_{\frac{1}{2}}$- and k-values were obtained for three soils--Dakota loam, Plano silt loam and Plainfield sand--under sterile and nonsterile conditions so that we could establish whether the degradation pathway was mediated by chemical or microbiological reactions. For all compounds in all soils $t_{\frac{1}{2}}$ was much lower in nonsterile than sterile soil indicating that degradation was controlled largely by microorganisms. For all herbicides--alachlor, metolachlor and atrazine--degradation was fastest in the Dakota loam but surprisingly similar for the Plano silt loam and Plainfield sand. Rates of degradation were most rapid for alachlor but similar for metolachlor and atrazine. It takes five half-lvies to reduce the amount of herbicide to 3% of the amount added. Thus, in Dakota loam 140 days is required to decompose 97% of added alachlor, 185 days for metolachlor and 190 days for atrazine. For the other two soils the time required to reduce the amount of herbicide to 3% of the amount added is 190 days for alachlor about 310 days for metolachlor and 340 days for atrazine.

For development of any mathematical model for use in regulating herbicide use, degradation rates and adsorption coefficients are the most important parameters required. We also made appreciable headway in separating the degradation products of the three herbicides. There are 1 to 6 alachlor metabolites depending on incubation time and soil type, 1 to 6 metolachlor metabolites and 4 to 8 atrazine metabolites. From a toxicological point of view it is essential to identify degradation products because they may be more or less toxic than the parent herbicide. To date we have identified hydroxyatrazine, deethylatrazine and deisopropylatrazine as degradation products of atrazine. It should be remembered that these degradation products appear in very low concentrations (fractions of a part per billion) and are difficult to extract and identify. In our review articles we have been able to draw detailed degradation pathways for the herbicides. These pathways will steer investigators to the compounds to look for in metabolite identification experiments.

We have also described a theoretical model of metolachlor decomposition by a microbial consortium and developed a routine microbial assay of pesticide-contaminated soils.

The three literature reviews on environmental fate of herbicides and their toxicology have been enthusiastically received and can form the basis for future invstigations on these compounds. We have already had discussions with a director of the Nutrient and Pest Management Program to have these reviews redrafted into formats understandable by decisionmakers and the general public. It is our earnest desire to see this accomplished quickly and to have broad statewide distribution.

Finally, a paper entitled "Mobility of ^{14}C-atrazine, alachlor and metolachlor in greenhouse lysimeters," was presented at the Annual Soil Science Society of America Proceedings was held in October, 1989 in Las Vegas, Nevada.

A second paper was presented by the research group at the Las Vegas meetings entitled "Fate of three herbicides in Wisconsin soils."

DEGRADATION OF ATRAZINE, ALACHLOR, METOLACHLOR IN SOILS AND AQUIFER MATERIALS

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In pursuit of the project objectives, we initiated experiments to determine the degradation and transport of alachlor, metolachlor and atrazine in soils. Some of the findings to date from these experiments were presented at an international symposium on groundwater held at Stanford University in the following format shown as section I. Other experiments and review documents are presented as sections II, III and IV.

I. FATE AND TRANSPORT OF ALACHLOR, METOLACHLOR AND ATRAZINE IN LARGE SOIL COLUMNS

ABSTRACT

^{14}C ring-labelled atrazine, alachlor, and metolachlor were surface-applied at 3.14 kg a.i./ha in greenhouse lysimeters containing two soils. Bromide (Br) -- a conservative tracer -- at 6.93 kg/ha as KBr and nitrate-nitrogen (NO_3-N) at 112 kg/ha as KNO_3 were mixed with each herbicide and surface-applied. Growth of Red top (*Agrostis alba*) was established in each column (105 cm long and 29.4 cm i.d.). The experiment consisted of 12 columns (2 soils x 3 herbicides x 2 replicates) each fitted with four sampling ports for leachates, a volatilization chamber, and an aeration and irrigation system. Volatile materials were trapped directly in solvents. One column replicate was dismantled for soil and plant analyses. Columns of Plainfield sand and Plano silt loam treated with alachlor and metolachlor were sampled after 23 and 28 weeks, respectively; the atrazine columns after 35 weeks. Herbicide residues were determined by liquid scintillation counting, extracted and separated by thin-layer chromatography using autoradiographic detection. Volatilization was < 0.01% of the amount of herbicide applied. The order of herbicide mobility was alachlor > metolachlor >> atrazine. As many as 8 to 12 alachlor metabolites and 2 to 6 metolachlor metabolites were separated in leachates.

Keywords

Alachlor; metolachlor; atrazine; herbicides; soil columns; leaching; volatilization; degradation; nonpoint pollution; herbicide mass-balance.

INTRODUCTION

The United States Environmental Protection Agency (USEPA) provide criteria for identifying and evaluating the source and spread of nonpoint pollution. Nonpoint pollution is the movement of pollutants from diffuse sources into surface or subsurface waterbodies and is among the leading causes of water quality problems (Chesters and Schierow, 1985). Pesticides are among the most important nonpoint pollutants carried from agricultural fields to surface waters or infiltrated into groundwaters. In the last decade, the occurrence of pesticides in ground water has been confirmed (Cohen et al.,

1984; Cohen et al., 1986; USEPA, 1986). The USEPA's Office of Ground Water Protection compiled a list of 17 pesticides in 23 states found in groundwater due to normal land applications (USEPA, 1986). Pesticides in groundwater cause public concern about the quality of drinking water, which has led to renewed pesticide monitoring and research and the call for stricter health advisories and water quality standards (Ehart et al., 1986). Pesticides may also escape to the atmosphere by volatilization (Taylor, 1978; Jury et al., 1980).

In several regions of Wisconsin, evidence shows the presence of a variety of herbicides in groundwater including atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine], metolachlor [2-chloro-N-[2-ethyl-6-methylphenyl]-N-2(2-methoxy-1-methyl) acetamide], and alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide]. They are extensively used in corn, potato and soybean production (WDATCP, 1986). A groundwater pesticide monitoring program in Wisconsin has shown detectable amounts of alachlor in 75 of 830 wells sampled, of metolachlor in 59 of 510 wells, and of atrazine in 152 of 752 wells (WDNR, 1988). Under normal agricultural conditions, 4 of 14 wells were contaminated with alachlor, 4 of 10 with metolachlor and 5 of 13 with atrazine (Postle, 1987). Groundwater contamination by these herbicides has led to increased public pressure to regulate their use because of potential adverse effects on human health; alachlor is listed as a probable human carcinogen while metolachlor and atrazine have shown indications of carcinogenicity to rats. Recently, groundwater enforcement standards for alachlor, atrazine, and metolachlor were set for Wisconsin at 0.5, 3.5, and 15 µg/l (ppb), respectively.

Alachlor, metolachlor and atrazine are now under close scrutiny by the state of Wisconsin and federal agencies with regard to their environmental fate and toxicological significance to humans, livestock, and ecosystems. The objective of this investigation is to evaluate transport of the herbicides and include volatilization, sorption, plant uptake, and degradation in a mass balance.

MATERIALS AND METHODS

Soil Types

Two Wisconsin soil types were used: Plainfield sand (Typic Udipsamment) obtained from a farm in Plover, WI; and Plano silt loam (Typic Argiudoll) obtained from the Rock County Experimental Farm near Janesville, WI. Pits (1 m^2) were dug for each soil to a depth of 1 m. Each soil was separated by horizons. Five horizons were recognized for the Plainfield soil and four for the Plano soil. The soils were air-dried, passed through a 6 mm screen and used for column packing after each horizon was well mixed; large rocks and roots were removed from the soils. Representative samples from each soil horizon were analyzed (Table 1).

Soil Columns

The experimental set-up shown in Figure 1 consists of twelve polypropylene columns (105 cm long x 29.4 cm internal diameter with 1.7 cm wall thickness). Controversy exists about the best way to construct columns for

Table 1. Description of surface and subsurface soils

Property	Plainfield sand, depth (cm)					Plano silt loam, depth (cm)			
	0-18	18-25	25-58	58-79	79-100	0-25	25-36	36-51	51-100
pH	5.8	6.0	5.7	5.8	5.9	6.5	6.4	5.9	5.6
Organic matter, %	2.0	0.9	0.3	0.3	<0.1	3.5	1.5	1.0	0.6
Total Kjeldahl N, mg/kg	690	390	220	160	90	1,800	920	590	440
Available P, mg/kg	79	45	55	82	64	38	15	24	48
Ca, mg/kg	350	340	160	40	50	1,700	1,500	1,700	1,900
Mg, mg/kg	80	70	50	20	10	630	640	880	870
K, mg/kg	61	31	26	11	7.0	210	140	190	200
Na, mg/kg	4.0	3.0	2.0	2.0	2.0	13	19	19	20
NH_4-N, mg/kg	<0.5	<0.5	<0.5	<0.5	<0.5	6.5	4.0	2.0	<0.5
NO_3-N, mg/kg	0.5	0.5	0.5	0.5	0.5	7.5	<0.5	<0.5	<0.5
Cl, mg/kg	<0.5	<0.5	<0.5	<0.5	<0.5	2.5	1.5	1.0	0.5
Br, mg/kg	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
CEC,[a] meq/100 g	2.6	2.4	1.3	0.4	0.3	14	13	16	17
Sand, %	91	89	90	93	97	9	12	10	7
Silt, %	6	6	5	4	1	70	62	57	62
Clay, %	3	5	5	3	2	21	26	33	31

[a]Cation exchange capacity estimated from cation composition of soil.

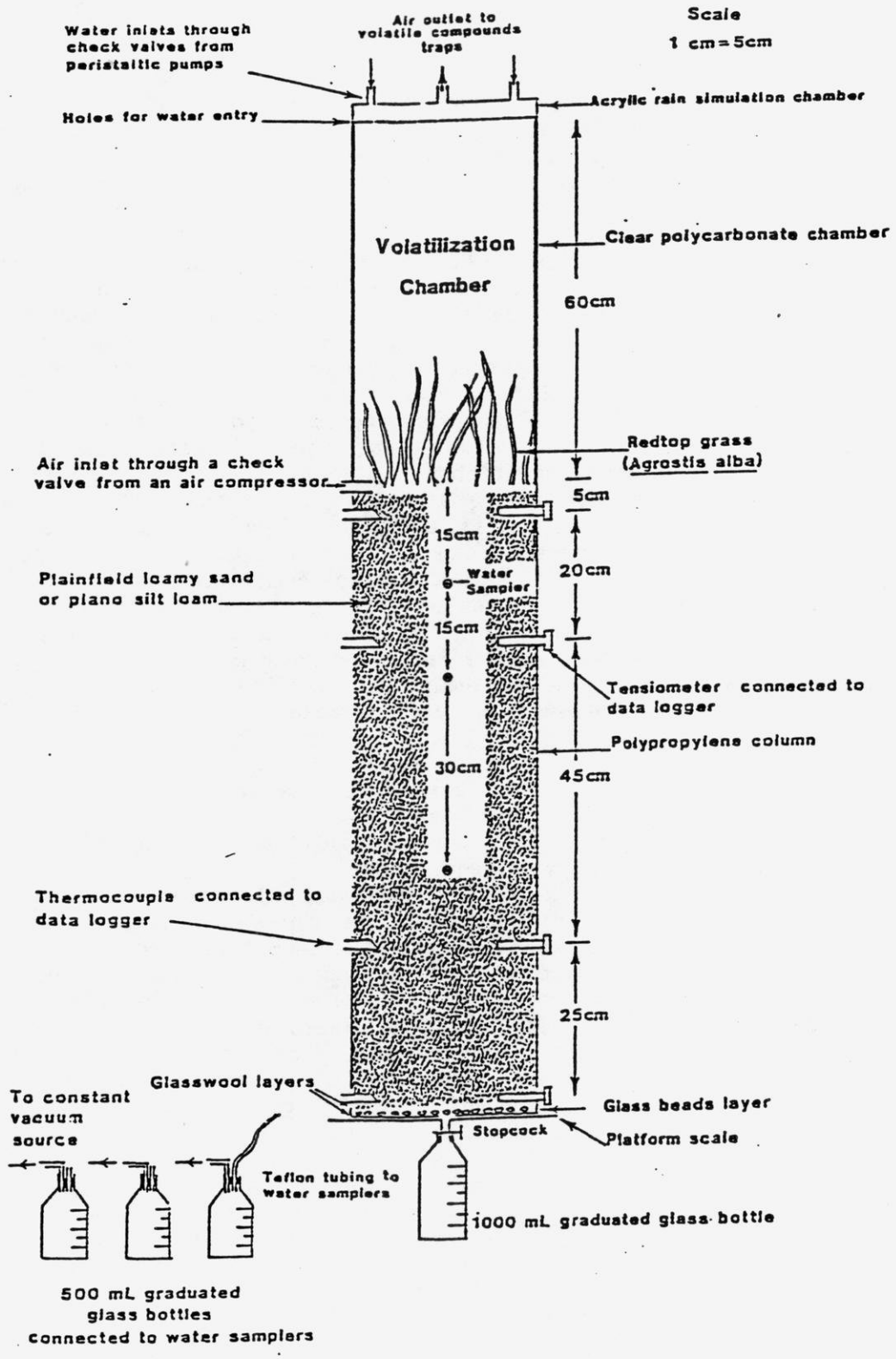

Figure 1. Column set-up.

transport experiments and so details are provided of the materials and dimensions used. Pure polypropylene is a rigid material slightly less inert than teflon but much less expensive; and far more inert to sorption than polyethylene, polyvinyl chloride, and silicone rubber (Barcelona et al., 1985).

Pure polypropylene plates (35.5 cm x 35.5 cm with 1.7 cm wall thickness) were cut and securely attached to the bottom of each column using stainless steel screws mounted into holes die-threaded into the plates and the edges of the columns. The plates were tightly sealed to the columns by placing a teflon ring cut to form a gasket seal along the walls of the columns and plates. Tap-threaded pure polypropylene adapter tubes of 3.2 mm internal diameter were attached to a hole die-threaded into the center of each plate. The adapter tubes were connected to stopcocks to control the free flow of leachate. Teflon thread seal ribbon was used to seal the tap- and die-thread combinations. To further insure air-tight conditions, hot glue was applied with a heat gun to all sealing surfaces. A hot air stream from a coil-heated air gun was used to form a smooth layer of plastic glue that hardens in 1 minute. A layer of fiber glass wool was placed at the bottom of each column above the adapter tube to prevent soil being washed from the columns. Solid glass beads (4 mm in diameter) were layered at a depth of 1 cm above the bottom of each column to create a saturated boundary. Another layer of fiber glass wool was placed on top of the glass beads to separate the beads from the soil. The soil horizons were carefully packed to their original field bulk density.

To draw water samples from several depths of the soil columns, vacuum-pressure sampling systems were designed and tested. Each sampling system was constructed by attaching a fritted (porous) glass disc in the form of a tube to a vacuum pump with an adjusted pressure of 0.1 bar. The diameter of the fritted disc was 3 cm with nominal pore size of 10 to 15 µm and 18 cm long with a 0.8 cm internal diameter tube reinforced by passing through rigid teflon tubing (1 cm I.D. and 2 mm wall thickness) to withstand the pressures from soil packing. A fritted glass lysimeter was developed and tested for soil water sampling because glass is known for its relative inertness compared to other materials. Water samplers were placed at 15, 30, and 60 cm below the soil surface. Prior to packing the soil, holes of 13 mm in diameter were drilled to mount the samplers. Care was taken to securely mount each glass sampler tube encased in rigid teflon tubing to the column wall with teflon film and hot glue to ensure complete sealing.

To provide for evapotranspiration, Redtop (*Agrostis alba*) grass was grown on the columns. Redtop is a long-lived perennial grass growing to a height of up to 1 m. Its root system consists of shallow, vigorous root stalks--5 to 15 cm long--that form a loose, coarse turf. It is one of the best wetland tame grasses. It can remain underwater for considerable periods of time without damage, yet it adapts to dry conditions and acid or alkaline soils. It is used as a soil binder along waterways.

Each herbicide was evenly sprayed on the soil surface with irrigation water at 3.14 kg a.i./ha (21.3 mg herbicide/column) after establishment of the grass cover. ^{14}C-labelled herbicides with the highest specific activities available were used; 72.65, 79.3 and 103.2 µCi/mg for alachlor, metolachlor, and atrazine, respectively. Application solutions consisted of 32, 30 and 23%

^{14}C labelled alachlor, metolachlor, and atrazine, respectively. Application amounts were at field recommended rates. Bromide (Br) -- a conservative tracer -- was applied to each column at 6.93 kg/ha as KBr. The soils were fertilized with NO_3-N at 112 kg/ha as KNO_3.

The columns were fed intermittently with distilled water to simulate rainfall. Distilled water was applied according to a precipitation schedule based on rainfall averages from 30 year records for Hancock and Janesville, WI compiled from National Oceanic and Atmospheric Administration records at the National Climatic Data Center, Ashville, North Carolina. Water was added to each column at regular intervals. The amount of water added accommodated the rainfall and sampling volumes which was an amount similar to the irrigation at the Hancock Experimental Farm.

Temperature and water potential profiles for the columns were determined using thermocouple junctions and tensiometers with pressure transducers. Four columns representing duplicates of the two soil types were monitored for temperature and water potential changes at four soil depths: 5, 25, 70, and 95 cm below the soil surface. Another thermocouple was placed at the soil surface-atmosphere interfaces. The thermocouples were teflon insulated. Each tensiometer was designed and constructed from a fritted glass disc, attached to a glass tube and interfaced with a pressure transducer. The fritted disc had a diameter of 3.0 cm and nominal porosity of 4.0 to 5.5 μm. Such a disc was tested and found to withstand very low tensions. Porous ceramic cups are not recommended for use because they sorb large amounts of organic compounds. The thermocouples and pressure transducers were attached to a Model 21X data logger (Campbell Scientific Inc., Logan, UT).

Evapotranspiration measurements or water loss from the soil surface and grass for four columns representing duplicates of the two soil types were monitored closely by placing these columns on scales (Fairbanks Scales, St. Johnsbury, VT) serving as continuous weight control devices. Evapotransporation measurements were taken prior to each scheduled precipitation by weighing the columns. Evapotranspiration was determined by keeping records of irrigation inputs, drainage and change in water storage.

Clear polycarbonate cylinders (0.16 cm wall, 30.5 cm I.D. and 60 cm long) were placed on top of each column, joined by a teflon ring, and sealed air-tight with hot-melt glue. Rain simulator chambers were constructed by sealing two plexiglass plates (0.34 cm wall x 36.5 cm x 36.5 cm) to sandwich a plexiglass cylinder (0.32 cm wall, 30.5 cm I.D., and 5 cm long). Holes (19 gauge) were evenly made in the bottom plate. Two pairs of plexiglass tubes (0.32 cm I.D. and 5 cm long) were installed in the upper plate to provide an inlet for water and outlet for air, respectively. The water inlets distribute water evenly across the area into the rain simulator chambers. The air outlet tubes connect the outside of the column to the rain simulator, and volatilization chambers to allow free entry of water between the volatilization chamber and the soil surface. The rain simulator chambers were sealed to the polycarbonate cylinders of each column with acrylic glue. Water is delivered to the chamber through plexiglass tubes passing by check valves using Masterflex multichannel peristaltic pumps controlled by a programmable electronic timer. The air outlets are merged by a T-shaped glass connector attached to a check valve and through a series of a back trap and two solvent traps containing a 1:1 mixture of hexane:acetone and another back trap and two

$^{14}CO_2$ traps (Simsiman and Chesters, 1975; Marinucci and Bartha, 1979). The traps were changed weekly.

The atmosphere contained in the volatilization chamber was changed four times a day by passing fresh air through a check valve sealed to a hole made into the polycarbonate cylinder 5 cm above the polypropylene column. Air was delivered from an oilless air compressor. Every 6 hours the pump was turned on and off by a programmable Chron Trol electronic timer (Lindburg Enterprises, San Diego, CA). The air flow was measured by a Gilmont air flowmeter. It was calculated that total air volume of the volatilization chamber is exchanged by running the pump for 5 minutes.

Sampling and Analyses

Water samples from the three soil depths were drawn at week 1, 2, 3, 4, 6, 8, 11, 15, and 23 by applying a low vacuum to the fritted glass lysimeter at each sampling port. The samples were collected in 500 ml glass bottles. The leachate from the bottom of each column was sampled at regular intervals in 1,000 ml glass bottles through stopcocks. The glass bottles were all new, graduated and fitted with teflon caps. They were washed three times with methanol before sampling. After recording the volume, herbicides were extracted from water samples by extraction with ethyl acetate immediately after sampling. The 1:1 hexane:acetone mixture air traps and the $^{14}CO_2$ traps were sampled weekly. Water and volatile materials were quantified by liquid scintillation counting (LSC) and all samples were refrigerated at 4°C.

One replicate of each herbicide treatment was dismantled and the other remained intact for later evaluation. Columns were dismantled removing volatilization and irrigation chambers. Shoots and roots of the grass were harvested separately. Columns were drained by gravity before soil sampling and the last leachate volume was extracted with ethyl acetate and evaluated by LSC. Samples of plant roots and shoots were collected from each column for herbicide residue analysis of plant tissue. Soil samples from four layers of each column were collected manually at 0-15, 15-30, 30-60, and 60-100 cm. In the process, every layer was sampled in three locations using borehole sampling with a long-reach spatula at the center of the column, near column walls, and between the center and the walls of columns. Each layer was mixed thoroughly and a fourth sample was taken from the mixed (bulk) layer. Plant tissue samples were carefully separated from soil particles by thoroughly washing with distilled water, and moisture contents of soil and plant tissue were measured. Total $^{14}CO_2$ in soil and plant samples were determined by combustion using biological oxidizer model OX600 (R. J. Harvey Instrument Corp., Hillsdale, NJ). The soil and water samples were also extracted with ethyl acetate for analysis by thin layer chromatography (TLC).

Herbicide Residue Extraction, Separation and Identification

Aliquots of 100 ml of water samples were extracted twice with ethyl acetate (100 ml). The extracts were dried by filtration through anhydrous sodium sulfate (Na_2SO_4) and evaporated to dryness in a stream of air at 45°C in a Rotovac rotary evaporator. The residues were redissolved in 2 ml ethyl acetate and stored in the freezer.

Extraction of herbicide residues was accomplished by adding 50 ml of ethyl acetate to approximately 50 g of herbicide-treated soil in a wide-mouth 240 ml glass bottle fitted with a teflon cap. The soil samples were shaken overnight, filtered through a Buchner funnel fitted with a 9 cm diameter Whatman glass microfiber filter 934-AH and 20 g oven-dried sodium sulfate. The samples were reextracted two times with 50 ml portions of ethyl acetate, shaken for 1 hr and filtered each time through the Buchner funnel. The combined filtrate, evaporated to dryness using a Rotovac rotary evaporator, was redissolved in 2 ml of ethyl acetate and stored in a freezer.

The herbicide residues in ethyl acetate extracts from water and soil samples were separated and analyzed by TLC using autoradiographic detection. Aliquots (200 µl) of the extracts were spotted on commercial silica gel plates and developed with the proper solvent mixtures for each herbicide. The solvent mixtures used were benzene:methanol (19:1) for alachlor, hexane:chloroform:ethyl acetate (3:1:1) for metolachlor, and benzene:acetic acid:water (60:40:3) for atrazine. Radioactive areas on the TLC plates were detected by placing Kodak SB-5 X-ray films on the plates. The radioactive spots representing the herbicide residues were scraped from the plates and transferred in 2 ml high purity methanol into teflon capped 8 ml vials. Aliquots (0.1 ml) were transferred to scintillation vials and radioactivity counted. Alternatively, radioacativity was quantified using a TLC-scanner.

RESULTS AND DISCUSSION

The cumulative amount of herbicide volatilized (organic volatile residues and $^{14}CO_2$) from soil columns is shown for 11 weeks in Table 2. The amounts were small possibly because the herbicides were applied with irrigation water and quickly leached below the soil surface. $^{14}CO_2$ also may have been dissolved by soil water and transported deeper into the profile. Volatilization may have been unusually low because conditions were essentially stagnant and the effect of wind was ignored. $^{14}CO_2$ was in general slightly greater than other trapped ^{14}C volatiles.

Amounts of applied ^{14}C herbicide recovered from soil and leachate are recorded as total herbicide residues (Table 3). The order of herbicide mobility is alachlor > metolachlor >> atrazine. More than twice as much alachlor as metolachlor leached to the bottom of the Plainfield sand columns while five times as much alachlor as metolachlor reached 1 m in the Plano soil columns. ^{14}C atrazine levels were low in the leachates from both soils. Continuation of the leaching experiments using the remaining columns will provide further information with respect to the mobility of herbicide residues. Based on determination of ^{14}C in leachates and $^{14}CO_2$ release from soil samples by combustion, a quantitative mass balance can be achieved for the three herbicides in Plainfield sand. However, in Plano silt loam only 71-87% of the ^{14}C can be accounted for (Table 4). This suggests that the herbicides and/or their degradation products are trapped in the interlayer spaces of expanding clay minerals in the Plano silt loam. The evidence is preliminary and the dimensions of the interlayer spacings need further investigation.

Radiolabelled herbicide distributions in the profiles of the two soils are shown in Table 4. The three herbicides and/or their metabolites were

Table 2. Cumulative ^{14}C volatilization of herbicide residues and CO_2 from soil columns in 11 weeks[a]

Soil	^{14}C (DPM)			^{14}C (%)		
	Volatiles	CO_2	Total	Volatiles	CO_2	Total
			Alachlor			
Plainfield sand	5×10^{3}	9×10^{3}	14×10^{3}	4.1×10^{-4}	8.2×10^{-4}	1.2×10^{-3}
Plano silt loam	26×10^{3}	24×10^{3}	50×10^{3}	2.3×10^{-3}	2.2×10^{-3}	4.5×10^{-3}
			Metolachlor			
Plainfield sand	24×10^{3}	92×10^{3}	116×10^{3}	2.2×10^{-3}	8.2×10^{-3}	1.0×10^{-2}
Plano silt loam	6×10^{3}	5×10^{3}	11×10^{3}	5.5×10^{-4}	4.3×10^{-4}	9.8×10^{-4}
			Atrazine			
Plainfield sand	13×10^{3}	78×10^{3}	91×10^{3}	1.2×10^{-3}	6.9×10^{-3}	8.1×10^{-3}
Plano silt loam	8×10^{3}	115×10^{3}	123×10^{3}	7.1×10^{-4}	1.0×10^{-2}	1.1×10^{-2}

[a]Values are means from two columns for each herbicide in disintegrations per minute (DPM) and percentage of total herbicide applied. Initial ^{14}C added was 1.0993×10^{9}, 1.1249×10^{9}, and 1.1224×10^{9} DPM as alachlor, metolachlor, and atrazine, respectively.

Table 3. Mass balance of ^{14}C in columns

Herbicide	Time (Weeks)	Leachate ^{14}C (%) at Depth (cm) 15	30	60	100	^{14}C Recovered from Soil by Combustion (%)	Total Recovery (%)
				Plainfield Sand			
Alachlor	23	0.21	-	4.9	17	76	98
Metolachlor	23	0.32	0.12	1.2	7.3	95	104
Atrazine	35	1.4	0.22	0.04	0.12	102	104
				Plano Silt Loam			
Alachlor	28	-	0.88	1.0	4.9	64	71
Metolachlor	28	1.5	1.0	0.47	0.92	83	87
Atrazine	35	1.8	0.32	0.13	0.08	72	74

- No sample.

Table 4. Distribution of ^{14}C in columns

% Alachlor at Depth (cm)				% Metolachlor at Depth (cm)				% Atrazine at Depth (cm)			
0-15	15-30	30-60	60-100	0-15	15-30	30-60	60-100	0-15	15-30	30-60	60-100
					Plainfield Sand						
43	15	9.1	8.8	46	21	15	13	85	9.3	3.9	3.3
					Plano Silt Loam						
33	15	8.9	6.7	49	18	10	5.9	48	13	7.5	3.6

concentrated in the top layer (0-15 cm) of both soils and levels decreased with increasing depths in the soil profile.

Herbicide residues were detected by TLC in extracts of leachate from the bottom ports of the six sacrificed columns. Atrazine did not leach in amounts large enough to allow extraction of quantities measurable by TLC except at the upper sampling port. Detectable amounts of alachlor and metolachlor and their metabolites moved completely through the columns. In Plainfield sand, alachlor was not detected before week 16; it appeared at and after week 16. In Plano silt loam, alachlor was not detected at or before week 16, was detected at week 17 but not at week 19. Metolachlor was detected in leachate from Plano silt loam at and after week 17, and at and after week 19 in Plainfield sand.

For the Plainfield sand, alachlor showed as many as 12 metabolites leaching from the bottom of the column after 23 weeks of which 4 were judged to be present in major amounts; for metolachlor it was 6 metabolites with 2 judged to be major. For the the Plano silt loam alachlor displayed 8 (2 of them major) metabolites and metolachlor showed 2 (1 major) metabolite. There was insufficient leaching of atrazine residues to make these types of separations. TLC indicates that some metabolites of alachlor and metolachlor were more mobile than the parent compounds. The mobility of alachlor was slower in Plano silt loam than in Plainfield sand, but alachlor seems to be degraded faster in Plano silt loam than in Plainfield sand.

Separation of herbicide metabolites from water and soil samples will continue by TLC and HPLC. Identification of major metabolites of alachlor, metolachlor, and atrazine is being attempted using separation, purification and MS techniques. A data base is being constructed for movement of the three herbicides to the 1 m depth with time. Attempts will be made to extrapolate the data to greater soil depths. The data base will be used to calibrate and verify such available pollutant movement models as the Leaching Estimation and Chemistry Model (Wagenet and Hutson, 1987) and the U.S. EPA Pesticide Root Zone Model (Carsel et al., 1984).

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II. DEGRADATION EXPERIMENTS

Evidence is accumulating on the presence of a variety of pesticides in the groundwater of several regions of Wisconsin. Among the pesticides of major concern are the herbicides alachlor, metolachlor and atrazine. They are extensively used in corn production and substantial amounts are used on potatoes and soybeans (WDATCP, 1986). The WDNR's Groundwater Pesticide Monitoring Program (WDNR, 1989) has shown detectable amounts of alachlor (in 79 of 834 wells sampled) of metolachlor (in 62 of 513 wells) and of atrazine (in 166 of 766 wells). Under normal agricultural conditions, a monitoring study revealed that 4 of 14 wells were contaminated with alachlor, 4 of 10 with metolachlor and 5 of 13 with atrazine (Postle, 1987). Groundwater contamination by these herbicides has led to increased public pressure to regulate their use because of potential adverse effects on human health; alachlor, metolachlor and atrazine have all shown indications of carcinogenicity to rats. Recently, groundwater enforcement standards for alachlor, atrazine, and metolachlor were set for Wisconsin at 0.5, 3.5, and 15 µg/l (ppb), respectively.

Transformations of pesticides depend on the interplay of many factors involving the physico-chemical properties of the pesticide, the chemical and biological properties of the soil, and other environmental variables. Most information on pesticide degradation has been accumulated for surface soils and only recently has interest been shown in the fate of pesticides in the vadose zone (the soil below the root zone) and in groundwater. To fully understand the fate of pesticides and delineate areas where leaching to groundwater is likely to be prevalent, the degradation rates and products for these compounds must be elucidated.

Degradation of alachlor, metolachlor, and atrazine was investigated in surface layers of three Wisconsin soil series displaying a wide range of properties. This investigation provides 1. rates of degradation and half-lives of the herbicides; 2. the degradation pathways they follow; and 3. the metabolites produced.

Chemical degradation is differentiated from microbial transformations by use of sterile and nonsterile soil systems. Wherever possible, overall detoxification rates for the herbicides (i.e., transformations to nonharmful products) will be established.

As part of this investigation we determined the suitability of a variety of solvents for extracting the herbicides from soils.

Materials

Soils. Surface soils, 0 to 25 cm (0 to 10 in), were collected at three Wisconsin locations: Plainfield sand from Plover in Portage County, Plano silt loam from Janesville in Rock County, and Dakota loam from Sauk City in Sauk County. The soils have a wide range of chemical and physical properties (Table 5).

Table 5. Descriptions of surface soils

Property	Plainfield sand	Plano silt loam	Dakota loam
pH	5.8	6.5	6.9
Organic matter, %	2.0	3.5	1.8
Total Kjeldahl N, %	0.069	0.18	0.097
Available P, mg/kg	79	38	58
CEC,[a] meq/100 g	2.6	14	6.3
Exchangeable ions, mg/kg			
Ca	350	1,700	760
Mg	80	630	220
K	61	210	250
Na	4.0	13	8.0
NH_4-N	<0.5	6.5	<0.5
NO_3-N	0.50	7.5	<0.5
Cl	<0.5	2.5	1.5
Soil separates, %			
Sand	91	9	49
Silt	6	70	41
Clay	3	21	10

[a]Estimated cation exchange capacity.

Herbicides. Standards of nonlabeled alachlor, metolachlor, and atrazine were obtained from the USEPA Pesticides and Industrial Chemicals Repository, Research Triangle Park, NC. ^{14}C ring-labeled analytical grade alachlor (3.5 mCi) was provided by Monsanto Co., St. Louis, MO. It has a specific activity of 73.2 µCi/mg and a purity of 97%. ^{14}C ring-labeled metolachlor (4.5 mCi) and atrazine (4.5 mCi) were provided by Ciba-Geigy, Greensboro, NC. Metolachlor has a specific activity of 79.3 µCi/mg and a purity of 97.1% while atrazine has a specific activity of 103.3 µCi/mg and a purity of 98.3%.

Solvents. Solvents selected to determine which were most suitable for herbicide extraction from soils are shown in Table 6. Each of the solvents--four for each herbicide--were of pesticide grade.

Table 6. Solvents used to extract the herbicides

Herbicide	Solvents			
	1	2	3	4
Alachlor	Ethyl acetate	Benzene:isopropanol 2:1 v/v	Methanol	Hexane: acetone 9:1 v/v
Metolachlor	Ethyl acetate	20% Acetonilrile in propanol	Methanol	Dichloromethane
Atrazine	Ethyl acetate	Acetonitrile	Diethyl-ether	Dichloromethane
Metribuzin	Ethyl acetate	Methanol	Benzene	Dichloromethane

The soil used in the investigation was Plano silt loam because it has the highest organic matter, clay and silt contents and should have the highest adsorptive capacity of the three soils used in the investigation.

Methods

Herbicide extraction. To test the suitability of various solvents as pesticide extractants, the following experiment was conducted.

Soil samples (50 g) were placed in each of 32x225 ml (8 oz) prescription bottles. Sets of eight soil samples were treated with one of the four herbicides (alachlor, metolachlor, atrazine and metribuzin) at a concentration of 2 µg/kg equivalent to 4 kg/ha. Duplicate samples from each herbicide-treated soil were extracted with the four solvents shown in Table 6. Choice of solvents was based on a literature evaluation.

The extraction was conducted by adding solvent (50 ml) to the herbicide-treated soil. The soil samples were shaken for 8 hours, filtered and the extracts transferred to 500 ml flasks. The samples were again extracted twice with 50 ml portions of each solvent and shaken for 1 hr. The combined extracts were filtered through a Buchner funnel fitted with a 9 cm diameter Whatman glass microfiber filter 934-AH and 20 g oven-dried sodium sulfate. The filtrate was evaporated to dryness using a Rotovac rotary evaporator, and redissolved in 5 ml of ethyl acetate. The final pesticide solution was analyzed by gas chromatography on a Hewlett-Packard 438A instrument using a 91 cm (3.0 ft) 2 mm i.d. column packed with 5% apiezon and 0.125% DIGS on 100 to 120 Supelcoport (Scientific Products, McGraw Park, IL., 60085) using a nitrogen/phosphorus detector. The injector and detector temperatures were 250°C and the column temperature 190°C. Nitrogen was used as carrier gas at a flow rate of 67 ml/min.

Herbicide transformations. A factorial experimental design was selected to study herbicide degradation. Three herbicides were used with soils from three locations. Soil samples from each location were either sterilized or nonsterilized to evaluate the difference between microbial and chemical degradation mechanisms.

Sterilization of half of the soil samples was accomplished by exposing them to γ-radiation generated by a ^{137}Cs source of 5 Mrd (Isomedic Co., Waukegan, IL).

Each incubation system was prepared as follows: Soil (50 g) was placed in a 225 ml glass bottle and covered with a slip-on membrane cap (Biomedical Polymers, Inc., Leominster, MA) permeable to gases but impermeable to water and microorganisms. To trap CO_2, a layer of soda lime (2 g) was placed on top of one cap and then covered with another cap (Figure 2). Herbicides dissolved in water were filter sterilized and added to the soil samples to provide a moisture content equivalent to 80% of field moisture capacity (FMC). Each pesticide in a ratio of 1:1 labeled:nonlabeled was added to the soil samples at a level of 2 µg/g equivalent to 4 kg/ha.

Soils were sampled after 0, 4, 8, 32, 64, 96, 128, 192 and 256 days of incubation. Triplicate samples from each of the three soils (sterile and nonsterile) for each pesticide were sacrificed at each scheduled sampling time

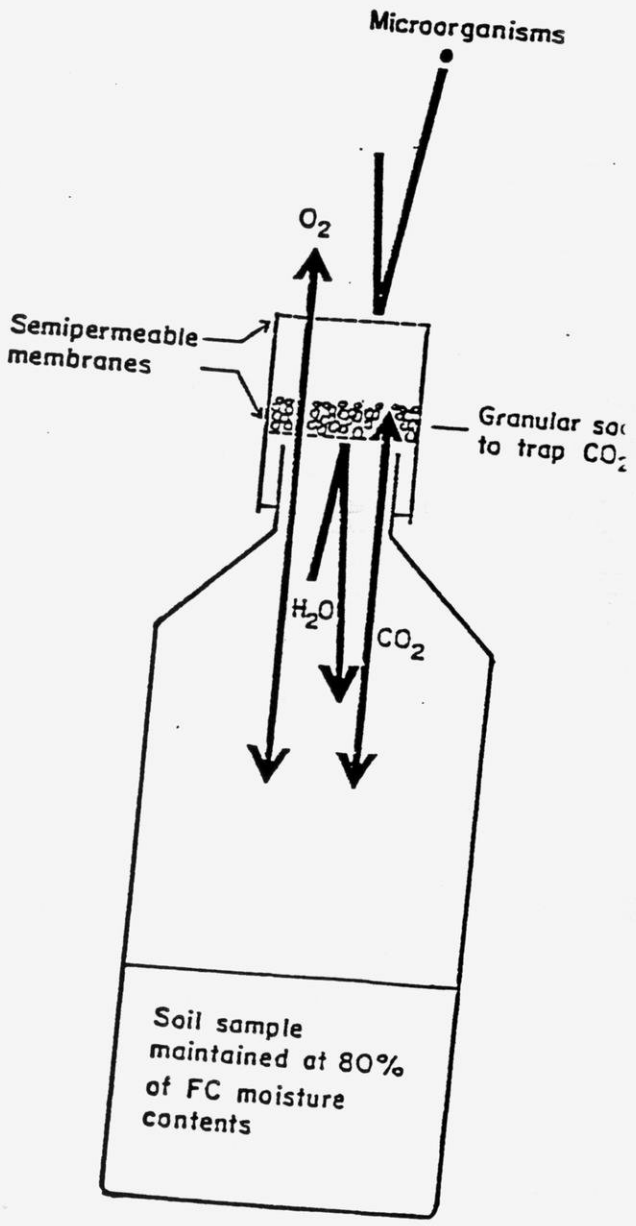

Fig. 2. The incubation system.

thus requiring 60 samples (including controls) at each sampling. Soil samples from each set of treatments were extracted with ethyl acetate using the procedure described earlier.

Several procedures were used to separate the herbicides and their metabolites to allow quantitative determination and identification.

The herbicides and their metabolites in concentrated organic solvent extracts were separated and analyzed by thin layer chromatography (TLC) using autoradiographic detection. Aliquots (10 to 100 µl) of the extracts were spotted on commercial silica gel plates which were developed with the proper solvents for each herbicide (Table 7). Radioactive areas on the TLC plates were detected by placing Kodak SB-5 X-ray films on the plates. The radioactive areas on the plates were removed and transferred to scintillation vials and radioactivity was quantified by liquid scintillation counting. Alternatively, radioactivity was quantified using a TLC-scanner instead of TLC-autoradiography.

Table 7. Solvent mixtures for developing TLC plates

Herbicide	Elutant
Alachlor	Benzene:methanol (95:5)
Metolachlor	Hexane:chloroform:ethyl acetate (3:1:1)
Metolachlor	Dichloromethane:acetone (95:5)
Atrazine	Benzene:acetic acid:water (60:40:3)

A gas chromatograph fitted with a N/P detector as mentioned previously in this report and a high-performance liquid chromatograph equipped with an ultraviolet and radioactive flow detectors were used to separate and quantify the herbicides and their metabolites.

Isolation of the herbicide and their metabolites from the concentrated organic solvents was conducted as follows: 1 ml of liquot of the concentrated organic extract was applied to a 20 cm x 20 cm silica gel TLC plate that had predeveloped in MeOH. Standards of each herbicide was applied too. The plates were developed in the proper TLC system for each herbicide (Table 7). After the plate dried, the bands on the plate were visualized by UV lamp and then by autoradiography as described earlier. The bands were scrapped and placed in vials. The silica gel scrappings from the bands were extracted with two 5-ml of methanol. The filtrate was concentrated to 0.2 ml by blowing a gentle nitrogen stream into the vials. The isolates were analyzed by mass spectrometry and identified as atrazine (Figure 3); deethylatrazine (Figure 4); and deisopropylatrazine (Figure 5).

The metabolites were identified by mass spectrometry.

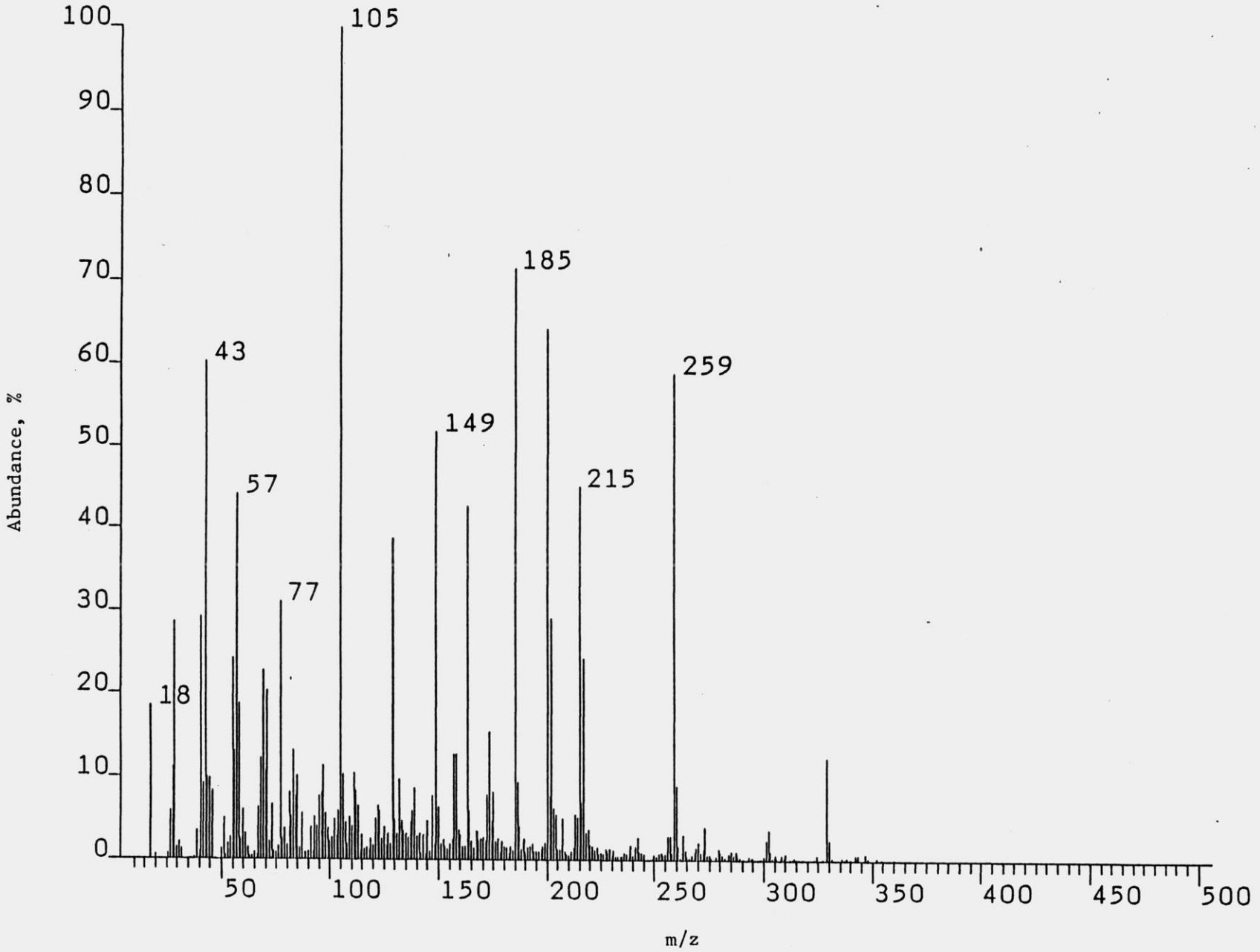

Figure 3. Mass spectrum of atrazine identified by 215 mass peak.

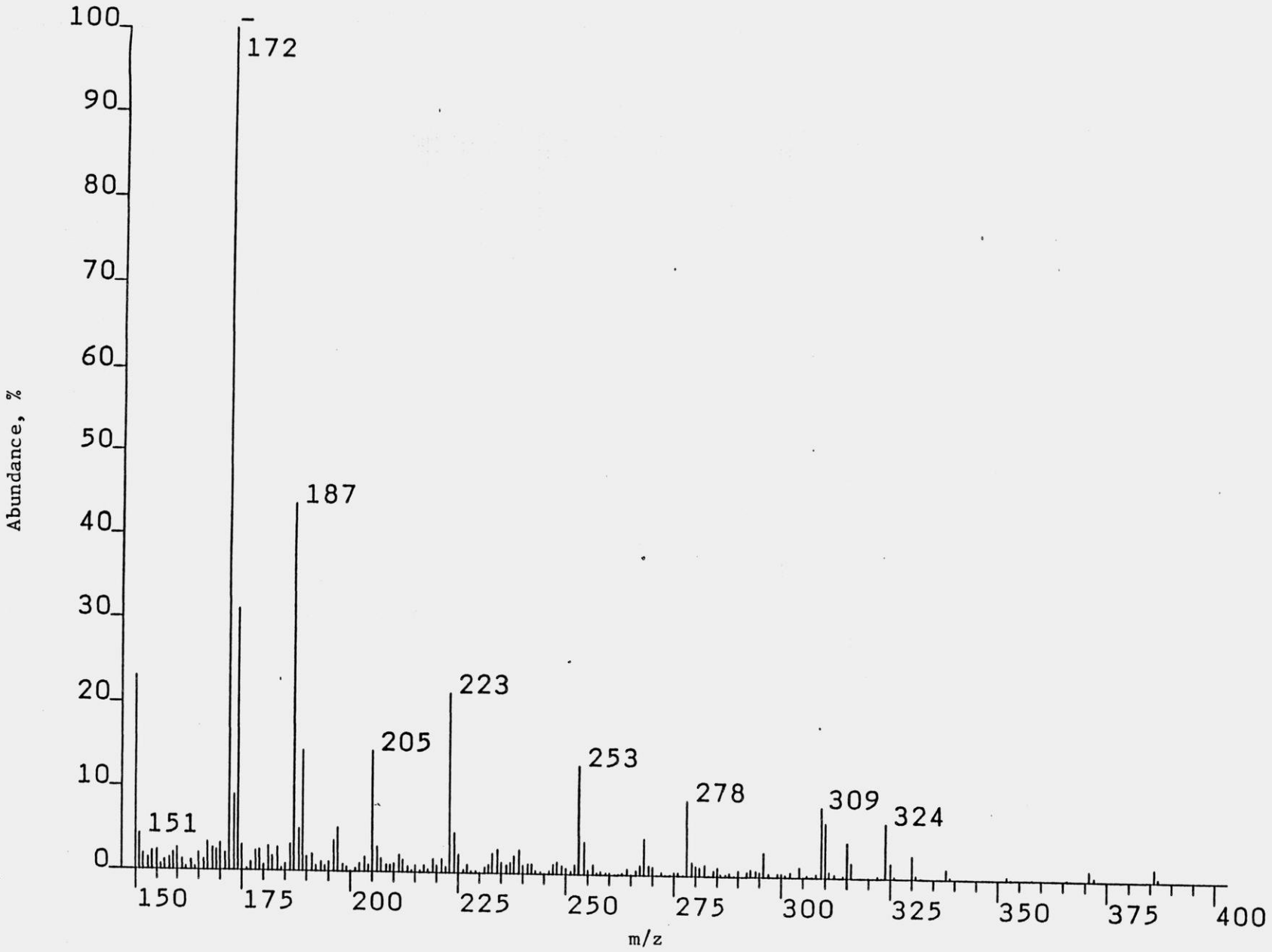

Figure 4. Mass spectrum of deethylatrazine identified by 187 mass peak.

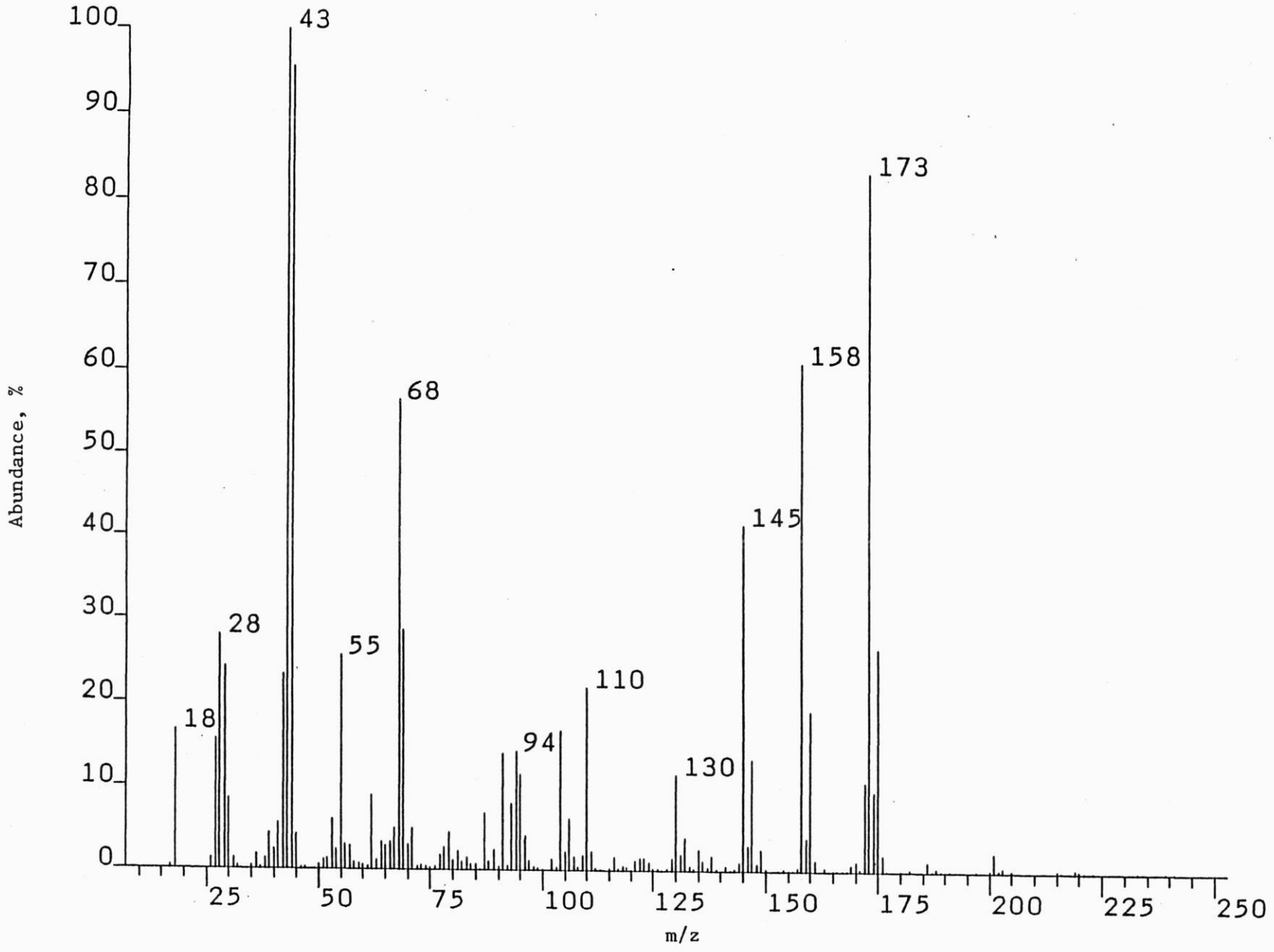

Figure 5. Mass spectrum of deisopropylatrazine identified by 173 mass peak.

Table 9. Degradation rates (k) and half-lives ($t_{\frac{1}{2}}$) of herbicides in surface soils under nonsterile and sterile conditions

Soil [a]	k [b]		$t_{\frac{1}{2}}$ (days) [b]	
	Nonsterile	Sterile	Nonsterile	Sterile
		Alachlor		
Dakota loam	0.0250	0.0061	28	114
Plano silt loam	0.0179	0.0036	38	192
Plainfield sand	0.081	0.0028	38	250
		Metolachlor		
Dakota loam	0.0186	0.0054	37	131
Plano silt loam	0.0102	0.0047	67	148
Plainfield sand	0.0125	0.0018	56	369
		Atrazine		
Dakota loam	0.0180	0.0056	38	122
Plano silt loam	0.0099	0.0084	67	83
Plainfield sand	0.0100	0.0068	69	102

[a]Organic matter contents of Dakota loam, Plano silt loam and Plainfield sand were 1.8, 3.5 and 2.0%, respectively; pH 6.9, 6.5, and 5.8, respectively.
[b]Mean of 3 replicates.

Data obtained by TLC separation showed the presence of 1 to 6, 1 to 6, and 4 to 8 metabolites produced during degradation of alachlor, metolachlor and atrazine, respectively (Table 10). The number and perhaps types of metabolites varied with time under nonsterile and sterile conditions. Identification of metabolites is difficult because of the unavailability of standard compounds and because metabolite concentrations are extremely low. However, an attempt is being made to identify the major products using the mass spectrophotometer of the U.W.-Madison Biochemistry Department. It is anticipated that metabolites produced in the soil incubation systems will include some of the products depicted in the degradation pathways compiled from the literature (Figs. 6, 7, 8 and Tables 11, 12, 13). Preliminary results showed that the major products of atrazine degradation in soils are hydroxyatrazine, deethylatrazine and deisopropylatrazine at the label concentrations recommended for agricultural use.

If some metabolites can be identified and their toxicology assessed from the literature an attempt will be made to apply a degradation model to the system in which degradation rate is calculated to the point that all residues are essentially nontoxic. This model will be similar to that described for aldicarb (Fathulla et al., 1989).

Vadose and aquifer materials have been collected in the same location where the surface soils were obtained in case analyses of these samples will help enhance data interpretation.

Table 10. Number of metabolites isolated by TLC technique

Soil	Days of incubation											
	1		4		8		16		32		64	
	NS[a]	S[a]	NS	S	NS	S	NS	S	NS	S	NS	S
					Alachlor							
Dakota loam			3	2	3	3	4	4	3	2	4	6
Plano silt loam			4	1	2	2	3	3	2		4	4
Plainfield sand			3	1	2	2	2	3	3	2	4	4
					Metolachlor							
Dakota loam	3	1	3	3	4	3	4	5	3	4	3	4
Plano silt loam	2	1	3	2	5	2	6	3	4	5	3	4
Plainfield sand	3	2	3	2	3	2	5	6	3	5	5	3
					Atrazine							
Dakota loam	4	7	4	6	5	6	5	4	6	7	5	4
Plano silt loam	5	8	5	5	5	5	4	5	6	6	7	4
Plainfield sand	5	6	6	5	5	7	6	4	4	5	4	4

[a]NS is nonsterile soil; S is sterilized soil.

Decomposition by a microbial consortium

Professor Harris and his collaborators have prepared two abstracts for presentation of posters at the Soil Science Society of America Annual Meetings. The first entitled "Theoretical Model of Aerobic Metolachlor Decomposition by a Microbial Consortium" was presented in November 1988 in Annaheim, California. The paper dealt with providing a mechanistic basis for developing and using communities (consortia) of soil microorganisms for bioremediation of soils and waters contaminated with organics of environmental concern that are known to be resistant to biodegradation by naturally-occurring single species of microorganisms. A steady state, continuous culture computer model was presented describing the mass balance kinetics of complete metolachlor decomposition by an aerobic microbial consortium as a function of environmental conditions and the ecophysiological properties of the trophic groups constituting the consortium. Supplemental organic substrate amendments and cometabolic and noncometabolic pathways are considered. Sensitivity analysis and experimental validation of the relative importance of the determinants of the nature and boundary conditions for complete metolachlor decomposition to target concentrations are greatly simplified by the hierarchal spread sheet structure of the computer model.

The second paper to be presented in Las Vegas in October 1989 is entitled "Routine Microbial Assay of Pesticide-Contaminated Soils." A plate dilution frequency approach will be described for routine assay of gross differences in the predominant and selected physiological types of microorganisms in soils contaminated with pesticides. A six-set dilution series is inoculated onto three agar plates as eight replicate 0.01 ml subsamples (0.08 ml aliquot) per

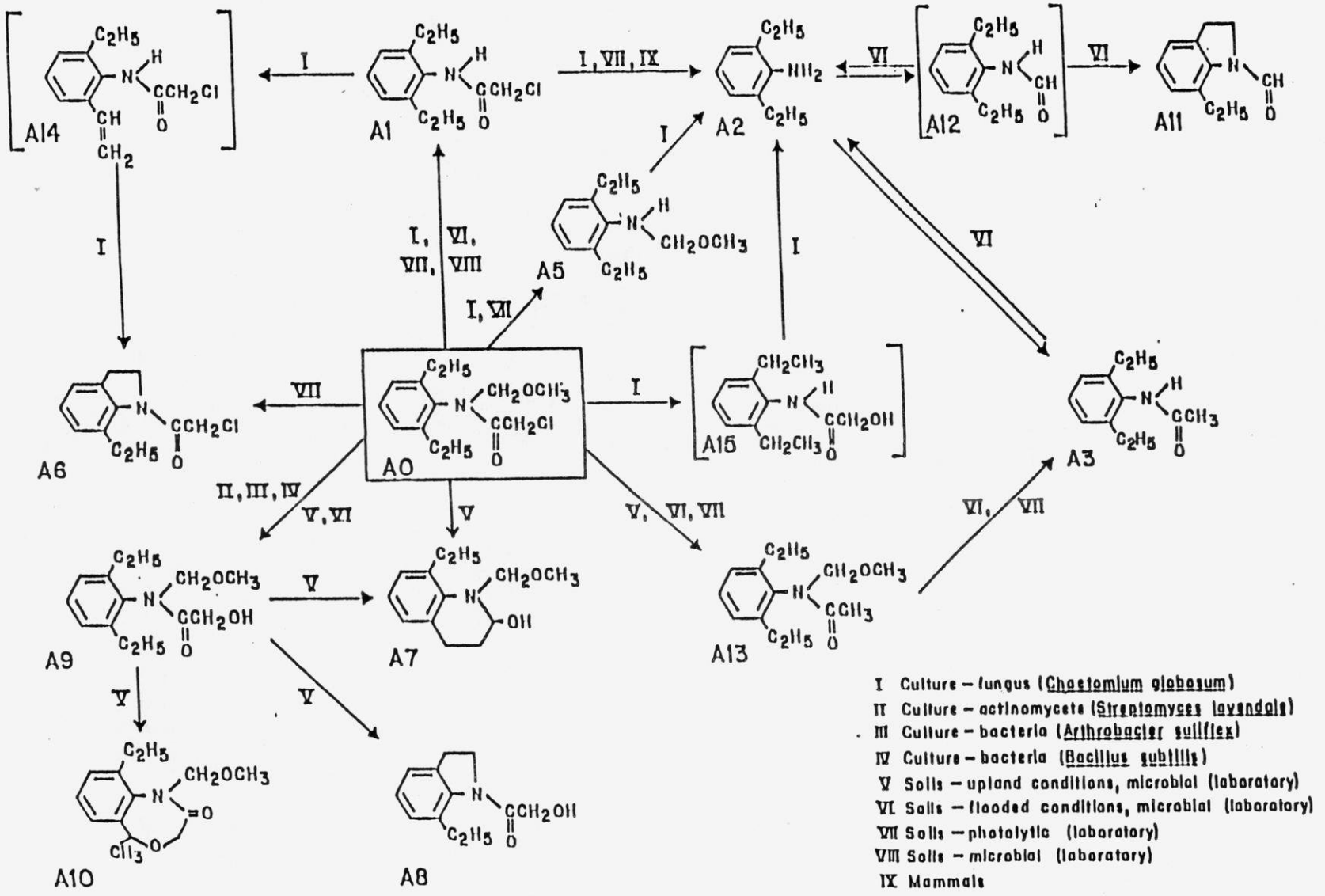

Fig. 6. Degradation pathways of alachlor (A0) (after Chesters et al., 1989).

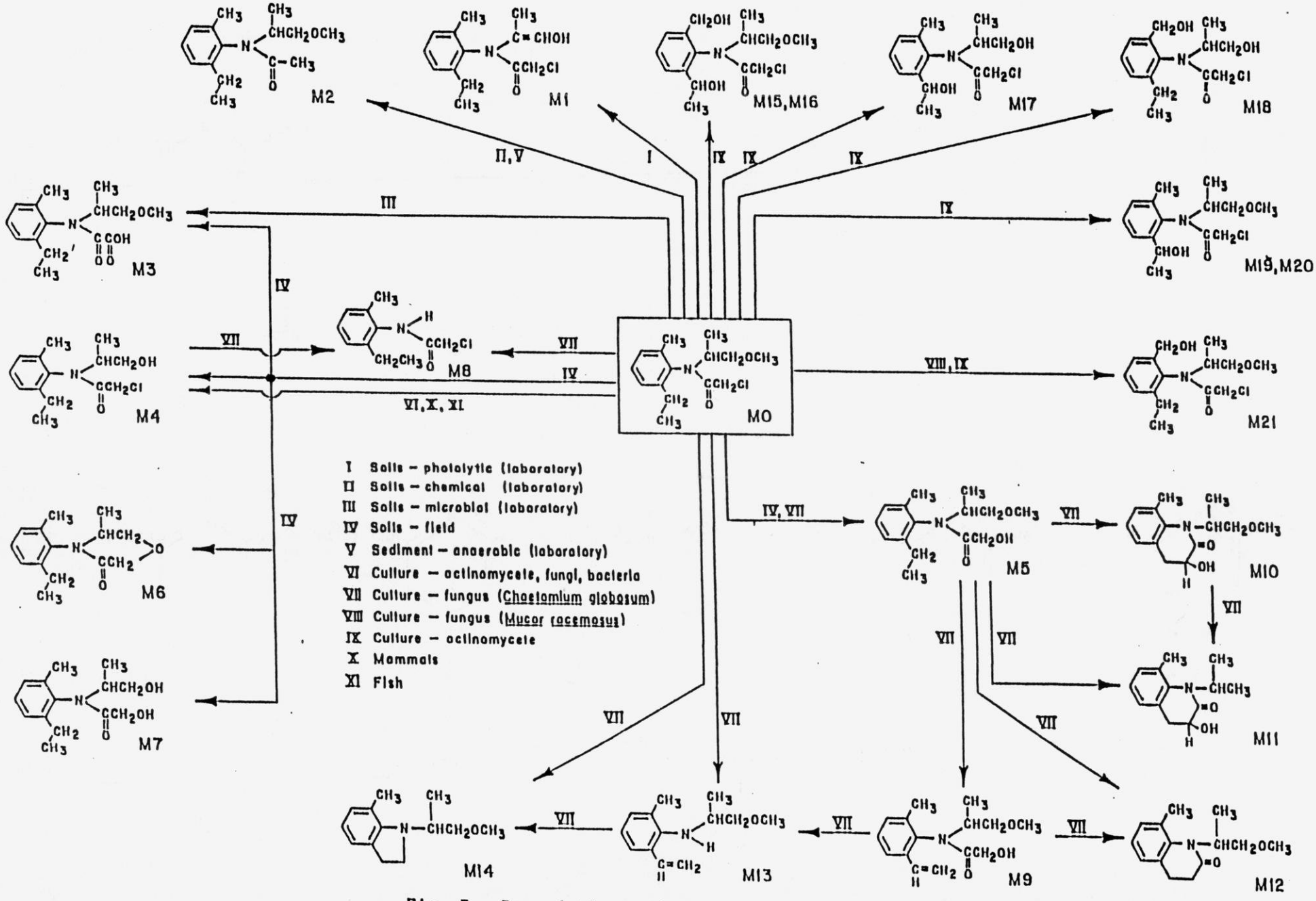

Fig. 7. Degradation pathways of metolachlor (M0) (after Chesters et al., 1989).

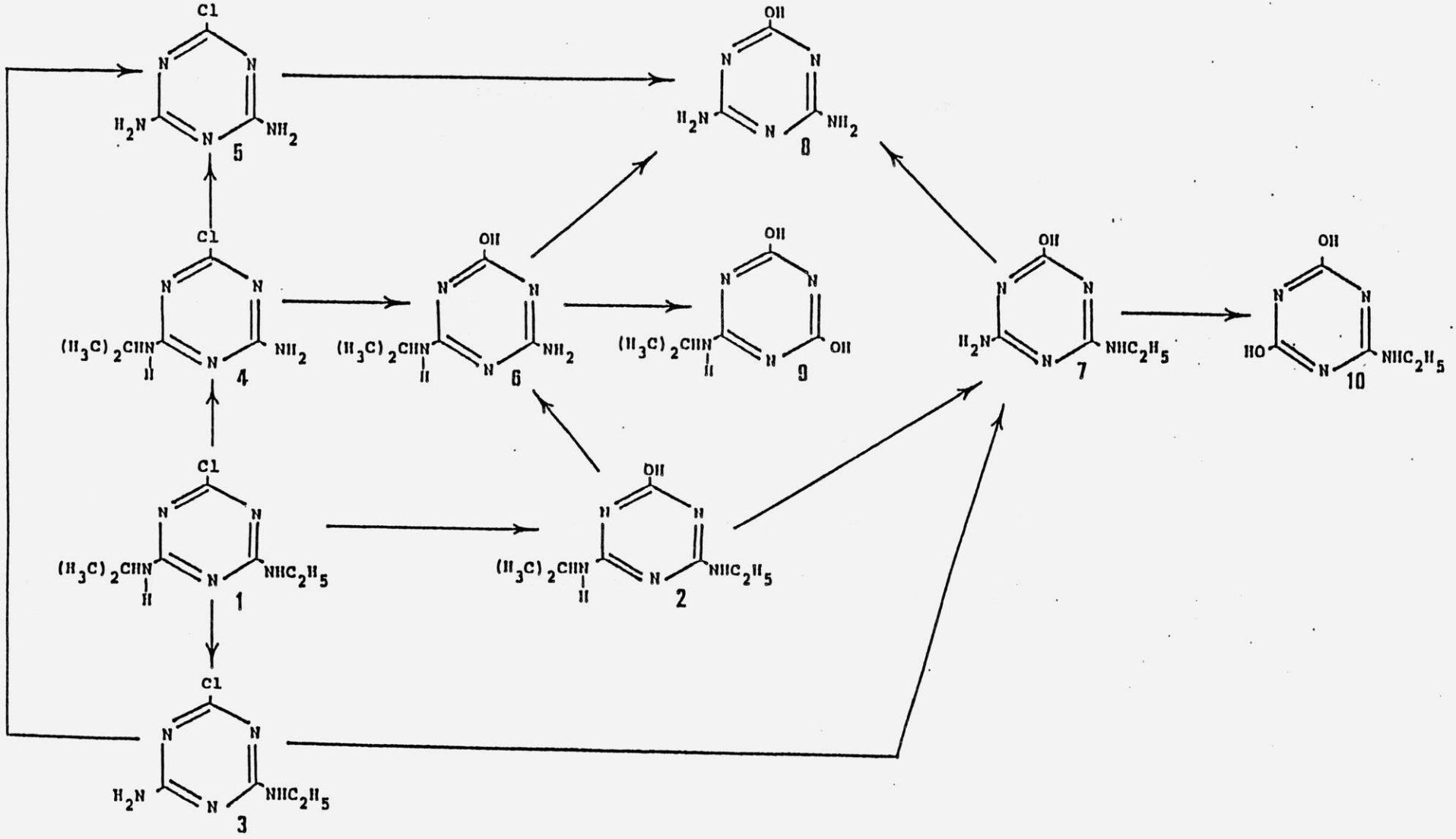

Fig. 8. Metabolic pathways of atrazine (1).

Table 11. Metabolites of alachlor (AO) identified in various environments (after Chesters et al., 1989)

Code no.	Chemical name	Identified in
AO	2-Chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide	Parent compound
A1[a-e]	2-Chloro-2',6'-diethylacetanilide	Soils, photolytic and microbial (lab); culture, fungus
A2[a,d,f]	2,6-Diethylaniline	Soils, photolytic (lab); culture, fungus; soil (flooded), microbial (lab); rat liver
A3[a,f]	2',6',-Diethylacetanilide	Soils, photolytic (lab); soils (flooded) microbial (lab)
A4[a]	Chloroacetic acid	Soils, photolytic (lab)
A5[a,d,g]	2',6'-Diethyl-N-methoxymethylaniline	Soils, photolytic (lab); culture, fungus
A6[a,c,d]	1-Chloroacetyl-2,3-dihydro-7-ethylindole	Soils, photolytic and microbial (lab); culture, fungus
A7[g]	8-Ethyl-2-hydroxy-1-methoxymethyl-1,2,3,4-tetrahydroquinoline	Soils (upland), microbial (lab)
A8[g]	7-Ethyl-1-hydroxyacetyl-2,3-dihydroindole	Soils (upland),microbial (lab)
A9[f,g]	2',6'-Diethyl-N-2-hydroxy(methoxymethyl)acetanilide	Soils (upland and flooded), microbial (lab); culture-actinomycete, bacteria
A10[g]	9-Ethyl-1,5-dihydro-1-(methoxymethyl)-5-methyl-4,1-benzoxazepin-2-(3H)-one	Soils (upland),microbial (lab)
A11[f]	1,2,3-Dihydro-1-formyl-7-ethylindole	Soils (flooded), microbial (lab)
A12[f]	2,6-Diethylformamide	Soils (flooded), microbial (lab)-intermediate
A13[f]	2',6'-Diethyl-N-(methoxymethyl)acetanilide	Soils (flooded), microbial (lab)
A14[d]	2-Chloro-2'-ethyl,6'-etheneacetanilide	Culture, fungus-intermediate
A15[d]	2-Hydroxy-2',6'-diethylacetanilide	Culture, fungus-intermediate
A16[h]	Mercapurate conjugate of A15	Monkey urine
A17[h]	Mercapturate conjugate of AO	Monkey urine
A18[h]	Cysteine conjugate of AO	Monkey urine
A19[h]	Thioacetic acid conjugate of AO	Monkey urine
A20[h]	Glucuronide conjugate of demethoxyalachlor	Monkey urine
A21[h]	Glutathione conjugate of AO	Red blood of mouse, monkey, and human
A22[h]	N-[(2,6-Diethyl)phenyl]-N-methoxymethyl-2-(hydroxy)acetamide	Rats
A23[h]	2',6'-Diethyl-N-(methoxymethyl)oxanilic acid, sodium salt	Rats
A24[h]	N-[(2,6-Diethyl)phenyl]-N-methoxymethyl-2-amino-2-oxaethanesulfonic acid, sodium salt	Rats
A25[h]	3-[N-(2,6-Diethyl)phenyl-N-methoxymethyl-2-amino-2-oxoethane-sulfinyl]-2-hydroxypropanoic acid, sodium salt	Rats
A26[h]	N-[(2-Ethyl-6-(1-hydroxyethyl)phenyl]-N-methoxymethyl-2-methylsulfonyl acetamide	Rats
A27[h]	N-(Methoxymethyl)-2-(methylsulfonyl)-2',6'-diethylacetanilide	Rats
A28[h]	N-(Methoxymethyl)-2-(methylsulfinyl)-2',6'-diethylacetanilide	Rats
A29[h]	2-(Methylsulfinyl)-2',6'-diethylacetanilide	Rats
A30[h]	N-2-Ethyl-6-(1-acetoxyethyl)-phenyl-2-(methylsulfonyl)acetamide	Rats
A31[h]	N-[2-Ethyl-6-(1-hydroxylethyl)-phenyl]-2-(methylsulfonyl)acetamide	Rats
A32[h]	N-(Methoxymethyl)-2-(methylthio)-2',6'-diethylacetanilide	Rats
A33[i]	2,6-Diethylnitrosobenzene	Rat liver

[a]Pang (1977); [b]Hargrove and Merkle (1971); [c]Chou (1977); [d]Tiedje and Hagedorn (1975); [e]Sethi and Chopra (1975); [f]Lee (1984); [g]Lee (1986); [h]USEPA (1986); [i]Kinnel et al. (1986)

Table 12. Metabolites of metolachlor (M0) identified in various environments (after Chesters et al., 1989)

Code no.	Chemical name	Identified in
M0[a]	2-Chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(methoxyprop-2-yl)acetamide	Parent compound
M1[b,c]	*N*-Chloroacetyl-*N*-(hydroxyprop-1-en-2-yl)-2-ethyl-6-methylaniline	Soils, photolytic (lab)
M2[b-e]	*N*-(2-Ethyl-6-methylphenyl)-*N*-(methoxyprop-2-yl)acetamide	Soils, chemical (lab); sediment, anaerobic (lab)
M3[b]	*N*-(2-Ethyl-6-methylphenyl)-*N*-(methoxyprop-2-yl)oxalamide	Soils, microbial (lab); soils (field)
M4[b,e-h]	2-Chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(hydroxyprop-2-yl)acetamide	Soils (field); culture-actinos., fungi, bact.; rat urine; fish
M5[b,c,e,f]	2-Hydroxy-*N*-(2-ethyl-6-methylphenyl)-*N*-(methoxyprop-2-yl)acetamide	Soils (field); water, photolytic (lab); culture, fungus; fish
M6[b]	4-(2-Ethyl-6-methylphenyl)-5-methyl-3-morpholinone	Soils (field)
M7[b]	2-Hydroxy-*N*-(2-ethyl-6-methylphenyl)-*N*-(hydroxyprop-2-yl)acetamide	Soils (field)
M8[f]	2-Chloro-*N*-(2-ethyl-6-methylphenyl)acetamide	Culture, fungus
M9[f]	2-Hydroxy-*N*-(2-methyl-6-vinylphenyl)-*N*-(methoxyprop-2-yl)acetamide	Culture, fungus
M10[f]	3-Hydroxy-8-methyl-*N*-(methoxyprop-2-yl)-2-oxo-1,2,3,4-tetrahydroquinoline	Culture, fungus
M11[f]	3-Hydroxy-*N*-isopropyl-8-methyl-2-oxo-1,2,3,4-tetrahydroquinoline	Culture, fungus
M12[f]	*N*-(Methoxyprop-2-yl)-8-methyl-2-oxo-1,2,3,4-tetrahydroquinoline	Culture, fungus
M13[f]	*N*-(Methoxyprop-2-yl)-*N*-(2-methyl-6-vinyl)aniline	Culture, fungus
M14[f]	1-(Methoxyprop-2-yl)-7-methyl-2,3-dihydroindole	Culture, fungus
M15,M16[g]	Isomers of 2-Chloro-*N*-[2-(1-hydroxyethyl)-6-hydroxymethylphenyl]-*N*-(methoxyprop-2-yl)acetamide	Culture, actinomycete
M17[g]	2-Chloro-*N*-[2-(1-hydroxyethyl)-6-methylphenyl]-*N*-(hydroxyprop-2-yl)acetamide	Culture, actinomycete
M18[g]	2-Chloro-*N*-(2-ethyl-6-hydroxymethylphenyl)-*N*-(hydroxyprop-2-yl)acetamide	Culture, actinomycete
M19,M20[g]	Isomers of 2-Chloro-*N*-[2-(1-hydroxyethyl)-6-methylphenyl]-*N*-(methoxyprop-2-yl)acetamide	Culture, actinomycete
M21[g,h]	2-Chloro-*N*-(2-ethyl-6-hydroxymethylphenyl)-*N*-(methoxyprop-2-yl)acetamide	Culture-actinomycete, fungus
M22[b,c]	4-(2-methyl-6-ethylphenyl)-5-methylmorpholine	Water, photolytic (lab)
M23[d]	*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylthio)acetamide	Sediment, anaerobic (lab)
M24[b,i,j]	Glutathione conjugate of M0	Corn, rape
M25[i,j]	Cysteine conjugate of M0	Corn, rape
M26[i]	Malonylcysteine conjugate of M0	Peanut
M27[i]	Sulfoxide of M26	Peanut
M28[j]	Thiolactic acid conjugate of M0	Corn, rape
M29[j]	Sulfoxide of M28	Corn, rape
M30[j]	Glucoside of M28	Corn, rape
M31[e]	*N*-(2-Ethyl-6-methylphenyl)-2-hydroxyacetamide	Rat urine
M32[e]	*N*-(2-Ethyl-6-methylphenyl)-*N*-(hydroxyacetyl)-DL-alanine	Rat urine and feces
M33[e,k]	2-Ethyl-6-methylaniline	Rat liver
M34[k]	2-Ethyl-6-methylnitrosobenzene	Rat liver

[a]M0 is often named 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide.
[b]USEPA (1980); [c]Dynamac (1986a); [d]McGahen (1982); [e]Dynamac (1986b); [f]McGahen and Tiedje (1978); [g]Krause et al. (1985); [h]Saxena et al. (1987); [i]Lamoureux and Rusness (1983); [j]Blattmann et al. (1986); [k]Kimmel et al. (1986).

Table 13. Metabolites of atrazine (1) in soils

Code no.	Chemical name
1	2-Chloro-4-ethylamino-6-isopropylamino-s-triazine
2	2-Hydroxy-4-ethylamino-6-isopropylamino-s-triazine
3	2-Chloro-4-ethylamino-6-amino-s-triazine
4	2-Chloro-4-amino-6-isopropylamino-s-triazine
5	2-Chloro-4,6-diamino-s-triazine
6	2-Hydroxy-4-amino-6-isopropylamino-s-triazine
7	2-Hydroxy-4-ethylamino-6-amino-s-triazine
8	2-Hydroxy-4,6-diamino-s-triazine
9	2,4-Dihydroxy-6-isopropylamino-s-triazine
10	2,6-Dihydroxy-4-ethylamino-s-triazine

dilution. A dispenser holding 1 ml allows inoculation of up to ten different media from one dispensation. A control medium inoculated with the first and last (tenth) aliquot from one or more dispensations allows a check of aliquot reproducibility. Incubated plates are monitored for positive growth responses, and total counts derived from MPN tables. Counts of morphologically distinct colonies are obtained similarly, and representative isolates removed for further characterization. A flow diagram scheme based on routinely-measurable taxonomic properties is presented for rudimentary characterization of aerobic heterotrophic bacteria. Examples are presented of the use of the approach to evaluate herbicide impact on predominant and herbicide tolerant and degrading, aerobic heterotrophs in soil.

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III. REVIEWS OF FATE OF HERBICIDES IN THE ENVIRONMENT

A. A detailed review of the Environmental Fate of Alachlor and Metolachlor has been prepared to provide us with existing information of these compounds. The review is being published by Reviews of Environmental Contamination and Toxicology and will appear in volume 10 as pages 1-74, published by Springer-Verlag in New York, Berlin, Heidelberg, and Tokyo. The article received excellent peer reviews and preprints have been distributed to investigators in the University of Wisconsin-System, and the state departments of Natural Resources and Agriculture, Trade and Consumer Protection. The document has been used as the basis for establishing future experiments. Because of its length only the Table of Contents is included overleaf.

B. Three presentations were prepared by G. Chesters for a Summer School held in Venice, Italy in June 1989. Negotiations are presently underway by the Academy of Sciences, Arts and Letters of Veneto to have these papers published in book form by Elsevier Press. The three Chesters presentations dealt with the Environmental Fate of Alachlor, Metolachlor, Aldicarb and Atrazine and are briefly described as:

1. Adsorption and Movement--Covers state-of-the-art information on the adsorption, movement and leaching of each of the four herbicides in the environment. The chapter is 14 single spaced pages in length.

2. Transformations--Details current knowledge on the degradation, metabolic products and breakdown rates of each of the herbicides in soils, aquatic systems, plants, mammals and fish. The chapter is 20 pages.

3. Toxicology--This chapter summarizes what is known about the toxicity of each of the herbicides to humans, animals, avian and aquatic organisms. The animal experiments have been most extensive in scope and were divided for discussion purposes into sections on acute toxicity, chronic toxicity, reproductive effects, teratogenicity, mutagenicity, and carcinogenicity. The article is 15 pages.

Each of the three articles can be used by researchers and by personnel of the state agencies in regulatory fields as a major source of information on environmental contamination. They will be provided to Profs. Wyman and Binning Co-directors of the Nutrient and Pest Management Program in the Center for Integrated Agriculture, for modification to a less technical format for presentation to the public. Chesters and Wyman have already discussed this and will move expeditiously to have the documents rewritten for the understanding of the general public and decisionmakers.

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Environmental Fate of Alachlor and Metolachlor

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