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Environmental Toxicology and Chemistry, Vol. 18, No. 10, pp. 2161–2168, 1999
Printed in the USA
0730-7268/99 $9.00 + .00

HYDROXYATRAZINE IN SOILS AND SEDIMENTS

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(*Received* 24 *August* 1998; *Accepted* 8 *January* 1999)

Abstract—Hydroxyatrazine (HA) is the major metabolite of atrazine in most surface soils. Knowledge of HA sorption to soils, and its pattern of stream water contamination suggest that it is persistent in the environment. Soils with different atrazine use histories were collected from four sites, and sediments were collected from an agricultural watershed. Samples were exhaustively extracted with a mixed-mode extractant, and HA was quantitated using high performance liquid chromatography with UV detection. Atrazine, deethylatrazine (DEA), and deisopropylatrazine (DIA) were also measured in all samples. Concentrations of HA were considerably greater than concentrations of atrazine, DEA, and DIA in all soils and sediments studied. Soil concentrations of HA ranged from 14 to 640 μg/kg with a median concentration of 84 μg/kg. Sediment concentrations of HA ranged from 11 to 96 μg/kg, with a median concentration of 14 μg/kg. Correlations of HA and atrazine concentrations to soil properties indicated that HA levels in soils were controlled by sorption of atrazine. Because atrazine hydrolysis is known to be enhanced by sorption and pH extremes, soils with high organic matter (OM) and clay content and low pH will result in greater atrazine sorption and subsequent hydrolysis. Significant correlation of HA concentrations to OM, pH, and cation exchange capacity of sediments indicated that mixed-mode sorption (i.e., binding by cation exchange and hydrophobic interactions) was the mechanism controlling HA levels in sediment. The presence of HA in soils and stream sediments at the levels observed support existing hypotheses regarding its transport in surface runoff. These results also indicated that persistence of HA in terrestrial and aquatic ecosystems is an additional risk factor associated with atrazine usage.

Keywords—Hydroxyatrazine Atrazine Stream sediments Mixed-mode sorption Persistence

INTRODUCTION

Hydroxyatrazine (2-hydroxy-4-ethylamino-6-isopropylamino-*s*-triazine) (HA) has often been identified as the major atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) degradation product formed in surface soils [1–5]. In addition, most laboratory incubation studies report that 20 to 70% of the added ^{14}C-atrazine forms bound residues [1,4–9]. In an Iowa soil incubated for 120 d, Kruger et al. [9] reported that 73% of the added ^{14}C-atrazine formed bound residues, based on exhaustive extraction with aqueous methanol. The ^{14}C-bound residues in this soil were then extracted with a mixed-mode extractant (3:1 0.5 M KH_2PO_4, pH 7.5:CH_3CN, v/v) designed to disrupt both cation exchange and hydrophobic interaction binding mechanisms [10]. As much as 60% of the ^{14}C-bound residues were recovered by mixed-mode extraction, and HA accounted for 74% of this fraction [10]. Thus, HA formation in other soils likely has been underestimated because of its mixed-mode sorption and the inability of commonly used extractants to effectively disrupt these binding mechanisms [10].

Formation of HA in the environment occurs by chemical, biological, or photolytic hydrolysis of atrazine in soils and water [1–5,11–15]. Atrazine hydrolysis in soils is enhanced by sorption to soil colloids, dissolved organic matter (OM), and extremes in pH. In water, HA formation via photolytic hydrolysis has been demonstrated under a variety of laboratory conditions, but the environmental significance of this pathway remains questionable [16].

Hydroxyatrazine is an important surface water contaminant throughout the corn belt [16–19]. Concentrations of HA in surface water typically range from 0.2 to 3 μg/L, with the highest concentrations observed under postplant conditions [16,18,19]. Hydroxyatrazine contributed a significant proportion of the total atrazine load (i.e., concentration of atrazine plus metabolites) in 95 northern Missouri (USA) streams sampled in 1994 and 1995. Under preplant conditions, HA contributed 39% of the median atrazine load of 0.64 μg/L, and under postplant conditions, HA contributed 14% of the median atrazine load of 4.6 μg/L [16]. In an additional 46 midwestern streams under postplant conditions, detection frequency of HA was similar to that of atrazine, deethylatrazine (2-chloro-4-amino-6-isopropylamino-*s*-triazine) (DEA), and deisopropylatrazine (2-chloro-4-ethylamino-6-amino-*s*-triazine) (DIA), but its median concentrations were much lower [16]. In a 2.5-year monitoring study of Goodwater Creek, Missouri, USA, HA was detected in 100% of the samples [18]. In this study, HA concentrations during spring runoff events were 12 to 81 times lower than peak atrazine levels and 4 to 16 times lower than peak DEA levels. However, median concentrations for the study were similar: 0.38 μg/L for HA, 0.57 μg/L for atrazine, 0.36 μg/L for DEA, and 0.21 μg/L for DIA [18]. The greater persistence and sorption of HA compared to atrazine, DEA, and DIA [6,20–24] results in attenuated HA concentrations during spring runoff events, but more consistent year-round concentrations and high frequencies of detection

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Table 1. Atrazine use history for each field site

Location	Site	Crop rotation[a]	No. atrazine applications	Time since last application[b] (months)	Atrazine rates[c] (kg/ha)	Total atrazine applied (kg/ha)
Iowa	IA-1	C-C	20 since 1972	24	0.3–3.4	37
	IA-2	C-C	14 since 1972	12	0.4–3.2	19
Kansas	KS-1	C-S	15 since 1972	22	1.3–2.2	22
	KS-2	C-C	25 since 1972	10	1.3–2.2	38
Missouri[d]	MO-1	C-S	4 since 1988	13	1.6–2.2	8.3
	MO-2	C-S	4 since 1987	13	1.6–2.2	8.3
	MO-3	C-S	4 since 1986	13	1.6–2.2	8.3
Ohio[d]	OH-1	C-S	4 since 1987	24	1.2–3.4	8.1
	OH-2	C-S-W	4 since 1986	36	1.3–1.7	6.0
	OH-3	C-C	8 since 1985	12	1.1–3.4	17

[a] C-C = corn–corn; C-S = corn–soybean; C-S-W = corn–soybean–wheat.
[b] Relative to sample collection date.
[c] Atrazine rates and total atrazine applied expressed as kilograms of active ingredient per hectare.
[d] Crop rotations at the Ohio and Missouri sites were as indicated since 1991. Before 1991, various rotations of corn, soybean, sorghum, and wheat were used.

[16,18]. Combining knowledge of HA in relation to stream hydrology, persistence and sorption in surface soil, and formation in the environment, Lerch et al. [16] proposed two mechanisms that control HA levels in streams: dissolved-phase transport from atrazine-treated fields under runoff conditions, and desorption of HA from streambed sediments by groundwater under base flow conditions. Under runoff conditions, some HA is extracted from the soil and transported to streams in the dissolved phase. Runoff events will also transport soil containing sorbed HA, and subsequent deposition in the streambed provides a source of HA-contaminated sediments under base flow conditions.

The potential accumulation and persistence of HA in soils and stream sediments represents environmental risk factors associated with atrazine usage that have not been previously addressed. Belfroid et al. [25] identified five categories, one of which is persistence and accumulation in sediment, as the basis for risk assessment of the impact of pesticide metabolites on aquatic ecosystems. Ranking of risk within each category is based on the metabolite behavior relative to the parent compound. Given the year-round detections of HA in streams [16,18], HA must persist at significant levels in field soils and stream sediments for the hypothesized mechanisms of Lerch et al. [16] to be valid. However, no measurements of HA in field soils or sediments have been reported to date. Thus, the primary objective of this study was to attempt to measure HA in field soils and stream sediments. Fulfillment of this objective would provide evidence in support of the surface transport mechanisms proposed by Lerch et al. [16], and it would establish whether or not HA represents a potential risk to ecosystems via persistence in soils and stream sediments. A secondary objective was to use mixed-mode extraction, combined with HPLC, and tandem mass spectroscopy (MS-MS) to quantitate and confirm the presence of HA in soils and sediments.

MATERIALS AND METHODS

Chemicals and standards

Analytical standards of atrazine, HA, DEA, and DIA were ≥95% pure (Crescent Chemical, Hauppauge, NY, USA). Stock solutions containing 10 mg/L HA were made in 0.1 M HCl. Working standards for HA analysis were prepared in the mixed-mode extractant (3:1 0.5 M KH_2PO_4, pH 7.5:CH_3CN, v/v) at concentrations of 5 to 250 μg/L. Atrazine, DEA, and DIA stock solutions of 10 mg/L were prepared in CH_3OH, and working standards were prepared in 2:3 CH_3OH:H_2O (v/v) (40% CH_3OH) at concentrations of 2 to 500 μg/L. All solvents and KH_2PO_4 used for soil extractions or analyses were HPLC grade, except chloroform ($CHCl_3$), which was pesticide grade (Fisher Scientific, Pittsburgh, PA, USA). The KH_2PO_4 solutions were adjusted to pH 7.5 using reagent-grade NaOH (50 or 75% w/v solutions). The HCl used for HA stock solutions was reagent grade.

Soil and sediment sampling

Field soils were collected from 0 to 15 cm depth at four midwestern U.S. locations at varying times from 1995 to 1997. Site selection was based on three criteria: well-documented records existed regarding atrazine use, soils represented a broad range of times since the last atrazine application, and soils varied in their atrazine use history (Table 1). Three of the sites are associated with the Management Systems Evaluation Area (MSEA) regional project: Deep Loess Hills Research Station, Treynor, Iowa, USA (IA site); Missouri MSEA site, Centralia, Missouri, USA (MO site); and Ohio MSEA site, Piketon, Ohio, USA (OH site). The fourth site was the Kansas River Valley Experimental Field site near Silver Lake, Kansas, USA. Soils from Iowa, Kansas, and Ohio were collected from separate fields with different atrazine use histories. At the Iowa site, soils were only collected from the toeslope, and, therefore, they represent an area of net deposition. At the Missouri site, three transects representing a catena sequence within the same field were sampled to determine if spatial distribution of HA was similar to that of atrazine at this site [26]. Transect MO-1 is a summit position with little erosion and minimal slopes (0–1%). Transect MO-2 is a mid-slope position on slopes of 1 to 3%. This transect represents the most eroded portion of the field. Transect MO-3 is the toeslope position, representing an area of net deposition. The Kansas and Ohio soils were representative samples for each of the fields.

The predominant soil series for each field site are as follows: Iowa, Monoma (fine-silty, mixed-mesic Typic Hapludoll) and Napier (fine-silty, mixed, mesic Cumulic Hapludoll); Kansas, Eudora (coarse-silty, mixed, mesic Fluventic Hapludoll); Missouri, Adco (fine, montmorillonitic, mesic Albaquic Hapludalf) and Mexico (fine, montmorillonitic, mesic Udollic

Table 2. Soil and sediment characterization data

Sample type Location	Site designation	pH	CEC[a] (meq/100 g)	Organic matter (%)	Sand (%)	Silt (%)	Clay (%)	Texture[b]
Soils								
Iowa	IA-1	6.0	11.4	3.7	18	50	32	SiCL
	IA-2	6.3	13.2	3.1	20	50	30	SiCL
Kansas	KS-1	6.4	10.8	1.7	30	52	18	SiL
	KS-2	5.7	13.3	1.8	30	48	22	L
Missouri	MO-1	6.5	7.4	2.6	18	56	26	SiL
	MO-2	7.0	6.1	2.1	18	56	26	SiL
	MO-3	5.9	7.5	2.5	14	58	28	SiCL
Ohio	OH-1	6.3	13.2	3.2	12	46	42	SiC
	OH-2	6.3	9.9	2.9	22	38	40	CL
	OH-3	6.3	9.4	2.4	26	40	34	CL
Sediments								
Field erosion	FLD-1	6.5	11.6	2.4	10	60	30	SiCL
	FLD-2	6.7	12.1	2.4	14	56	30	SiCL
Stream bank	SBK-1	7.2	14.8	3.6	26	46	28	CL
	SBK-2	6.8	10.6	3.4	42	32	26	L
	SBK-3	7.2	6.9	1.3	64	20	16	SL
Streambed	SB-1	6.7	15.0	3.8	44	32	24	L
	SB-2	7.0	4.1	0.5	86	2	12	LS
	SB-3	7.0	5.9	1.4	72	14	14	SL
	SB-4	7.1	8.4	1.8	52	26	22	SCL

[a] CEC = cation exchange capacity.
[b] SiCL = silty clay loam; SiL = silt loam; L = loam; SiC = silty clay; CL = clay loam; SL = sandy loam; LS = loamy sand; SCL = sandy clay loam.

Ochraqualf); and Ohio, field OH-1, Huntington (fine-silty, mixed, mesic Fluventic Hapludoll), Lindside (fine-silty, mixed, mesic Fluvaquentic Eutrochrept), and Nolin (fine-silty, mixed, mesic Dystric Fluventic Eutrochrept) and fields OH-2 and OH-3, Huntington and Rossburg (fine-loamy, mixed, mesic Fluventic Hapludoll). An overview of soils and cropping systems has been detailed for the MSEA field sites [27], and additional information has also been documented at each site in Iowa [28], Kansas [29], Missouri [26], and Ohio [30]. Soils were stored refrigerated (2–4°C) at field moisture content (15.5–22.2%, wet weight basis) until analyses were performed, typically within 3 months of receipt. In preparation for extraction, large pieces of plant residue were removed, and soils were thoroughly mixed and large aggregates broken by hand to obtain uniform samples with aggregate sizes of approximately 8 mm or less.

Stream and field sediment samples were collected from Goodwater Creek, Missouri, USA, on October 24, 1996, 2 d after a runoff event in the watershed (Table 2). This watershed was chosen for three main reasons: it is a predominantly agricultural watershed; long-term water quality data regarding levels of atrazine, HA, DEA, and DIA exist; and the Missouri field site lies within this watershed; thus, the overall sampling scheme provides data representing a continuum from field soils to deposited stream sediments. The naturally formed claypan soils within Goodwater Creek watershed are predominantly of the Putnam–Mexico soil association. Approximately 85% of the land-use within this 7,250-ha watershed is agricultural, and 20% of the watershed (1,400 ha) is planted to corn or sorghum [18,31]. Using statewide average application rates and percentage of corn acres treated with atrazine, average atrazine inputs to the watershed have been approximately 1,730 kg/year from 1992 to 1996. Bed and freshly deposited bank sediment samples were collected at the watershed outlet (7,250-ha drainage area) and at a subwatershed (1,215-ha drainage area). Bed sediments were collected to a depth of 8 cm. Three cores were collected across the streambed and composited for each sample. Freshly deposited bank sediments were collected to a depth of 3 cm, with several composited subsamples from each deposition area. Freshly eroded field sediments were collected immediately below flume outlets from two corn plots at the Missouri MSEA site in the same way as the bank sediments. Atrazine use history of the plots was the same as described for the Missouri soil samples (Table 1). The most recent atrazine application to these plots was 20 weeks befor sampling of the eroded sediments.

All samples were stored saturated at 2 to 4°C until analyses could be performed. In preparation for extraction of the sediments, large pieces of plant material were removed from the saturated samples. The samples were then centrifuged for 30 min at 3,500 rpm and 4°C, the water supernatant discarded, and the samples air-dried in a chemical fume hood for 24 to 64 h. Air-dried samples were thoroughly mixed and large aggregates broken by hand to provide uniform samples with aggregate sizes of approximately 6 mm or less.

Extraction procedures

For HA, duplicate 25-g (dry weight) samples were extracted with 50 ml of the mixed-mode extractant (3:1 0.5 M KH_2PO_4, pH 7.5:CH_3CN, v/v) in 250-ml Teflon® screw-cap centrifuge tubes. Samples were extracted for 1 h at ambient temperature (22–25°C) using an end-to-end shaker at 200 oscillations/min and a stroke length of 4 cm. Samples were centrifuged for 30 min at 3,500 rpm and 10°C, and the supernatant was decanted and filtered through stacked 0.45-μm nylon and 0.2-μm Anotop (Whatman Intenational, Maidstone, UK) syringe filters. The filtered extract was then used, without further sample clean up, for HPLC analysis of HA (see below). Previous work using this procedure indicated that multiple extractions of a sample may be required for quantitative recovery of HA [10]. Spike

recovery experiments were not performed for HA because previous work with the Missouri field soils showed quantitative recovery of spiked ^{14}C-HA was achieved after two extractions [10]. However, six extractions were required before no HA was detected in the MO-3 sample. This indicated that fresh spike recoveries were not representative of the extraction conditions needed for quantitative recovery of field-aged HA residues. Therefore, each sample was sequentially extracted from two to seven times, as described above, such that no HA was detected in the last extract. The number of extractions required was directly proportional to the HA concentration. Each sequential extract was analyzed separately, and the total HA was reported as the sum of the HA concentrations in each extract, corrected to a dry weight basis.

For atrazine, DEA, and DIA, 50-g (dry weight) samples were sequentially extracted twice with 100 ml of 4:1 CH_3OH:H_2O (80% CH_3OH) in 250-ml Teflon screw-cap centrifuge tubes. Samples were extracted on an end-to-end shaker at about 200 oscillations/min for 1 h at ambient temperature and centrifuged for 30 to 60 min at 3,500 rpm and 10°C. The supernatants were then combined and evaporated to remove the CH_3OH using a Savant concentrator (Savant Instruments, Farmingdale, NY, USA). The remaining water was extracted three times with 50 ml of chloroform ($CHCl_3$). The chloroform was evaporated to 2 to 5 ml in the Savant concentrator, toluene was added as a keeper, and the remaining solution was brought to dryness under a stream of ultrapure N_2. The samples were then reconstituted in 2 ml of 40% CH_3OH and filtered through Anotop 0.2-μm syringe filters into chromatography vials. For all samples, duplicate unspiked and one spiked sample at 10 μg/kg of atrazine, DEA, and DIA were extracted. Samples were spiked with 5 ml of a 100 μg/L aqueous solution (containing 16% CH_3OH) and refrigerated for 16 to 24 h before extraction. Reported concentrations of atrazine, DEA, and DIA were the mean of the unspiked duplicates corrected for the spike recovery efficiency. Recoveries ranged from 73 to 95% for atrazine, 78 to 93% for DEA, and 76 to 105% for DIA.

HPLC analyses

Analysis of HA by octyl (C_8) reverse-phase HPLC was previously described by Lerch and Donald [32] and Lerch et al. [16,18]. The HPLC conditions were: column, 250 mm × 4.6 mm (inner diameter) octyl LC-8-DB (Supleco, Bellefonte, PA, USA); mobile phase, isocratic 45:55 CH_3OH:H_2O; flow rate, 1 ml/min; detection, 220 nm; injection volume, 40 μl; column temperature, 35°C. Using 25-g (dry weight) soil and sediment samples, the HA detection limit was 10 μg/kg. The HPLC analysis of atrazine, DEA, and DIA was performed as described above for HA, with the following exceptions: mobile phase, 0.005 M KH_2PO_4, pH 7 to 7.5:CH_3CN; and flow rate, 1.50 ml/min. Mobile phase step gradients were used with steps at 16% CH_3CN for 9 min, 24% CH_3CN for 5 min, 30% CH_3CN for 12 min, 70% CH_3CN for 10 min, and equilibration at 16% CH_3CN for 9 min. Gradients between steps were linear for 0.1 min giving a total run time of 45.5 min. Using 50-g (dry weight) soil or sediment samples, detection limits for atrazine, DEA, and DIA were 0.08 μg/kg.

MS-MS confirmation

The presence of HA was qualitatively confirmed by MS-MS or liquid chromatography–tandem mass spectrometry (LC-MS-MS) on fractions collected from the original mixed-mode extracts as described by Lerch et al. [18]. Using reverse-phase HPLC, as described above, HA fractions were collected with an ISCO Foxy 200 fraction collector (Lincoln, NE, USA) using a 1.5-min time window. Because the mixed-mode method involved sequential extractions, only the first extract was used for confirmation because it had the highest HA concentration. Twenty fractions of each sample, 10 from each of two replicates, were pooled by collection in glass culture tubes. The HA standards of 5 μg/L and 100 μg/L were collected in the same manner. The fractions were evaporated to dryness in the Savant concentrator, reconstituted in 5 ml of CH_3OH, evaporated to approximately 0.25 to 0.5 ml, and filtered through 0.2-μm Anotop syringe filters.

A Perkin-Elmer Sciex API 365 MS-MS (Norwalk, CT, USA) with an atmospheric pressure ionization (API) sample interface was used for the confirmation analyses. Confirmation by LC-MS-MS utilized multireaction monitoring (MRM) as described by Lerch et al. [16], except that HPLC separation was by reverse-phase and sample introduction to the mass spectrometer was by turbo ionspray API. Full-scan daughter ion spectra were obtained by MS-MS analysis with turbo ionspray API under the following conditions: mobile phase, isocratic 50:50 CH_3OH:H_2O, both containing 1.1% CH_3COOH; flow rate, 0.2 ml/min; injection volume, 20 μl. The MS-MS conditions were: positive ion mode; full scan daughter spectra, mass range (m/z) of 50 to 210; collision gas, ultrapure N_2; declustering potential, 50 V; collision energy, 16 V; dwell time, 0.5 ms with 2.0-ms pause. Under these conditions, the 5 μg/L HA standard sample, prepared by fraction collecting, was detected.

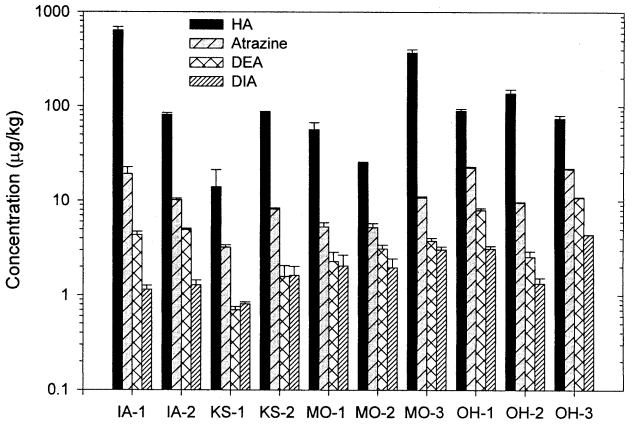

Fig. 1. Concentrations of hydroxyatrazine (HA), atrazine, deethylatrazine (DEA), and deisopropylatrazine (DIA) in soils. Bars denote concentration ± range (n = 2).

RESULTS

Soil and sediment concentrations

The HA concentrations were greater than atrazine, DEA, and DIA in all field soils studied (Fig. 1). Median concentrations were in the order HA > atrazine > DEA > DIA. All samples followed this order, except KS-1 and KS-2. For both KS samples, DIA was slightly greater than DEA, indicating possible use of other triazines, such as cyanazine or simazine, that also degrade to DIA. Median soil concentrations were HA, 84 μg/kg; atrazine, 9.9 μg/kg; DEA, 3.4 μg/kg; and DIA, 1.8 μg/kg. Concentration ratios of HA to atrazine ranged from 3 to 34, and median HA levels were 8 times greater than atrazine, 23 times greater than DEA, and 36 times greater than DIA (Table 3).

Table 3. Concentration ratios of hydroxyatrazine (HA) to atrazine, deethylatrazine (DEA), and deisopropylatrazine (DIA) in soils and sediments

	Soils			Sediments	
Site	HA:atrazine	HA:DEA	HA:DIA	Site	HA:atrazine
IA-1	33	146	554	FLD-1	51
IA-2	8	16	63	FLD-2	10
KS-1	4	20	17	SBK-1	5
KS-2	9	44	43	SBK-2	7
MO-1	11	25	28	SBK-3	43
MO-2	5	8	13	SB-1	17
MO-3	34	98	121	SB-2	15
OH-1	4	11	29	SB-3	7
OH-2	14	54	103	SB-4	12
OH-3	3	7	17	Mean	19
Mean	13	43	99	Median	12
Median	8	23	36		

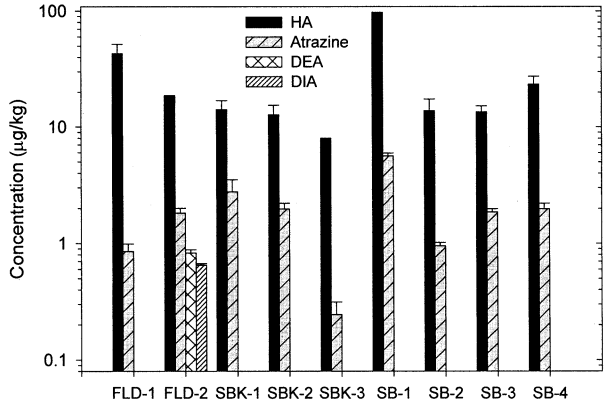

Fig. 2. Concentrations of hydroxyatrazine (HA), atrazine, deethylatrazine (DEA), and deisopropylatrazine (DIA) in sediments. Bars denote concentration ± range (n = 2).

In sediments, HA was also present at higher concentrations than atrazine, DEA, and DIA in all samples (Fig. 2). Concentrations of HA ranged from 11 to 96 µg/kg with a median concentration of 14 µg/kg. Compared to field soils collected within Goodwater Creek watershed, HA concentrations in sediment were similar to those in field soils MO-1 and MO-2, but much lower than in MO-3. Atrazine concentrations ranged from 0.25 to 5.6 µg/kg in sediments with a median concentration of 1.9 µg/kg. Deethylatrazine and DIA were detected only in the FLD-2 sample, an edge-of-field sediment, at levels of 0.83 and 0.65 µg/kg, respectively. Concentration ratios of HA to atrazine ranged from 5 to 51 with a median of 12 (Table 3). The median concentration ratio of HA to atrazine in sediment was 50% higher than in soils.

Mixed-mode extraction of HA from soils or sediments provided acceptable precision. Coefficients of variation (i.e., standard deviation/mean) for soils ranged from 1.6 to 24%, with 7 of 10 samples under 10%. The highest measurement variability for the soils was associated with the lowest concentration sample (KS-1) as HA levels approached the detection limit. For sediments, coefficients of variation ranged from 0.4 to 19%, with five of nine samples greater than 10%. The overall greater measurement variability for sediments compared to soils reflects the lower sediment HA concentrations. Five of the sediments had HA levels near the detection limit of 10 µg/kg.

Atrazine use history and soil concentrations of atrazine and metabolites

Atrazine use history of the field sites varied from as few as 4 applications to as many as 25 (Table 1). In addition, the most recent atrazine applications at the sites varied from 10 to 36 months before sampling. However, HA, atrazine, DEA, and DIA concentrations varied independently of these factors (Table 4). Of the sites with the most intensive atrazine use, three of the four (IA-2, KS-1, and KS-2) had HA levels at or below the median concentration. Conversely, two of the highest HA levels were observed for sites with only four atrazine applications (MO-3 and OH-2) over the previous 10 years. At the OH sites, HA concentration was inversely related to the number of atrazine applications since 1991 (six for OH-3, three for OH-1, and two for OH-2). Atrazine, DEA, and DIA had some of their highest levels in the OH-1 sample, which had received only four atrazine applications over the last 10 years. Conversely, the KS-2 sample, which had 25 atrazine applications over the last 25 years, had less than the median concentrations for atrazine, DEA, and DIA.

Correlations of concentration versus time since atrazine application were expected to show a relationship of increasing HA with time and corresponding decreases in atrazine, DEA, and DIA, because the latter compounds are known to be less stable in soils [21,22]. In general, this was not observed (Table 4). Samples KS-1 and OH-1 had very low HA concentration ratios (Table 3) despite about 2 years since atrazine application. For the KS samples, KS-1 had lower HA concentration ratios than KS-2, yet atrazine had not been applied to KS-1 in more than twice the time. At the MO site, all samples had an equal time since the last atrazine application, yet HA concentrations varied from 25 µg/kg in MO-2 to 370 µg/kg in MO-3, indicating that variations in soil properties as a function of landscape position were more important than time since application

Table 4. Correlations of hydroxyatrazine (HA), atrazine, deethylatrazine (DEA), and deisopropylatrazine (DIA) concentrations with atrazine use and soil physical and chemical characteristics[a]

Compound	Total atrazine applied	No. atrazine applications	Time since last application[b]	OM (%)[c]	CEC[d]	pH	Sand (%)	Silt (%)	Clay (%)
HA	0.39	0.30	0.23	0.72**	0.04	−0.55**	−0.45*	0.13	0.28
Atrazine	0.13	0.01	0.18	0.63**	0.40	−0.34	−0.31	−0.52*	0.73**
DEA	−0.14	0.24	−0.10	0.40	0.15	−0.02	−0.26	−0.46*	0.63**
DIA	−0.35	0.45*	−0.32	0.00	−0.21	−0.02	−0.26	−0.19	0.39

[a] Correlation coefficients (r) determined by linear regression analysis; levels of significance are: *$p \leq 0.05$; **$p \leq 0.01$ (n = 20 for each regression analysis).
[b] Based on atrazine use data from Table 2.
[c] OM = organic matter content of soils.
[d] CEC = cation exchange capacity of soils (meq/100 g soil).

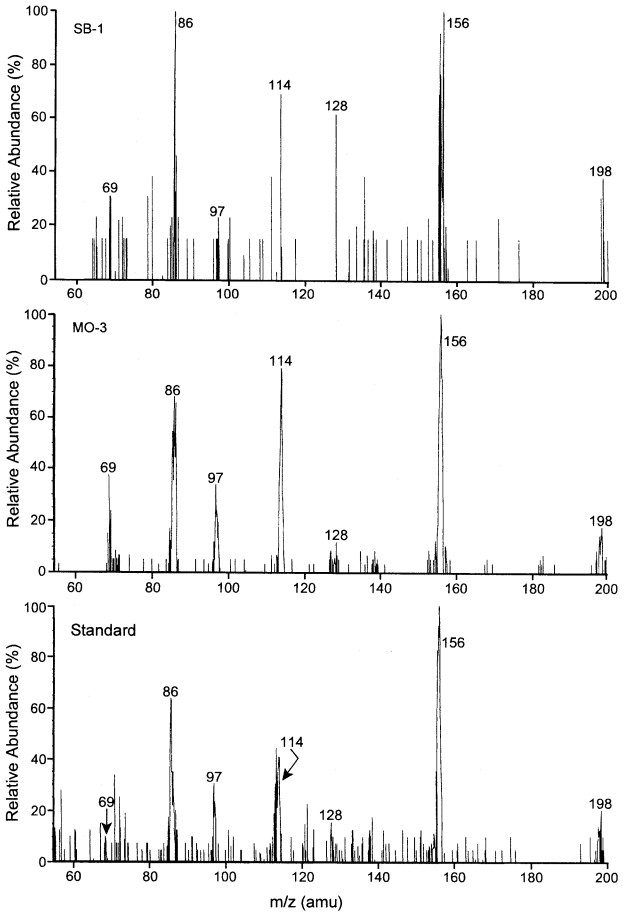

Fig. 3. Daughter ion spectra of hydroxyatrazine isolated from sediment (SB-1) and soil (MO-3) compared to a standard. Relative intensities of the base peak (*m/z* 156) were (in counts per second): SB-1, 703; MO-3, 4,180; and standard, 1,760.

in determining the concentrations of atrazine and its metabolites.

Confirmation of HA in soils and sediments

The presence of HA in soils and sediments was confirmed by LC-MS-MS and MS-MS methods. Four soils, one from each site, and five sediment samples, including at least one of each sediment type, were confirmed. The LC-MS-MS method utilized the multireaction monitoring technique, which provides three points of confirmation: HPLC retention time, presence of the protonated molecular ion (*m/z* 198, $[M + H]^+$), and presence of a diagnostic daughter ion (*m/z* 156, $[M - C_3H_7 + H]^+$). Because of its greater sensitivity compared to full-scan daughter ion MS-MS, this technique was used for confirmation of HA in the sediment samples. In order to obtain HA daughter ion spectra comparable to that presented in previous work [18,33], full-scan MS-MS was employed for the soil samples and the highest concentration sediment sample (SB-1) (Fig. 3). Daughter ion spectra for an HA standard, a soil sample (MO-3), and a sediment sample (SB-1) exhibited spectra with the same diagnostic daughter ions at *m/z* 198, 156, 128, 114, 97, 86, and 69 (see Lerch et al. [18] for daughter ion identification). These spectra were also in very close agreement with HA spectra obtained by electrospray LC-MS-MS [18] and by thermospray LC-MS-MS [33]. Moreover, the daughter ion spectrum reported by Lerch et al. [18] was obtained by isolating HA from Goodwater Creek water samples. Based on ^{14}C-atrazine degradation in soils, numerous publications have identified the formation of ^{14}C-HA [1–5]. However, the spectra presented here (Fig. 3) represent the first confirmation of HA in either field soils or stream sediments.

DISCUSSION

Factors controlling HA levels in soils

Our results indicated that management factors, such as atrazine use and time since application, exert less effect than soil chemical and hydrologic processes on the persistence of atrazine and its metabolites in soils. Ostrofsky et al. [30] reported that atrazine degradation in samples collected from OH-2, OH-3, and a nearby herbicide-free control site was dependent upon the frequency of atrazine application. The extent of atrazine mineralization in the three soils increased with increasing atrazine use frequency, with up to 80% mineralization in the OH-3 soil. Ostrofsky et al. [30] concluded that frequent atrazine use maintains an active atrazine degrading microbial community. Thus, if these results have general applicability, management of atrazine should influence the rate and extent of atrazine mineralization in soils. Sites with more extensive atrazine use should have more atrazine mineralized and less formation of stable metabolites, resulting in lower total atrazine residues (i.e., atrazine plus HA, DEA, and DIA) in soils. At the OH sites, total atrazine residues were inversely related to atrazine use, indicating a shift in the degradation pattern toward atrazine mineralization, as found by Ostrofsky et al. [30]. However, at the IA and KS sites, total atrazine residues were directly related to the number of atrazine applications. Overall, a significant negative correlation between atrazine concentrations and total atrazine applied or number of atrazine applications was not observed (Table 4). Within-field variability of atrazine concentrations for the MO samples also indicated that other processes such as mobility, sorption [26], and erosion are more important factors controlling the levels of atrazine and its metabolites in soils.

The higher HA concentrations relative to atrazine, DEA, and DIA observed in all soils were primarily controlled by a combination of sorption, aqueous-phase mobility, degradation, and erosion. Atrazine, HA, and DEA concentrations in soil were positively correlated to OM and/or clay content, and HA was also negatively correlated to pH (Table 4). Because these parameters relate to the sorption of these compounds [10,20,23], it was apparent that greater sorption resulted in higher soil concentrations because of less transport from surface soil via leaching or runoff. Compared to HA, the mobility of atrazine, DEA, and DIA is expected to be greater because of their lower sorption [20,23,24]. Reported sorption coefficients (K_d) for several soils range from 1.7 to 82 L/kg for HA, 0.1 to 14 L/kg for atrazine, 0.1 to 6.5 L/kg for DEA, and 0.4 to 8.6 L/kg for DIA [20,23,24]. For all of the soils studied, HA showed several times greater sorption than that of atrazine, DEA, and DIA. The inverse relationship between mobility (or water solubility) and sorption is well documented [23,34–36]. Kruger et al. [36] showed that atrazine, DEA, and DIA mobility in surface soils was inversely related to OM and clay content. Hydroxyatrazine has also been shown to be more stable in soils than atrazine, DEA, and DIA [8,21,22]. The lack of correlation to any soil physical or chemical parameters for DIA (Table 4) may have reflected its high rate of degradation [8,21,37]. The high HA concentrations relative to atrazine, DEA, and DIA resulted because it is less mobile and more

Table 5. Correlations of hydroxyatrazine (HA) and atrazine to sediment physical and chemical characteristics[a]

Compound	OM (%)[b]	CEC[c]	pH	Sand (%)	Silt (%)	Clay (%)
HA	0.50*	0.56*	−0.54*	−0.24	0.22	0.27
Atrazine	0.70**	0.66**	−0.21	−0.16	0.12	0.27

[a] Correlation coefficients (r) determined by linear regression analysis; levels of significance are: $*p \leq 0.05$; $**p \leq 0.01$ ($n = 18$ for each regression analysis).
[b] OM = organic matter content of sediments.
[c] CEC = cation exchange capacity of sediments (meq/100 g soil).

thermodynamically stable. In addition, the higher HA concentrations relative to DEA and DIA may also indicate that HA is formed to a greater extent in soils. Soil erosion also seems to contribute to higher HA concentrations. Two of the samples representing areas of net soil deposition (IA-1 and MO-3) had the highest HA concentrations. This indicated that in these field situations, transport of sorbed HA can be an important mechanism for its accumulation in lower landscape positions.

Hydroxyatrazine and atrazine correlations to soil properties suggested the importance of sorption as the key factor controlling absolute concentrations of HA in soil. Hydroxyatrazine has been shown to sorb to soils by pH-dependent cation exchange and hydrophobic interactions (mixed-mode sorption) [10], whereas atrazine sorption is primarily via hydrophobic interactions and hydrogen bonding [38,39]. Thus, the poor correlation of HA to cation exchange capacity (CEC), combined with significant correlations to OM and pH, suggests that HA levels in soils were largely controlled by sorption of the parent compound (Table 4). Because atrazine hydrolysis is known to be enhanced by sorption and pH extremes [11], soils with high OM and clay content and low pH will result in greater atrazine sorption and subsequent hydrolysis. Spatial distribution of HA and atrazine for the three MO soil samples also indicated the relationship between atrazine sorption and HA concentrations. Ghidey et al. [26] showed that the spatial distribution of atrazine at the MO site, up to 27 weeks following application, was inversely related to pH and directly related to OM content and/or CEC. Hydroxyatrazine concentrations exhibited the same trends in spatial distribution as observed by Ghidey et al. [26] for atrazine. Thus, soil conditions that result in greater sorption of atrazine will also tend to have higher rates of atrazine hydrolysis and greater HA concentrations.

Factors controlling HA levels in sediments

Concentrations of HA in sediments were directly correlated to OM and inversely correlated to pH, but unlike field soils, CEC was also directly correlated to HA concentrations (Table 5). This combination suggested that mixed-mode sorption controlled HA concentrations in sediment. During a runoff event, the greater sorption of HA results in relatively more HA retained on the sediments than atrazine. This would result in the observed increases in the HA to atrazine ratios of sediments compared to field soils (Table 3). Wauchope [35] summarized surface runoff data from numerous studies that showed about 80% of atrazine loss is in the aqueous phase. Given that the median HA to atrazine concentration ratio for sediments was 12, it is reasonable to assume that atrazine hydrolysis is no longer a significant source of HA in sediments. Therefore, the levels of HA in sediments are independent of the conditions that controlled its formation in field soils, and the chemical parameters related to mixed-mode sorption (OM, CEC, and pH) are manifested.

Stream sediments from the upper watershed had higher levels of HA and atrazine because of their greater OM, CEC, and clay contents compared to the watershed outlet (Fig. 2 and Tables 2 and 5). The extremely high variability in atrazine and HA concentrations in sediments reflected the variability in sediment deposition patterns with regard to the sorptive colloids and the source of the sediment. The general absence of DEA and DIA in sediments reflected that surface transport of these compounds is predominately in the dissolved phase. Roy and Krapac [40] reported low adsorption of atrazine and DEA to alluvial sediments from the Embarras River, Illinois, USA. The magnitude of the sorption coefficients indicated that atrazine and DEA would not be significantly retained by these sediments [40]. In contrast, sediments from Goodwater Creek consistently had detectable levels of atrazine, indicating that significant adsorption of atrazine to stream sediments can occur.

CONCLUSIONS

Hydroxyatrazine is a persistent and widespread contaminant that is present at significant levels in both field soils and stream sediments. In field soils, HA was detected in all soils at four different locations under different management practices and atrazine use histories. High levels of HA can persist for at least 24 to 36 months after atrazine application, and the consequences of its persistence on soil ecology are unknown. Hydroxyatrazine was present at greater concentrations than atrazine, DEA, and DIA in all soils. Absolute concentrations of HA in soils seem to be primarily controlled by soil conditions that promote sorption of the parent compound. Soils with elevated OM and clay content and low pH enhance hydrolysis of atrazine, resulting in greater HA levels. Concentrations of HA relative to atrazine, DEA, and DIA are mainly a function of differences in mobility and sorption, thermodynamic stability, and extent of formation in surface soils.

Hydroxyatrazine levels were greater than atrazine, DEA, and DIA in all sediments. Despite the heterogenous nature of sediment deposition in streams, HA was detected in all stream sediments, and in some cases, at levels greater than in field soils. Higher levels of HA relative to atrazine, DEA, and DIA reflected the greater sorption of HA to sediment during surface transport. The low levels of atrazine and the absence of DEA and DIA in sediments indicated that these compounds are primarily transported in the dissolved phase. Based on significant correlations of HA concentration to OM, CEC, and pH, mixed-mode sorption apparently controls HA levels in sediment.

Mixed-mode extraction was shown to be an effective means of quantitatively extracting HA from soils and sediments. The sequential extraction approach produced acceptable precision and sensitivity. However, further refinements of the methods are needed in order to standardize the extraction procedure, achieve sample clean up, and improve sensitivity for detection of other hydroxylated atrazine metabolites. Optimization of the extraction conditions and development of a solid-phase extraction clean-up procedure are currently underway.

The results presented provide unequivocal evidence in support of the hydrologic transport mechanisms controlling HA concentrations in stream water [16]. Two key pieces of evidence were required for these proposed transport mechanisms to have validity: long-term persistence of HA in surface soils,

and presence of HA in stream sediments. Contamination of stream sediments with a persistent metabolite is one of the relevant risk factors in assessing the impact of pesticides on aquatic ecosystems [25]. The presence of HA in stream sediments at the levels observed combined with the reported pattern of HA concentrations in stream water [18] indicates that HA is persistent in sediments. Therefore, an additional risk factor associated with atrazine usage is the potential impact of sediment-bound HA on aquatic ecosystems.

Acknowledgement—We wish to thank Ragu Ramanathan and Yong-Xi Li for their mass spectrometry expertise. Thanks to Norm Fausey, Larry Kramer, and Larry Maddux for their assistance in acquiring soil samples and obtaining atrazine use history data for the field sites. Thanks to Paul Brugmann for his technical support.

REFERENCES

1. Skipper HD, Gilmour CM, Furtick WR. 1967. Microbial versus chemical degradation of atrazine in soils. *Soil Sci Soc Am Proc* 31:653–656.
2. Winkelmann D, Klaine SJ. 1991. Degradation and bound residue formation of atrazine in a western Tennessee soil. *Environ Toxicol Chem* 10:335–345.
3. Sorenson BA, Koskinen WC, Buhler DD, Wyse L, Lueschen WE, Jorgenson MD. 1994. Formation and movement of C-14-atrazine degradation products in a clay loam soil in the field. *Weed Sci* 42:618–624.
4. Gan J, Becker RL, Koskinen WC, Buhler DD. 1996. Degradation of atrazine in two soils as a function of concentration. *J Environ Qual* 25:1064–1072.
5. Miller JL, Wollum AG, Weber JB. 1997. Degradation of carbon-14-atrazine and carbon-14-metolachlor in soil from four depths. *J Environ Qual* 26:633–638.
6. Capriel P, Haisch A, Khan SU. 1985. Distribution and nature of bound (nonextractable) residues of atrazine in a mineral soil nine years after the herbicide application. *J Agric Food Chem* 33:567–569.
7. Calderbank A. 1989. Occurrence and significance of bound pesticide residues in soil. *Rev Environ Contam Toxicol* 108:71–103.
8. Kruger EL, Somasundaram L, Kanwar RS, Coats JR. 1993. Persistence and degradation of ^{14}C-atrazine and ^{14}C-deisopropylatrazine as affected by soil depth and moisture conditions. *Environ Toxicol Chem* 12:1969–1975.
9. Kruger EL, Rice PJ, Anhalt JC, Anderson TA, Coats JR. 1997. Comparative fates of atrazine and deethylatrazine in sterile and nonsterile soils. *J Environ Qual* 26:95–101.
10. Lerch RN, Thurman EM, Kruger EL. 1997. Mixed-mode sorption of hydroxylated atrazine degradation products to soil: A mechanism for bound residue. *Environ Sci Technol* 31:1539–1546.
11. Armstrong DE, Chesters G, Harris RF. 1967. Atrazine hydrolysis in soils. *Soil Sci Soc Am Proc* 31:61–66.
12. Mandelbaum RT, Wackett LP, Allan DL. 1993. Rapid hydrolysis of atrazine to hydroxyatrazine by soil bacteria. *Environ Sci Technol* 27:1943–1946.
13. Pelizzetti E, Maurino V, Minero C, Carlin V, Pramauro E, Zerbinati O, Tosato ML. 1990. Photocatalytic degradation of atrazine and other s-triazine herbicides. *Environ Sci Technol* 24:1559–1565.
14. Minero C, Pramauro E, Pelizzetti E, Dolci M, Marchesini A. 1992. Photosensitized transformations of atrazine under simulated sunlight in aqueous humic acid solution. *Chemosphere* 24:1597–1606.
15. Torrents A, Anderson BG, Bilboulian S, Johnson WE, Hapeman CJ. 1997. Atrazine photolysis: Mechanistic investigations of direct and nitrate mediated hydroxy radical processes and the influence of dissolved organic carbon from the Chesapeake Bay. *Environ Sci Technol* 31:1476–1482.
16. Lerch RN, Blanchard PE, Thurman EM. 1998. Contribution of hydroxylated atrazine degradation products to the total atrazine load in midwestern streams. *Environ Sci Technol* 32:40–48.
17. Adams CD, Randtke SJ. 1992. Ozonation byproducts of atrazine in synthetic and natural waters. *Environ Sci Technol* 26:2218–2227.
18. Lerch RN, Donald WW, Li Y-X, Alberts EE. 1995. Hydroxylated atrazine degradation products in a small Missouri stream. *Environ Sci Technol* 29:2759–2768.
19. Cai Z, Monson SJ, Spalding RF. 1996. Determination of atrazine and hydroxyatrazine in agricultural runoff waters by liquid chromatography and fast atom bombardment-high resolution mass spectrometry. *J Assoc Off Anal Chem Int* 79:929–935.
20. Brouwer WWM, Boesten JJTI, Siegers WG. 1990. Adsorption of transformation products of atrazine by soil. *Weed Res* 30:123–128.
21. Winkelmann DA, Klaine SJ. 1991. Degradation and bound residue formation of four atrazine metabolites, deethylatrazine, deisopropylatrazine, dealkylatrazine, and hydroxyatrazine in a western Tennessee soil. *Environ Toxicol Chem* 10:347–354.
22. Baluch HU, Somasundaram L, Kanwar RS, Coats JR. 1993. Fate of major degradation products of atrazine in Iowa soils. *J Environ Sci Health B* 28:127–149.
23. Seybold CA, Mersie W. 1996. Adsorption and desorption of atrazine, deethylatrazine, deisopropylatrazine, hydroxyatrazine, and metolachlor in two soils from Virginia. *J Environ Qual* 25:1179–1185.
24. Moreau C, Mouvet C. 1997. Sorption and desorption of atrazine, deethylatrazine, and hydroxyatrazine by soil and aquifer solids. *J Environ Qual* 26:416–424.
25. Belfroid AC, van Drunen M, van Gestel CAM, van Hattum B. 1996. *Relative Risks of Transformation Products of Pesticides for Aquatic Ecosystems.* Free University of Amsterdam, Amsterdam, The Netherlands.
26. Ghidey F, Alberts EE, Lerch RN. 1997. Spatial and temporal variability of herbicides in a claypan soil watershed. *J Environ Qual* 26:1555–1563.
27. Ward A, Hatfield J, Lamb J, Alberts E, Logan T, Anderson J. 1994. The management systems evaluation areas program: Tillage and water quality research. *Soil Tillage Res* 30:49–74.
28. Saxton K, Spomer R, Kramer L. 1971. Hydrology and erosion of loessial watersheds. *J Hydraulics Div Am Soc Chem Eng* 11: 1835–1851.
29. Omay AB, Rice CW, Maddux LD, Gordon WB. 1997. Changes in soil microbial and chemical properties under long-term crop rotation and fertilization. *Soil Sci Soc Am J* 61:1672–1678.
30. Ostrofsky EB, Traina SJ, Tuovinen OH. 1997. Variation in atrazine mineralization rates in relation to agricultural management practice. *J Environ Qual* 26:647–657.
31. Blanchard PE, Donald WW. 1997. Herbicide contamination of groundwater beneath claypan soils in north-central Missouri. *J Environ Qual* 26:1612–1621.
32. Lerch RN, Donald WW. 1994. Analysis of hydroxylated atrazine degradation products in water using solid-phase extraction and high performance liquid chromatography. *J Agric Food Chem* 42:922–927.
33. Abian J, Durand G, Barcelo D. 1993. Analysis of chlorotriazines and their degradation products in environmental samples by selecting various operating modes in thermospray HPLC/MS/MS. *J Agric Food Chem* 41:1264–1273.
34. Chiou CT, Peters LJ, Freed VH. 1979. A physical concept of soil–water equilibria for nonionic organic compounds. *Science* 206: 831–832.
35. Wauchope RD. 1978. The pesticide content of surface water draining from agricultural fields—a review. *J Environ Qual* 7:459–472.
36. Kruger EL, Zhu B, Coats JR. 1996. Relative mobilities of atrazine, five atrazine degradates, metolachlor, and simazine in soils of Iowa. *Environ Toxicol Chem* 15:691–695.
37. Mills MS, Thurman EM. 1994. Preferential dealkylation reactions of *s*-triazine herbicides in the unsaturated zone. *Environ Sci Technol* 28:600–605.
38. Weber JB, Weed SB, Ward TM. 1969. Adsorption of *s*-triazines by soil organic matter. *Weed Sci* 17:417–421.
39. Cheng HH. 1990. Organic residues in soils: Mechanisms of retention and extractability. *Int J Environ Anal Chem* 39:165–171.
40. Roy WR, Krapac IG. 1994. Adsorption and desorption of atrazine and deethylatrazine by low organic carbon geologic materials. *J Environ Qual.* 23:549–556.