

Analysis of Selected Herbicide Metabolites in Surface and Ground Water of the United States

By Elisabeth A. Scribner, E.M. Thurman, and Lisa R. Zimmerman

ABSTRACT

One of the primary goals of the U.S. Geological Survey (USGS) laboratory in Lawrence, Kansas, is to develop analytical methods for the analysis of herbicide metabolites in surface and ground water that are vital to the study of herbicide fate and degradation pathways in the environment. Methods to measure metabolite concentrations from three major classes of herbicides—triazine, chloroacetanilide, and phenylurea—have been developed. Methods for triazine metabolite detection cover nine compounds; six compounds are detected by gas chromatography/mass spectrometry, one is detected by high-performance liquid chromatography with diode-array detection, and eight are detected by liquid chromatography/mass spectrometry. Two metabolites of the chloroacetanilide herbicides—ethane sulfonic acid and oxanilic acid—are detected by high-performance liquid chromatography with diode-array detection and liquid chromatography/mass spectrometry. Alachlor ethane sulfonic acid also has been detected by solid-phase extraction and enzyme-linked immunosorbent assay. Six phenylurea metabolites are all detected by liquid chromatography/mass spectrometry; four of the six metabolites also are detected by gas chromatography/mass spectrometry.

Additionally, surveys of herbicides and their metabolites in surface water, ground water, lakes, reservoirs, and rainfall have been conducted through the USGS laboratory in Lawrence. These surveys have been useful in determining herbicide and metabolite occurrence and temporal distribution and have shown that metabolites may be useful in evaluation of nonpoint-source contamination.

INTRODUCTION

Herbicides in surface and ground water are a major concern throughout the United States. Numerous studies have been completed by various government agencies, including the U.S. Geological Survey (USGS), and by chemical manufacturers to document occurrences of herbicides in ground water (Kolpin and others, 1996), rainfall (Goolsby and others, 1997; Pomes and others, 1998), and surface water (Thurman and others, 1992). However, there has been little study of the metabolites of these herbicides. Because metabolites are formed in the environment and transported to surface and ground water, it is important to understand herbicide use and the fate of herbicide metabolites.

Modern agricultural practices in the United States often require extensive use of herbicides for production of corn, soybeans, sorghum, and other row crops. Data compiled by Gianessi and Puffer (1991) indicate that about two-thirds of the 285 million kg (kilograms) of herbicides applied

annually in the United States are used in crop production in the Midwest. In 1990 and again in 1992, because of concern about water contamination, manufacturers voluntarily reduced the maximum recommended application rate by 50 percent for atrazine, the most used herbicide on corn and sorghum. These application-rate changes have affected the frequency of detection of some of the herbicide metabolites (Scribner and others, 1998).

In 1987, a laboratory for organic geochemistry research was established at the USGS in Lawrence, Kansas. Since that time, analytical methods have been and continue to be developed to measure metabolite concentrations from three major classes of herbicides—triazine, chloroacetanilide, and phenylurea. From studies of degradation, fate, and transport of herbicides and their metabolites in soil and aquatic environments, numerous journal articles, book chapters, and USGS reports have been published.

The principal purpose of this paper is to list the analytical methods that have been developed to

measure herbicide metabolites in surface and ground water. Listings of herbicide and metabolite surveys that have been conducted through the USGS laboratory in Lawrence also are summarized. Brief descriptions and illustrations depicting the degradation pathways for the parent herbicides also are presented.

ANALYTICAL METHODS DEVELOPED

Methods developed include use of gas chromatography/mass spectrometry (GC/MS), high-performance liquid chromatography with

diode-array detection (HPLC/DAD), liquid chromatography/mass spectrometry (LC/MS), and solid-phase extraction (SPE) and enzyme-linked immunosorbent assay (ELISA) for analysis of triazine, chloroacetanilide, and phenylurea herbicide metabolites as shown in table 1. Triazine metabolites include deethylatrazine (DEA), deisopropylatrazine (DIA), hydroxyatrazine (HA), cyanazine acid (CAC), cyanazine amide (CAM), deethylcyanazine (DEC), deethylcyanazine acid (DCAC), deethylcyanazine amide (DCAM), and deisopropylprometryn. Six triazine metabolites are analyzed by GC/MS according to procedures described by Thurman and others (1990) and Meyer and

Table 1. Methods of analysis for triazine, chloroacetanilide, and phenylurea herbicide metabolites at the U.S. Geological Survey laboratory, Lawrence, Kansas

[GC/MS, gas chromatography/mass spectrometry; HPLC/DAD, high-performance liquid chromatography with diode-array detection; LC/MS, liquid chromatography/mass spectrometry; SPE, solid-phase extraction; and ELISA, enzyme-linked immunosorbent assay]

Metabolite	Method			
	GC/MS	HPLC/ DAD	LC/MS	SPE/ ELISA
Triazine metabolites				
Deethylatrazine (DEA)	X		X	
Deisopropylatrazine (DIA)	X		X	
Hydroxyatrazine (HA)		X	X	
Cyanazine acid (CAC)			X	
Cyanazine amide (CAM)	X		X	
Deethylcyanazine (DEC)	X		X	
Deethylcyanazine acid (DCAC)			X	
Deethylcyanazine amide (DCAM)	X		X	
Deisopropylprometryn	X			
Chloroacetanilide metabolites				
Acetochlor ethane sulfonic acid (ESA)		X	X	
Acetochlor oxanilic acid (OXA)		X	X	
Alachlor ethane sulfonic acid (ESA)		X	X	X
Alachlor oxanilic acid (OXA)		X	X	
Metolachlor ethane sulfonic acid (ESA)		X	X	
Metolachlor oxanilic acid (OXA)		X	X	
Phenylurea metabolites				
3,4-dichloroaniline (DCA)	X		X	
3,4-dichlorophenylurea (DCPU)			X	
3,4-dichloromethylphenylurea (DCPMU)			X	
Trifluoromethylaniline (TFMA)	X		X	
Trifluoromethylphenylurea (TFMPU)	X		X	
Demethylfluometuron (DMFM)	X		X	

others (1993). Hydroxyatrazine is analyzed by both HPLC/DAD and LC/MS (Lerch and others, 1998). Since 1998, all triazine metabolites are analyzed by LC/MS except for deisopropylprometryn.

Two metabolites of the chloroacetanilide herbicides—ethane sulfonic acid (ESA) and oxanilic acid (OXA)—are detected by HPLC/DAD and LC/MS for acetochlor, alachlor, and metolachlor (Ferrer and others, 1997; Kalkhoff and others, 1998; Kolpin and others, 1998). Alachlor ESA is also analyzed by SPE and ELISA (Aga and others, 1994).

Phenylurea metabolites of diuron—3,4-dichloroaniline (DCA), 3,4-dichlorophenylurea (DCPU), and 3,4-dichloromethylphenylurea (DCPMU)—are analyzed by LC/MS. DCA is also analyzed by GC/MS. Phenylurea herbicide analysis also includes the metabolites of fluometuron—trifluoromethylaniline (TFMA), trifluoromethylphenylurea (TFMPU), and demethylfluometuron (DMFM)—which are detected by both GC/MS and LC/MS.

SURVEYS OF HERBICIDES AND METABOLITES

Surveys conducted in the Midwestern United States through the USGS laboratory in Lawrence are: 1989–90—surface-water runoff at 147 reconnaissance sites in a 10-State area (Scribner and others, 1993); 1990–91—rainfall samples from 81 collection sites (Goolsby and others, 1995); 1990–92—storm-runoff samples from nine stream basins (Scribner and others, 1994); 1991–98—samples from 303 well sites (Kolpin and others, 1993); 1992–93—samples collected from 76 reservoirs (Scribner and others, 1996); 1993—samples from the Mississippi River during flood stage (Goolsby and others, 1993); and 1994–95 and 1998—samples from 53 streams to help determine if changes in herbicide use resulted in a change in herbicide concentrations since the 1989–90 reconnaissance study (Scribner and others, 1998).

Further surveys of herbicides and their metabolites in the cotton-growing areas of the United States have been made to relate herbicide use to occurrence in streams during 1995–97 (Coupe and others, 1998). In 1996, surface-water samples were collected at 64 sites in the Mississippi Embayment and analyzed in conjunction with the

USGS National Water-Quality Assessment (NAWQA) Program. Special emphasis in the 1996 survey was placed on streams in the Mississippi Delta as described in Thurman and others (1998).

DEGRADATION PATHWAYS

Concentrations of metabolites in water commonly may be equal to or even exceed concentrations of parent compounds. It has been found that metabolite concentrations in ground water often exceed parent compound concentrations for both triazine and chloroacetanilide herbicides, whereas in surface water the parent compound is most abundant after application of herbicide in the spring and is replaced gradually with metabolites throughout the growing season. In the fall, the metabolite concentrations may exceed concentrations of the parent compound (Kolpin and others, 1996, 1998; Kalkhoff and others, 1998).

Degradation of the phenylurea herbicides of diuron and fluometuron is similar. Both herbicides degrade by N-demethylation under aerobic conditions to metabolites (Ahrens, 1994; Field and others, 1997).

Triazine Metabolites

The triazine herbicides—atrazine, cyanazine, simazine, and propazine—are four compounds that have been used on corn and sorghum in the Midwestern United States. Application amounts of these herbicides in 1995 were 20 million kilograms of atrazine, 11 million kilograms of cyanazine, and 0.3 million kilograms of simazine. Propazine use has been discontinued (Gianessi and Puffer, 1991). Triazine herbicides degrade by various pathways to a series of metabolites. Atrazine degrades in soil through both biotic and abiotic reactions to the dealkylated metabolites, DEA and DIA, and the hydroxylated metabolite, HA (fig. 1). DEA may further degrade to the dealkylated hydroxymetabolites of didealkylatrazine (DDA), hydroxydeethylatrazine (HDEA), and hydroxydeisopropylatrazine (HDIA). DIA may further degrade to the hydroxymetabolites of DDA and HDIA. HA may degrade to dealkylated HDIA and HDEA (Lerch and others, 1998). The atrazine degradation pathway includes further dealkylation of DEA, DIA, and HA to the opening of the ammeline ring and eventual



Figure 1. Pathways for degradation of atrazine.

mineralization to carbon dioxide and nitrogen gas (Gunther and Gunther, 1970). The degradation pathway of atrazine generally is well known and studied extensively.

Field-dissipation studies of the four chlorinated parent triazine herbicides atrazine, cyanazine, simazine, and propazine have found that they all degrade in soil in similar fashion and form at least one of two common dealkylated metabolites, DIA and (or) DEA. Figure 2 summarizes triazine degra-

dation pathways described in Thurman and others (1994), which documents work on all four triazine compounds using field-dissipation studies.

Cyanazine is used primarily on corn in the upper Midwest. The work done by the USGS laboratory in Lawrence is the first major integrated research conducted on the geochemistry of cyanazine in surface water of the Midwest. Methods development, field-dissipation, and regional studies by laboratory personnel indicate that cyanazine is



Figure 2. Pathways for degradation of atrazine, cyanazine, propazine, and simazine to DEA and DIA.

more labile than atrazine and that they both have a common metabolite, DIA (Meyer, 1994). Cyanazine degrades by deethylation to DEC, which degrades rapidly to DCAM. DCAM, in turn, degrades to DCAC, which further degrades to DDA. CAM is an important metabolite of cyanazine that is readily detected. CAM degrades to DCAM and CAC. CAC then degrades to DCAC and DIA, which further degrade to DDA. CAC and DCAC may be rapidly transported through the unsaturated zone (Meyer, 1994). Structures and degradation pathways for cyanazine and its metabolites are shown in figure 3.

Various studies in the Midwestern United States have demonstrated the importance of the two triazine metabolites, DEA and DIA, which were found to occur in water that has received parent triazine herbicides. These studies show that DEA has atrazine as its major source (98 percent) and only trace levels are derived from propazine (fig. 2). DIA has atrazine as its major source (75 percent) and cyanazine as a secondary source (25 percent). Trace amounts of DIA are contributed by simazine. Propazine and simazine do not contribute substan-

tially to the DIA-to-DEA ratio in surface water (Thurman and others, 1994). When DEA is the major metabolite in the unsaturated zone, the deethylatrazine-to-atrazine ratio (DAR) may be used to document the first major runoff of herbicides from nonpoint-source corn fields to surface water (Thurman and Fallon, 1996). The DAR in soil water quickly decreases from about 0.5 to less than 0.1 upon application of herbicide and the first major runoff occurrence in a basin. The DAR then gradually increases to values of approximately 0.4 to 0.6 during the harvest season. Atrazine and DEA have been reported frequently in ground water but less is known about the occurrence of HA in surface water. HA occurs at concentrations considerably less than atrazine or its metabolites, DEA and DIA (Lerch and others, 1998).

Other triazine herbicides analyzed by GC/MS include prometryn. Prometryn is a parent herbicide used in cotton-growing areas of the United States. Its dealkylated metabolite, deisopropylprometryn, also is analyzed by GC/MS. During a recent study in the Mississippi Embayment, prometryn was not detected in water samples at concentrations greater



Figure 3. Pathways for degradation of cyanazine.

than 1.0 microgram per liter ($\mu\text{g/L}$), and deisopropylprometryn was not detected at concentrations greater than the reporting level of $0.05 \mu\text{g/L}$ (Thurman and others, 1998). The less extensive use of

prometryn probably was responsible for the low concentrations in the samples analyzed (Coupe and others, 1998).



Figure 4. Pathways for degradation of acetochlor, alachlor, and metolachlor to ethane sulfonic acid and oxanilic acid.

Chloroacetanilide Metabolites

The chloroacetanilide herbicides—acetochlor, alachlor, and metolachlor—constitute the second major class of herbicides used in the United States. Together with triazine herbicides, chloro-

acetanilide herbicides account for the majority of herbicides applied to farmland in the Midwestern United States (Gianessi and Puffer, 1991). Alachlor and metolachlor have been used for more than 20 years (Thurman and others, 1996). Acetochlor was used for the first time in 1994. In general, chlo-



Figure 5. Pathways for degradation of diuron.

roacetanilide herbicides are known to degrade more quickly in soil than triazine herbicides, and typical half-lives of the chloroacetanilide herbicides range from 15 to 30 days (Leonard, 1988), compared to 30 to 60 days for triazine herbicides (Ferrer and others, 1997). Figure 4 shows the degradation pathway of the chloroacetanilide parent compounds to ESA and OXA. Chloroacetanilide herbicides have been analyzed by HPLC/DAD (Kolpin and others, 1998); however, since 1998, these metabolites also are analyzed by LC/MS at the USGS laboratory in Lawrence. SPE and ELISA are combined for the trace analysis of the herbicide alachlor and its major soil metabolite, ESA. The method is viable for the analysis of both surface- and ground-water samples and is comparable to GC/MS and HPLC analyses for alachlor and ESA (Aga and others, 1994).

Phenylurea Metabolites

The phenylurea herbicides, diuron and fluometuron, are used in the cotton-growing areas of

the United States. Diuron is also used in many other areas on fruit crops. The annual application of diuron is about 1.8 million kg of active ingredient (Gianessi and Anderson, 1995). It was ranked as the third most hazardous pesticide to ground-water resources (Newman, 1995). Diuron degrades by N-demethylation under aerobic conditions to metabolites including 3,4-dichloromethylphenylurea (DCPMU), 3,4-dichlorophenylurea (DCPU), and 3,4-dichloroaniline (DCA) (Dalton and others, 1966). The degradation pathway for diuron and its metabolites, DCPMU, DCPU, and DCA, is shown in figure 5.

Fluometuron is used primarily in Mississippi and in the eastern coastal plain (Gianessi and Anderson, 1995) as a preemergent herbicide for broadleaf and grass control in cotton; therefore, the timing of the highest concentrations of fluometuron in surface water is much different than for corn because application times are different (4.7 average annual applications on cotton versus 1.2 average annual applications on corn). Application of flu-

ometuron in the United States totaled about 1.5 million kg in 1996 at an average rate of approximately 0.81 kg per hectare of active ingredient (National Agricultural Statistics Service, 1997). Three fluometuron metabolites that have been analyzed are TFMA, TFMPU, and DMFM. In a study using GC/MS (Coupe and others, 1998), analyses showed that DMFM was the most common and was present in the highest concentration. TFMPU was not detected in any of the samples, and TFMA was detected infrequently and in low concentrations (Coupe and others, 1998). The degradation pathways of fluometuron and its metabolites are shown in figure 6.

SUMMARY

The U.S. Geological Survey laboratory in Lawrence, Kansas, was established in 1987 to enhance scientific knowledge in the field of organic geochemistry. Special emphasis has been on

water-quality analysis as related to problem areas involving contamination of surface and ground water. Analytical methods continue to be developed to assess the nature, amount, and movement of herbicides and their metabolites in soil and water. This scientific work is important to define water quality and its relation to nonpoint-source contamination.

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Figure 6. Pathways of degradation of fluometuron.

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AUTHOR INFORMATION

Elisabeth A. Scribner and E.M. Thurman, U.S. Geological Survey, Lawrence, KS (scribner@usgs.gov and ethurman@usgs.gov)

Lisa R. Zimmerman, University of Kansas Center for Research, Inc., Lawrence, KS (lzimmer@usgs.gov)