

Hydroxylated Atrazine Degradation Products in a Small Missouri Stream

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This research assessed the occurrence of hydroxylated atrazine degradation products (HADPs) in streamwater from Goodwater Creek watershed in the claypan soil region of northeastern Missouri. Streamwater was sampled weekly from June 1992 to December 1994 at a V-notch weir used to measure streamflow for this 7250-ha watershed. Filtered water samples were prepared by cation exchange solid-phase extraction and analyzed for hydroxyatrazine (HA), deethylhydroxyatrazine (DEHA), and deisopropylhydroxyatrazine (DIHA) by high-performance liquid chromatography with UV detection. HADPs were confirmed by mass spectrometry and an alternative HPLC/UV method. Frequency of HADP detection was 100% for HA, 25% for DEHA, and 6% for DIHA. Concentrations ranged from 0.18 to 5.7 $\mu\text{g L}^{-1}$ for HA, from <0.12 to 1.9 $\mu\text{g L}^{-1}$ for DEHA, and from <0.12 to 0.72 $\mu\text{g L}^{-1}$ for DIHA. These results establish that HADPs can contaminate surface water and that HA contamination of surface water is a significant fate pathway for atrazine in this watershed.

Introduction

Widespread contamination of surface waters by atrazine and its N-dealkylated degradation products in the corn production areas of the United States and Canada has been well established over the last 20 years (1–7). However, surface water contamination by hydroxylated atrazine degradation products (HADPs; hydroxyatrazine, deethylhydroxyatrazine, and deisopropylhydroxyatrazine) has never been thoroughly assessed. Although HADPs are unlikely to contaminate groundwater (8–11), their potential to contaminate surface water has received little consideration. In a study of ozonation treatment of atrazine-contaminated surface waters, HA was detected at 1–2 μg

L^{-1} from two reservoir samples (12). Although these findings were confirmed by alternative methods, the sites were sampled only once. Atrazine degradation in a small Iowa stream was postulated to degrade to HA via photolysis, but direct proof of HA in the stream was not provided (13). Using a polyclonal immunoassay, HA was not detected in any environmental water samples (14). However, long-term monitoring for HA was not conducted. To date, no long-term monitoring studies of HADPs in surface water have been published.

Hydroxylated atrazine degradation products (HADPs) are chemically distinct from their chlorinated analogues. They have lower solubility in water and organic solvents, and HADPs are stronger bases with dissociation constants near 10^{-5} compared to about 10^{-2} for most chlorinated s-triazines (15–18). Furthermore, HADPs are tautomeric compounds with the stability of the enol, keto, and ionic forms dependent upon pH (19–21). Because more than one stable form exists, HADPs are inherently difficult to isolate from environmental samples unless pH is adjusted to shift the tautomeric equilibrium so that only one form predominates (16). HADP formation is generally thought to detoxify atrazine because HADPs are not toxic to plants or aquatic photosynthetic microorganisms (15, 22, 23). HA is toxic to rats with an acute LD_{50} of >3000 mg kg^{-1} , but it is not mutagenic or teratogenic. HA toxicity in rats resulted from mechanical kidney damage (nephrotoxicity) caused by HA crystallization (D. D. Sumner, Ciba-Geigy Corp., personal communication). The overall impact of HADPs in terrestrial or aquatic ecosystems has not been assessed to date.

Laboratory analyses of HADPs in soil and water have usually used radioisotopes and thin-layer or high-performance liquid chromatography for separation (8–11, 24–27). In general, reverse-phase high-performance liquid chromatography (HPLC) methods are best suited for the separation and quantitation of HADPs because of their moderate nonpolarity and low volatility (16, 28, 29). Early HPLC methods were useful for separating a large number of triazines and their metabolites, but they were not often applied to environmental samples (17, 30, 31). Recently, HPLC was used to determine unlabeled atrazine and its metabolites in soil and water (16, 28, 29, 32). In addition, enzyme-linked immunoassays were developed for HA in soil and water (14, 33). Thus, methods for HADP analysis in environmental samples have only recently become available.

HPLC/mass spectrometry (MS) can directly confirm HADPs in environmental matrices without derivatization. However, the design of currently available HPLC/MS systems fails to provide sufficient fragmentation (34). HPLC/MS with a direct liquid introduction interface operated in the negative and positive ion modes was used for analysis of a number of triazines (35). This system facilitated some fragmentation, but it lacked abundant diagnostic ions other than the protonated or deprotonated parent ions. More recently, thermospray HPLC/MS/MS was used to analyze chlorotriazines and their degradation products, including the HADPs (34). In this work, daughter ion spectra were obtained to confirm the formation of HADPs from laboratory photolysis experiments.

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HA formation in the environment occurs abiotically via chemical or photochemical hydrolysis of atrazine (8, 15, 19, 34, 36–43). In water, atrazine hydrolysis to HA is pH dependent, and the presence of humic and fulvic acids increases the reaction rate (37, 44–46). In soils, chemical degradation of atrazine to HA is generally the major degradation pathway with lesser amounts of the biologically formed products, deethylatrazine (DEA) and deisopropylatrazine (DIA) (8, 19, 37–39). HA formation in soils has been attributed to chemical hydrolysis of adsorbed atrazine (19, 37). Presumably, colloidal soil surfaces are sufficiently acidic, compared to bulk soil solution, to catalyze the hydrolysis of adsorbed atrazine (37). Photolytic hydrolysis of atrazine to HA in water and on solid surfaces proceeds indirectly from $\cdot\text{OH}$ radical-generating photosensitizers, such as humic acid, NO_3^- , polycyclic aromatic hydrocarbons, and biological reactions (22, 43, 47–49).

The N-dealkylated HADPs, deethylhydroxyatrazine (DEHA) and deisopropylhydroxyatrazine (DIHA), are usually minor soil degradation products of atrazine or HA in short-term studies (24, 25). However, in a long-term field study of atrazine fate in soil, N-dealkylated HADPs comprised a significant proportion of the bound residues in bulk soil and humic acid fractions (50). While atrazine hydrolysis occurs abiotically (51), biological hydrolysis of DEA, DIA, and dideethylatrazine to their respective hydroxy analogues by *Pseudomonas* species was significant in culture (51) and in [^{14}C]atrazine-treated soils incubated for 1 year (26). DEHA and DIHA were also shown to be the major degradation products from photolysis of DEA and DIA in water (34).

HA is more persistent than atrazine or its chlorinated degradation products in soil (8, 24, 27). After 5 months incubation in two soils, less than half the applied [^{14}C]HA was degraded compared to 65–89% of added [^{14}C]atrazine (24). Following 180 d in soil microcosms, HA half-life was 121 d compared to 21–26 d for atrazine and its chlorinated degradation products (8, 27). In surface soil of pH 5.5, HA formed rapidly (detected within 4 d), was present in greater concentration than atrazine after 180 d, and persisted longer than DEA or DIA (8). Furthermore, Winkelmann and Klaine (8) concluded that annual atrazine application would result in HA accumulation in surface soil. To date, persistence of the N-dealkylated HADPs has not been studied.

In many soils, HA adsorbed to soil colloids more than atrazine or chlorinated atrazine degradation products (9, 52). In two silt loam soils differing mainly in pH, HA adsorption was 5.7–10 times greater than atrazine (9). HA adsorption was markedly increased by low pH, increasing about 2-fold at pH 4.0–4.5 compared to pH 6.1. Because HA is a stronger base than atrazine, more HA was in cationic form at pH 4.0–4.5, thus increasing adsorption (9). In this same study, essentially no HA desorbed from either soil after 3 d. HA adsorbed more than atrazine, DEA, and DIA in four different soils covering a wide range of textures, pH, and organic matter content (52). Distribution coefficients of organic matter, K_{om} ($\text{dm}^3 \text{kg}^{-1}$), were 103–590 for HA, 44–102 for atrazine, 24–46 for DEA, and 31–62 for DIA (52). Because it adsorbed strongly to soil, HA was concluded to show little potential for leaching through soils (8, 9). Column and field leaching studies support this conclusion with little or no HADPs leached to depths up to 60 cm (10, 11).

Based on the known behavior of HADPs in soil, their accumulation in surface soils receiving repeated atrazine

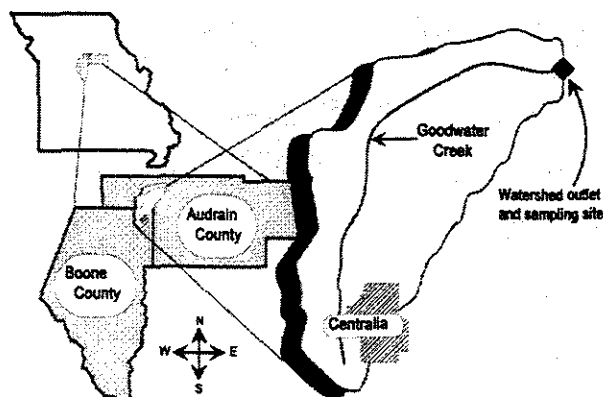


FIGURE 1. Site location for Goodwater Creek sampling.

application is likely. Thus, HADPs in surface runoff could contaminate streamwater. Furthermore, atrazine contamination of surface waters is well documented, and subsequent degradation to HADPs in streamwater may be possible. The overall environmental fate of atrazine is currently incomplete without direct study of HADPs in surface water of agricultural watersheds. Methodology for direct, routine analysis of HADPs in surface water is now available, but full-scan or daughter ion mass spectra of HADPs in surface water have not been published to date. Therefore, the objectives of this research were (1) to conduct long-term monitoring of HADPs in streamwater of Goodwater Creek watershed in the claypan soil region of northeastern Missouri and (2) to confirm their presence using HPLC/UV, thermospray HPLC/MS, direct probe MS, and electrospray HPLC/MS/MS methods.

Experimental Procedures

Chemicals and Standard Materials. Hydroxyatrazine (HA) [2-hydroxy-4-ethylamino-6-isopropylamino-*s*-triazine], deethylhydroxyatrazine (DEHA) [2-hydroxy-4-amino-6-isopropylamino-*s*-triazine], deisopropylhydroxyatrazine (DIHA) [2-hydroxy-4-ethylamino-6-amino-*s*-triazine] were 94–99% pure (Ciba-Geigy Corp., Greensboro, NC). Atrazine [2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine] was 98% pure (Ultra Scientific, North Kingstown, RI), and deethylatrazine (DEA) [2-chloro-4-amino-6-isopropylamino-*s*-triazine] and deisopropylatrazine (DIA) [2-chloro-4-ethylamino-6-amino-*s*-triazine] were 97% pure (Crescent Chemical Co., Inc., Hauppauge, NY). All solvents used were HPLC grade. All chemicals used for the routine analysis of the HADPs by cation exchange solid-phase extraction and C_8 reverse-phase HPLC were described by Lerch and Donald (16). Ammonium acetate (NH_4OAc) was HPLC grade, and $\text{NH}_4\text{-OH}$ and ammonium formate (NH_4CHO_2) were reagent grade (J. T. Baker Chemical Co., Phillipsburg, NJ). Formic acid (CHOOH) was supplied by Sigma Chemical Co. (St. Louis, MO).

Stream Sampling and Site Description. Goodwater Creek was sampled at a V-notch weir used to measure daily streamflow for the entire 7250-ha Goodwater Creek watershed, located in Audrain and Boone Counties of Missouri (latitude, $39^\circ 18' \text{N}$; longitude, $92^\circ 03' \text{W}$) (Figure 1). About 70% of the watershed is cropped with approximately 23% of the cropped acreage (990 ha) planted with corn or sorghum in 1992 and 1993 (L. K. Heidenreich, University of Missouri, personal communication). Naturally formed claypan soils (argillic horizon derived from clay loess parent material) of the Mexico-Putnam soil

association predominate in the watershed. These soils are somewhat poorly to poorly drained on slopes of 0–3% with silt loam surface horizons and clay loam subsoils containing 40–60% montmorillonitic clays. Ranges of surface soil chemical properties are as follows: pH, 5.0–6.0; organic matter content, 10–30 g kg⁻¹; and cation exchange capacity, 10–20 cmol(+) kg⁻¹. The slow infiltration and percolation of water resulting from the claypan combined with an erosive silt loam surface and gently rolling topography make Goodwater Creek very susceptible to herbicide contamination by surface runoff.

Two to five 1-L grab samples were collected in amber glass jars with Teflon-lined screw caps on a weekly basis or as permitted by streamflow from June 1992 to December 1994. Collection bottles were cleaned by detergent solution followed by rinsing with HPLC-grade water, methanol, and acetone before being baked at ≥ 300 °C for 8 h. All samples were filtered through 0.45- μ m nylon filters to remove suspended sediment and then combined and mixed to provide a uniform sample for each sample day. HADPs showed negligible adsorption to the nylon filters (16). Field samples were stored at 2–4 °C before analysis. Samples were filtered within 2 d of receipt and usually analyzed within 10 d but always within 40 d. Stream samples containing measurable levels of HA and detectable levels of DEHA were re-analyzed after as much as 109 d storage at 2–4 °C. The results were within 2–18% of the original quantitation for HA, and detectable levels of DEHA were confirmed for all samples.

Routine Analyses

Hydroxylated Atrazine Degradation Products. Analysis of HADPs by propylbenzenesulfonic acid cation exchange (SCX) solid-phase extraction (SPE) and C₈ reverse-phase HPLC with UV detection (C₈-HPLC/UV) was previously described (16). For the analysis of DEHA and DIHA, detection was changed to 210 nm, and the column temperature was changed to 35 °C in January 1994. Detection of these analytes was improved nearly 2-fold because of their higher absorbance at 210 nm compared to 220 nm (29). Because this change was made near the completion of the study, the original reported limits of detection (0.12 μ g L⁻¹) and quantitation (0.40 μ g L⁻¹) for DEHA and DIHA were used. For HA, the limit of detection was 0.04 μ g L⁻¹, and the limit of quantitation was 0.13 μ g L⁻¹. Quality control samples included lab blanks and spikes at either 0.5, 1.0, or 5.0 μ g L⁻¹ for field samples (field spikes) and HPLC-grade water (lab spikes). Spike concentrations were based on HADP background levels. Field spike recovery data were used to develop quality control charts for evaluating method precision, accuracy, and reproducibility over time (16). All (untreated) stream samples were analyzed in duplicate or triplicate along with a corresponding set of quality control samples. Reported HADP concentrations represent the average concentration of replicates corrected for the corresponding field spike recovery.

Atrazine and Chlorinated Metabolites. Sample cleanup for the determination of atrazine, DEA, and DIA was performed by C₁₈ SPE (53). Surrogate (terbutylazine) spiked 100-mL samples were passed through conditioned Varian LRC 500-mg C₁₈ SPE cartridges (Varian, Inc., Harbor City, CA) followed by sorbent drying under a vacuum of about 25–40 cm of Hg for 1–2 h. SPE cartridges were eluted with 2 mL of ethyl acetate and analyzed by gas chromatography

(GC). GC analysis was performed on a Varian 3400 equipped with a N-P detector, an 8200 autosampler, a split less injector, and a Restek XTI-5 fused-silica capillary column (30 m \times 0.53 mm) with a 5% diphenyl/95% dimethyl polysiloxane stationary phase (Restek Corp., Bellefonte, PA). Typical column conditions were as follows: He flow rate, 6 mL min⁻¹; temperature program, 75 °C held for 2 min to 145 °C at 20 °C min⁻¹ to 187 °C at 3 °C min⁻¹ to 215 °C at 5 °C min⁻¹ to 280 °C at 25 °C min⁻¹ hold for 1 min; sample injection volume, 4 μ L. Limits of detection were 0.04 μ g L⁻¹ for atrazine and 0.05 μ g L⁻¹ for DEA and DIA. Quality control samples included laboratory and field blanks and spikes, field duplicates, and independently analyzed splits. Splits were quantitatively and qualitatively confirmed for atrazine using GC/MS (Huntingdon Engineering and Environment, Inc., St. Paul, MN). GC/MS confirmation of DEA and DIA are pending; however, quality control charts of duplicate samples showed that duplicate precision was 20% or less for concentrations above the limit of quantitation (0.15 μ g L⁻¹). Reported concentrations of these compounds were corrected for surrogate recovery, and DEA and DIA were also corrected for lab spike recovery.

Characterization of Streamwater. Chemical characterization of water samples for pH, dissolved organic C (DOC), NO₃N, NH₄N, total dissolved PO₄P, dissolved Ca²⁺ and Mg²⁺, and total alkalinity were previously described (16). Averages (\pm standard deviation) of streamwater chemical properties were as follows: pH, 7.9 \pm 0.3; DOC, 8.8 \pm 2.6 mg L⁻¹; NO₃N, 0.82 \pm 0.65 mg L⁻¹; NH₄N, 0.19 \pm 0.15 mg L⁻¹; PO₄P, 0.13 \pm 0.09 mg L⁻¹; Ca²⁺, 35.2 \pm 12.3 mg L⁻¹; Mg²⁺, 7.2 \pm 3.0 mg L⁻¹; and total alkalinity, 97 \pm 39 mg L⁻¹.

Confirmation Analyses

HADPs were qualitatively confirmed using cation exchange HPLC with UV detection (SCX-HPLC/UV), thermospray HPLC/MS, direct probe MS, and electrospray reverse-phase HPLC/MS/MS. All confirmation analyses were conducted by Analytical Bio-Chemistry Laboratories, Inc. (Columbia, MO) on either a portion of the original filtered field samples or on HPLC fractions supplied to them as described below.

SCX-HPLC/UV Confirmation. Approximately 20% of all samples were confirmed for HADPs by SCX-HPLC/UV. A 100-mL portion of the original filtered field samples was used. Samples were prepared on a vacuum manifold using 3 mL reservoir and 500 mg SCX SPE cartridges (J. T. Baker Chemical, Co., Phillipsburg, NJ) as follows: (1) condition cartridges by sequentially adding 5 mL each of 75% CH₃-OH:20% H₂O:5% NH₄OH mixture, CH₃OH, and H₂O; (2) pass a 100-mL sample, acidified to pH 2.5 with 0.2 mL of formic acid, through cartridge at a flow rate of 5–10 mL min⁻¹ until cartridge is dry; (3) wash cartridge with 5 mL of CH₃OH; (4) elute with 3 mL of 75% CH₃OH:20% H₂O:5% NH₄OH mixture into a silanized collection tube; (5) evaporate eluant to dryness under N₂ in a maximum 30 °C water bath; (6) reconstitute in 1 mL of 25% CH₃OH:74.5% H₂O:0.5% CHOOH mixture for HPLC analysis.

SCX-HPLC/UV analysis employed a Shimadzu (Columbia, MD) system consisting of LC-6A pumps capable of binary gradient separations, a SIL-6A injector using 150- μ L sample injection, a SPD-6A UV spectrophotometer set to 240 nm, and a SCL-6A controller. A Spherisorb SCX, 5 μ m, 15 cm by 4.6 mm (i.d.) (Phase Separations, Inc., Norwalk, CT) column was used. Mobile phases were as follows: A, 25% CH₃OH:75% H₂O containing 1 g L⁻¹ NH₄OAc and 5 mL

L⁻¹ HOAc, pH 3.5–4.0; B, 16 g L⁻¹ NH₄OAc, pH 7.0–7.4. Two different methods employing mobile phases A and B at flow rates of 2 mL min⁻¹ were used:

time	mobile phase A (%)	mobile phase B (%)
DEHA and DIHA Separation Conditions		
0.00	100	0
12.00	100	0
13.00	0	100
18.00	0	100
19.00	100	0
24.00	100	0
HA Separation Conditions		
0.00	50	50
7.00	50	50
8.00	0	100
13.00	0	100
14.00	50	50
19.00	50	50

The retention times (t_r) under these conditions were approximately 9.5 min for DIHA, 12 min for DEHA, and 6.5 min for HA. Limits of detection were 0.1 $\mu\text{g L}^{-1}$ for all three analytes.

Thermospray HPLC/MS. HA was confirmed by thermospray HPLC/MS in the same samples described above for SCX-HPLC/UV. DEHA and DIHA were confirmed by thermospray HPLC/MS in a subset of these samples containing the highest concentrations (6% of all samples collected). Samples were prepared by SCX SPE (see above) followed by thermospray HPLC/MS. HPLC conditions were described above for SCX-HPLC/UV with some exceptions. Sample injection volume was 100 μL , and mobile phase flow rate was 0.7 mL min⁻¹. For DIHA and DEHA, the mobile phase composition (described above) was isocratic 90% A/10% B resulting in a t_r of 7.5 min for DIHA and of 8.8 min for DEHA. For HA, the separation conditions were isocratic 10% A/90% B resulting in a t_r of 8.3 min. A Zorbax 300 SCX, 6 μm , 15 cm by 4.6 mm (i.d.) column (Mac-Mod Analytical, Inc., Chadds Ford, PA) was used. Typical thermospray interface and MS conditions were as follows: mass spectrometer, VG-TRIO 3 (Fisons Instruments, Inc., Danvers, MS); MS mode, thermospray positive ion; thermospray capillary temperature, 320 °C; MS electrode, 185 V; MS collector multiplier, 500 V; MS scan time, 5.00 s. Relative ion counts of each HADP were performed in selective ion monitoring (SIM) mode for masses corresponding to the $[M + H]^+$ parent ions as follows: 156 for DIHA, 170 for DEHA, and 198 for HA.

Electrospray HPLC/MS/MS. The daughter ion mass spectra of HA and DEHA were obtained using fractions collected from samples prepared for routine analysis by SCX SPE and C₈-HPLC/UV. The Beckman HPLC system (San Ramon, CA) used for routine analysis was equipped with an ISCO Foxy 200 fraction collector (Lincoln, NE), and fractions of DEHA and HA were collected from six different samples representing about 6% of the samples collected for the entire study. Twenty 1-mL fractions were pooled into a glass culture tube for each sample and analyte. Fractions were also collected from standards of DEHA and HA. The fractions were evaporated to 1–2 mL in a Savant Speedvac SS-4 evaporator (Farmingdale, NY) at 60 °C and about 670 Pa and further evaporated just to dryness under a stream of N₂(g) at ambient temperature (22–25 °C). Samples were reconstituted in 5 mL of CH₃OH, by repeated vortex mixing, and evaporated under a stream of N₂(g) to

about 100 μL for DEHA and to about 250 μL for HA. Standard samples were analyzed by HPLC, and overall efficiencies for the evaporation procedure were about 80% for both DEHA and HA. Final concentrations of the fractions were estimated to be 28–150 $\mu\text{g L}^{-1}$ for DEHA and 267–1002 $\mu\text{g L}^{-1}$. DIHA was omitted from these analyses because its final concentrations were below the MS/MS detection limits of 10–12 $\mu\text{g L}^{-1}$. A Finnigan TSQ-7000 MS/MS system (San Jose, CA) and a Thermo Separation Products CM4100 HPLC system (San Jose, CA) with an electrospray ionization interface (electrospray HPLC/MS/MS) were used to obtain daughter ion spectra from the fraction samples of DEHA and HA. HPLC separations were performed using a Hypersil microbore C₁₈, 25 cm by 1.0 mm (i.d.) column, sample injection volume of 5 μL , and a flow rate of 75 $\mu\text{L min}^{-1}$. Mobile phase A was 2% aqueous CH₃CN with 0.1% CHOOH/NH₄CHO₂, pH 3, and mobile phase B was 10% H₂O:80% CH₃CN:10% tetrahydrofuran (C₄H₈O) with 0.1% CHOOH/NH₄CHO₂, pH 3. Mobile phase gradient separation conditions were

time	mobile phase A (%)	mobile phase B (%)
0.00	90	10
3.00	90	10
25.00	10	90
30.00	10	90
32.00	90	10
40.00	90	10

These conditions resulted in a t_r of 12.9 min for HA and of 23.3 min for DEHA. MS/MS conditions for $[M + H]^+$ masses of 198 (HA) and 170 (DEHA) were as follows: electrospray ionization voltage, 4.5 kV; capillary temperature, 225 °C; scan time, 1 s; scan range, 50–225 amu; collision energy, 25 eV; collision gas, Ar; multiplier voltage, 1200 V.

Direct Probe MS. Full-scan mass spectra were obtained by direct probe MS for DEHA fractions described in the Electrospray HPLC/MS/MS section. AHP 5989A MS engine (Palo Alto, CA) was used under the following conditions: N₂ head pressure, 0.55 MPa; electron impact source temperature, 250 °C; quadrupole temperature, 100 °C; scan range, 40–250 amu; repeller voltage, 7 V; electron energy, 70 eV; multiplier voltage, 2906 V; MS mode, positive ion detection. A 2- μL sample was put in a capillary tube and placed on the probe tip. The probe tip was held at 40 °C for 1 min, then heated to 120 °C at 30 °C min⁻¹, and held for 1 min.

Results and Discussion

Monitoring of HADPs in Streamwater. HADPs were consistently detected in Goodwater Creek over the 2.5-yr monitoring period reported (Figure 2). Concentrations ranged from 0.18 to 5.7 $\mu\text{g L}^{-1}$ for HA, from <0.12 to 1.9 $\mu\text{g L}^{-1}$ for DEHA, and from <0.12 to 0.72 $\mu\text{g L}^{-1}$ for DIHA. Total runoff to the stream varied greatly during the study with 15 cm in 1992, 75 cm in 1993, and 30 cm in 1994 compared to the 20-yr (1971–91) annual average of 29 cm. Despite the extreme variability in runoff, DEHA and HA showed similar seasonal concentration changes from year to year. Their concentrations increased during the spring, reached their highest levels in June or July, slowly dissipated from late summer to fall, and reached very low or non-detectable levels in the winter. This same general pattern has been reported for other herbicides and metabolites in surface water (6, 7) except that the extent of dissipation by fall was greater than that observed for HA and DEHA.

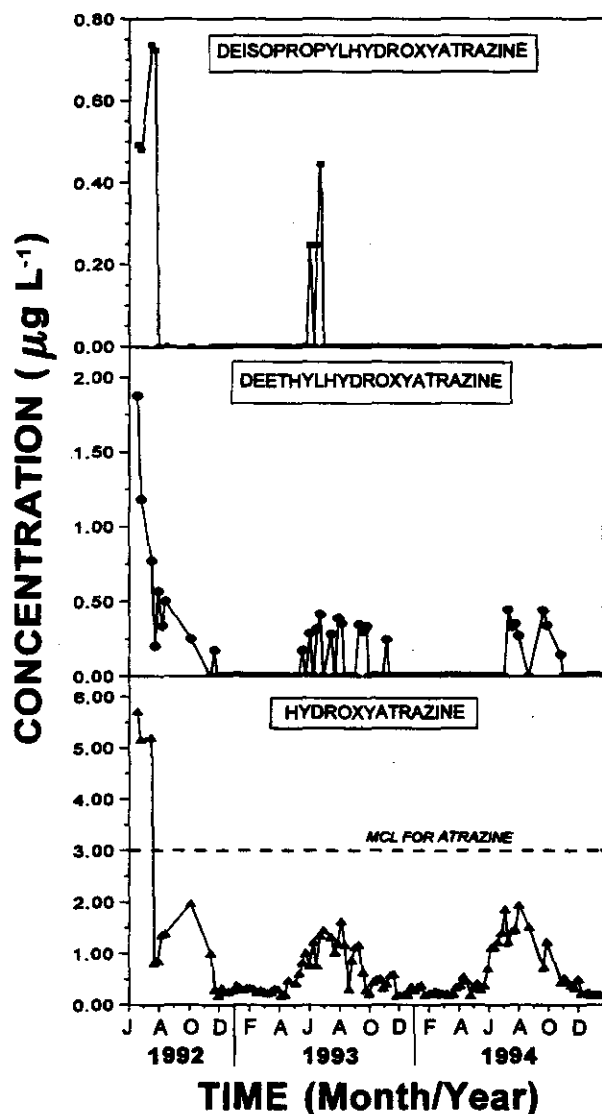


FIGURE 2. Concentrations of hydroxyatrazine (HA), deethylhydroxyatrazine (DEHA), and deisopropylhydroxyatrazine (DIHA) in Goodwater Creek from June 1992 to December 1994.

Concentrations of HA and DEHA varied inversely relative to streamflow throughout the study (Figures 2 and 3). For example, a small runoff event in mid-July 1992 reduced HA concentration from 5.2 to 0.82 $\mu\text{g L}^{-1}$ in 1 week. Thereafter, HA concentrations steadily increased over the next 70 d to 2.0 $\mu\text{g L}^{-1}$, during which time there were no significant runoff events (Figure 3). This relationship was also observed in 1993 and 1994 when several major runoff events led to lower concentrations of HA or DEHA followed by increases in concentration under baseflow (i.e., low streamflow) conditions. Thus, for this watershed, HA or DEHA concentrations decreased due to dilution by the surface runoff. The rise in HADP concentrations during baseflow conditions indicates that there was a source of HADPs in the creek during these periods. Quarterly samples (June, September, and December 1994 and March 1995) of shallow groundwater in the creek's alluvium indicated that no HADPs were present, and tile drainage is not used in the watershed. Thus, soluble HADPs in baseflow originated from other sources such as desorption from sediment and chemical, photochemical, or microbial hydrolysis of atrazine in the stream.

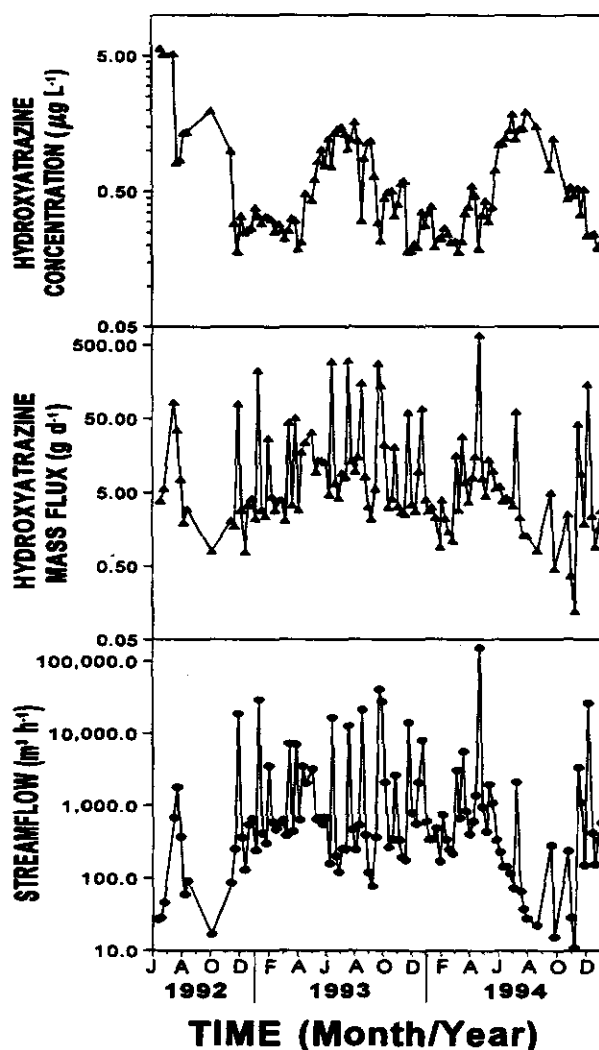


FIGURE 3. Hydroxyatrazine (HA) concentration and mass flux in relation to Goodwater Creek streamflow from June 1992 to December 1994.

In contrast to the concentration data, the mass flux of HA was directly related to streamflow (Figure 3). During surface runoff events, the total mass of HA increased even though its concentration decreased in the creek. Therefore, the increased mass of HA in the stream was a direct consequence of HA transported during runoff events. This strongly suggests that one of the mechanisms by which HA reached the streamwater was desorption from soils and subsequent transport in solution by surface runoff. Some transported HA may have come from the field soil solution levels maintained by the equilibrium between adsorbed and solution HA. However, the soil solution is unlikely to be a significant source of HA transported during runoff events because this equilibrium strongly favors adsorption (9, 52). Photolytic hydrolysis of atrazine on soil and plant surfaces is another potential source of soluble HA transported by surface runoff.

Comparison of HADPs to Chlorinated Analogues. Atrazine and DEA generally showed higher concentrations in spring and early summer and lower concentrations in late summer and fall than HA (Figure 4). In the first 2–3 months after atrazine application, atrazine and DEA showed large concentration spikes while HA concentrations remained low. The highest concentration spikes were 112

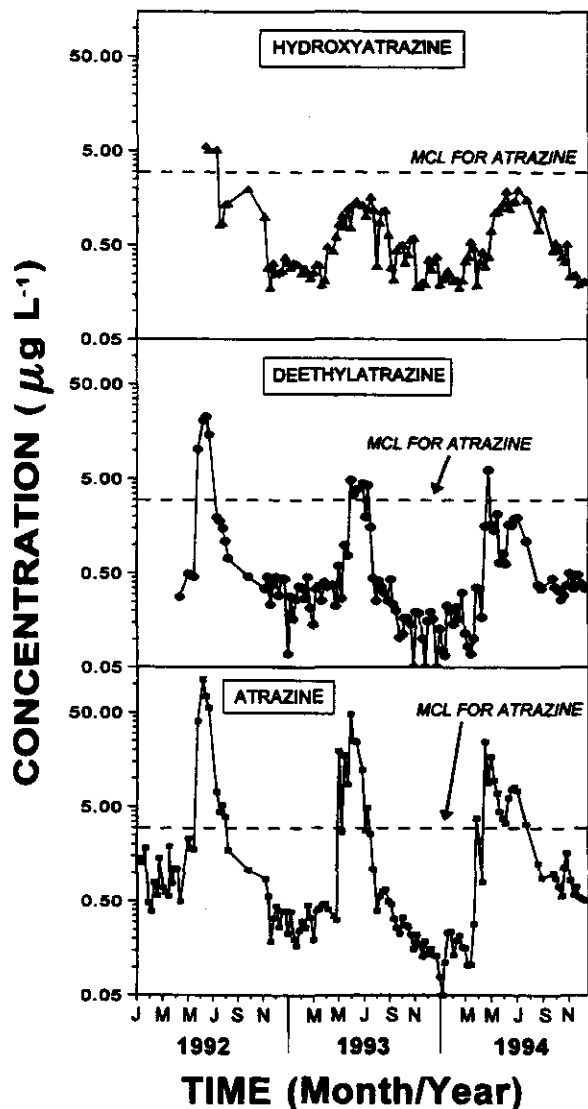


FIGURE 4. Concentrations of hydroxyatrazine (HA), deethylatrazine (DEA), and atrazine in Goodwater Creek from January 1992 to December 1994.

$\mu\text{g L}^{-1}$ for atrazine, $23 \mu\text{g L}^{-1}$ for DEA, and only $5.7 \mu\text{g L}^{-1}$ for HA. In 1993 and 1994, levels of atrazine and DEA in the stream peaked during surface runoff events from May through July. These same events diluted HA. Streamwater concentrations of atrazine and DEA resulting from surface runoff events decreased with increasing time after atrazine application. In contrast, HA levels generally were higher than either atrazine or DEA by late August of 1992 and 1993, and it remained higher until November of both years, except during significant runoff events. During the winters of 1992–1993 and 1993–1994, atrazine, DEA, and HA were usually similar in concentration ($0.1\text{--}0.3 \mu\text{g L}^{-1}$) except DEA, which decreased to very low levels on occasion. In 1994, dry conditions during the growing season led to very low amounts of atrazine and DEA transported from soil, and therefore their levels in the stream remained as high or higher than HA through the fall.

The greater persistence of HA in the stream relative to atrazine and DEA primarily resulted from four processes. First, atrazine and DEA have lower soil adsorption than HA; thus, more atrazine and DEA were available for transport in runoff during late spring to early summer. By fall, less atrazine and DEA remain available in the surface

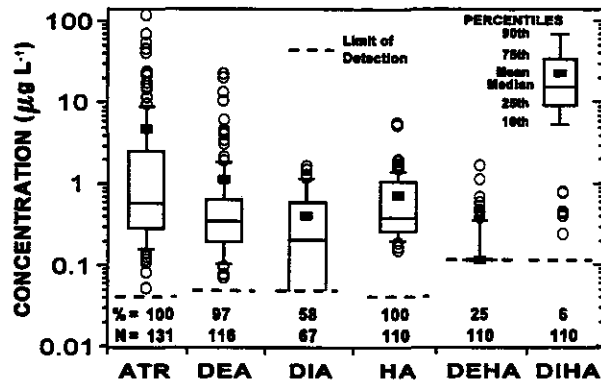


FIGURE 5. Summary of concentrations and frequency of detection (expressed as percent) of atrazine and its degradation products in Goodwater Creek from June 1992 to December 1994 (N = sample size).

soil for transport. In 1993 and 1994, average mass transport during the 8-week period following atrazine application accounted for 89% of the atrazine, 57% of the DEA, and only 36% of the HA annually transported from the watershed. Second, as adsorption and bound residue formation of atrazine increased with time after application, atrazine hydrolysis would become progressively more significant (19, 37). Third, atrazine and DEA biologically degrade more than HA in agricultural soils (8, 27). Thus, the greater persistence and adsorption of HA resulted in proportionally more remaining in surface soil later in the season. Fourth, atrazine and DEA will both be subject to leaching to a greater extent than HA (10, 11), further increasing the proportion of HA to atrazine or DEA in the plow layer with time. Both atrazine and DEA leached to depths of 0.9 m in field plots equipped with pan lysimeters, and DEA has frequently been detected in groundwater wells to depths of 15 m within Goodwater Creek watershed (unpublished data).

Frequency of HADP detection in surface water samples was 100% for HA, 25% for DEHA, and 6% for DIHA (Figure 5). Atrazine, HA, and DEA were the most frequently detected surface water contaminants resulting from atrazine use in the watershed. As a group, the HADPs were detected less frequently than their chlorinated analogues, and the N-dealkylated HADPs were relatively insignificant surface water contaminants. Atrazine and DEA showed much broader concentration ranges and higher mean concentrations than HA. Because of a few high concentration spikes, mean concentrations of atrazine ($4.9 \mu\text{g L}^{-1}$) and DEA ($1.3 \mu\text{g L}^{-1}$) were greater than their respective 75th percentile concentrations. Because HA concentrations do not greatly fluctuate like those of atrazine and DEA, its mean ($0.74 \mu\text{g L}^{-1}$) and median ($0.38 \mu\text{g L}^{-1}$) concentrations were similar. The median HA concentration was slightly greater than DEA and only $0.19 \mu\text{g L}^{-1}$ lower than atrazine. The N-dealkylated HADPs were infrequently detected with both occurring at levels below $2 \mu\text{g L}^{-1}$. While DEHA was generally present from late spring through the fall, DIHA was detected only in late spring to early summer of 1992 and 1993. DEHA levels were not closely associated with those of DEA. Instead, DEHA concentrations in the stream were more related to HA concentrations showing a similar seasonal pattern and response to streamflow. DIHA was detected so infrequently that comparisons to the other metabolites were not valid. DIA analysis was limited to the last 1.5 years of the study, and it was primarily detected in the spring and summer. These results further confirm the

TABLE 1

Qualitative Confirmation of Hydroxylated Atrazine Degradation Products (HADPs) by SCX-HPLC/UV Compared to Quantitative Analysis by C₈-HPLC/UV

sample date	C ₈ -HPLC/UV (μg L ⁻¹)			SCX-HPLC/UV ^d		
	DIHA ^a	DEHA ^b	HA ^c	DIHA	DEHA	HA
6/22/92*	0.48	1.2	5.2	Y	Y	Y
7/13/92*	0.74	0.77	5.2	Y	Y	Y
8/10/92	ND ^e	0.50	1.4	N	Y	Y
9/29/92*	ND	0.25	2.0	N	Y	Y
11/9/92	ND	0.17	1.0	N	Y	Y
11/16/92	ND	ND	0.29	N	Y ^f	Y
12/29/92	ND	ND	0.38	N	N	Y
1/19/93	ND	ND	0.33	N	N	Y
2/22/93	ND	ND	0.26	N	N	Y
4/5/93	ND	ND	0.21	N	N	Y
4/12/93	ND	ND	0.49	N	N	Y
5/17/93	ND	ND	1.0	N	N	Y
6/14/93*	0.45	0.41	1.4	N ^g	Y	Y
6/28/93*	ND	0.69	1.5	Y ^h	Y	Y
7/14/93	ND	ND	1.0	N	Y ⁱ	Y
7/26/93*	ND	0.36	1.6	N	Y	Y
8/10/93	ND	ND	0.30	N	N	Y
8/30/93	ND	0.35	1.2	N	Y	Y
9/14/93	ND	0.34	0.29	N	Y	Y
10/4/93	ND	ND	0.49	N	N	Y

* Samples indicated by * were also confirmed for DIHA by thermospray HPLC/MS. ^b Samples indicated by * were also confirmed for DEHA by thermospray HPLC/MS, direct probe MS, and electrospray HPLC/MS/MS. ^c All samples listed were also confirmed for HA by thermospray HPLC/MS, and samples indicated by * were also confirmed by electrospray HPLC/MS/MS. ^d Presence confirmed indicated by Y (yes) or N (no). ^e ND, not detectable at 0.12 μg L⁻¹ for DIHA and DEHA. ^f Detected at 0.10 μg L⁻¹. ^g Presence confirmed by thermospray HPLC/MS. ^h Detected at 0.17 μg L⁻¹ and confirmed by thermospray HPLC/MS. ⁱ Detected at 0.36 μg L⁻¹.

presence of atrazine, DEA, and DIA in the surface water of the Corn Belt region, and atrazine and DEA are more frequently detected than DIA in watersheds with limited cyanazine use (6, 7, 54). Our data showed higher year-round prevalence of atrazine and DEA in streamwater than previously reported (6, 7), but no previous long-term studies to our knowledge have been published on HADPs in surface water.

Confirmation of HADPs in Streamwater. Separation of HADPs by SCX-HPLC/UV was used to confirm the HADPs in streamwater samples after initial quantitation by C₈-HPLC/UV (18) (Table 1). Samples used for confirmation represented a broad range of concentrations and times of year. Because of the lengthy storage time of some samples before analysis by SCX-HPLC/UV (up to 1.5 years), the data were only used for qualitative confirmation. SCX-HPLC/UV confirmed the C₈-HPLC/UV analysis, detecting HA in all 20 samples. For DEHA and DIHA, 18 of the 20 samples analyzed by SCX-HPLC/UV agreed with the C₈-HPLC/UV analysis. One discrepancy for DEHA was a sample reported to have 0.10 μg L⁻¹ by SCX-HPLC/UV, the detection limit of the method but below the detection limit of the C₈-HPLC/UV method. The other discrepancy for DEHA was a reported detection of 0.36 μg L⁻¹, yet the original analysis did not detect DEHA. SCX-HPLC/UV analysis of the sample collected on June 14, 1993, did not detect DIHA, but thermospray HPLC/MS analysis confirmed the presence of DIHA, supporting the original C₈-HPLC/UV analysis. Conversely, both SCX-HPLC/UV and thermospray HPLC/MS confirmed the presence of DIHA in the sample collected on June 28, 1993, but the C₈-HPLC/UV analysis did not detect DIHA. Overall, SCX-HPLC/UV results confirmed the

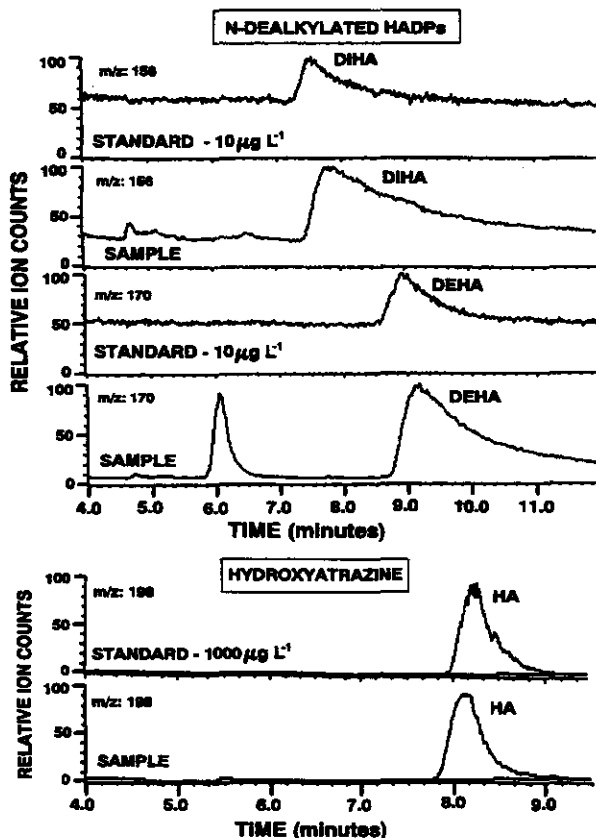


FIGURE 6. Relative ion chromatograms from thermospray HPLC/MS operated in selective ion mode (SIM) of hydroxyatrazine (HA), deethylhydroxyatrazine (DEHA), and deisopropylhydroxyatrazine (DIHA) standards and streamwater samples.

C₈-HPLC/UV analyses despite some discrepancies for N-dealkylated HADPs.

SIM mode thermospray HPLC/MS also confirmed the presence of HADPs for those samples indicated in Table 1. Relative ion chromatograms of [M + H]⁺ parent ions showed excellent agreement between retention times of standards and samples for each mass monitored (Figure 6). For HA, thermospray HPLC/MS confirmed the HPLC/UV results, detecting HA in all 20 samples. Confirmation of DIHA and DEHA also showed good agreement with the HPLC methods. DEHA was confirmed by thermospray HPLC/MS in all six samples analyzed and in all cases agreed with both HPLC/UV methods. DIHA analysis by thermospray HPLC/MS showed discrepancies with one of the six samples for each of the HPLC/UV methods (as discussed above). Overall, thermospray HPLC/MS detected DIHA in four of the six samples analyzed. Thermospray HPLC/MS was previously used to confirm HA in surface water (12). Qualitative mass spectral confirmation by thermospray HPLC/MS has the advantage of direct analysis of HADPs, and when used in conjunction with SCX-HPLC/UV, it can provide routine confirmation using the same sample prepared by SCX SPE (as described under the Confirmation Analyses Section). The major disadvantage is the lack of structural information needed for more definitive confirmation. Hence, more rigorous mass spectral methods were performed for confirmation of HA and DEHA. The low levels of DIHA in water samples prevented further analyses by such techniques.

Daughter ion mass spectra of HA and DEHA were obtained by electrospray HPLC/MS/MS via Ar collision of

TABLE 2

Relative Abundance and Tentative Identification of Parent and Daughter Ions from Mass Spectra of Hydroxyatrazine (HA) and Deethylhydroxyatrazine (DEHA) by Electrospray HPLC/MS/MS and Direct Probe MS

method	compound	molecular weight	observed parent and daughter ions		relative abundance (%) ^a
			<i>m/z</i>	tentative identification	
electrospray HPLC/MS/MS	hydroxyatrazine (HA)	197	198 ^b	[M + H] ⁺	9-18
			156	[M - C ₃ H ₇ + 2H] ⁺	100
			128	[M - C ₃ H ₇ - C ₂ H ₅ + 3H] ⁺	7-12
			113	[M - C ₃ H ₇ - C ₂ H ₅ - NH + 3H] ⁺	42-71
			97	[M - C ₃ H ₇ - C ₂ H ₅ - 2NH + 2H] ⁺	18-34
			86	[M - C ₃ H ₇ - C ₂ H ₅ - NH - HCN + 3H] ⁺	40-73
			69	[M - C ₃ H ₇ - C ₂ H ₅ - NH - HCN - OH + 3H] ⁺	11-16
			171 ^b	[M + 2H] ⁺	4-10
			129	[M - C ₃ H ₇ + 3H] ⁺	1-4
			101	[M - C ₃ H ₇ - CO + 3H] ⁺	8-15
direct probe MS	deethylhydroxyatrazine (DEHA)	169	73	[M - C ₃ H ₇ - CO - HCN + 2H] ⁺	36-72
			57	[M - C ₃ H ₇ - CO - HCN - NH ₂ + 2H] ⁺	100
			169 ^b	[M] ⁺	64-100
			97	[M - C ₃ H ₇ - 2NH + H] ⁺	18-23
			81	[M - C ₃ H ₇ - 2NH - OH + 2H] ⁺	11-44
			69	[M - C ₃ H ₇ - 2NH - OH - HCN + 2H] ⁺	60-100
			43	[M - C ₃ N ₄ OH ₃ - NH] ⁺	33-85

^a Range of observed abundances for each ion. ^b Parent ion.

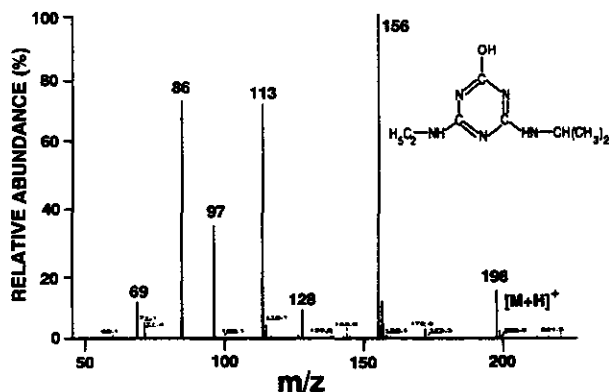


FIGURE 7. Electrospray HPLC/MS/MS daughter ion mass spectra of the corresponding [M + H]⁺ ion of hydroxyatrazine (HA) isolated from a streamwater sample (note: the molecular structure depicted represents the tautomeric form fragmented).

their corresponding [M + H]⁺ ions (Figures 7 and 8). The [M + H]⁺ parent ion at *m/z* 198 was observed for all HA samples, and tentative identification of all major daughter ions provided further evidence for identification of HA (Figure 7 and Table 2). The most abundant daughter ion for all HA samples was at *m/z* 156 formed by loss of the isopropyl group, but loss of the ethyl group was not observed for HA. For HA and other *s*-triazines, loss of the isopropyl group predominated over loss of the ethyl group (34). Loss of both alkyl groups was consistently observed, but at much lower abundance, at *m/z* 128 (Figure 7). The most diagnostic daughter ions were at *m/z* 128, 113, and 97 in which the dealkylated and increasingly deaminated 2-hydroxy-substituted triazine ring was present. Lower mass ions at *m/z* 86 and 69 represented triazine ring breakage via loss of an HCN group, and for *m/z* 69, the hydroxyl group was also removed. Based on our assignment of the daughter ion fragments, the chromatography conditions resulted in HA fragmentation in the enol form (as depicted in Figure 7). The HA mass spectra by electrospray HPLC/MS/MS very closely matched that produced by thermospray HPLC/MS/MS (34).

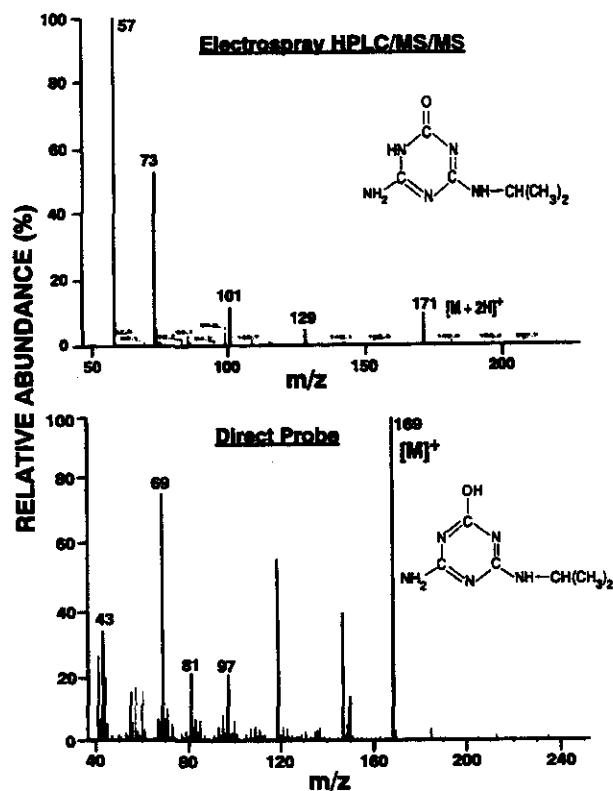


FIGURE 8. Mass spectra of deethylhydroxyatrazine (DEHA) isolated from a streamwater sample using electrospray HPLC/MS/MS via the corresponding [M + H]⁺ ion and direct probe MS (note: the molecular structures depicted represent the tautomeric forms fragmented).

Daughter ion mass spectra of DEHA by electrospray HPLC/MS/MS consistently showed the presence of the [M + 2H]⁺ parent ion (*m/z* 171) for all samples (Figure 8). Given the results of the HA mass spectra, this was an unexpected finding, but the formation of a [M + 2H]⁺ parent ion for several triazines by negative mode thermospray HPLC/MS has been reported (34). In addition, the assignment of all major daughter ions could be explained in

terms of $[M + 2H]^+$ parent ion fragmentation. The most abundant daughter ions were at m/z 57 with a relative abundance of 100% for all samples and at m/z 73 with relative abundances of 36–72% (Table 2). These ions were not, however, uniquely diagnostic of DEHA since they represented breakage of the triazine ring by removal of keto (C=O) and HCN groups. The most diagnostic daughter ion (i.e., possessing an intact triazine ring with an O at the 2 position) in the DEHA spectra was of very low relative abundance at m/z 129, formed by loss of the isopropyl group. Unlike HA, the assignment of daughter ion fragments for DEHA indicated loss of a keto group rather than loss of a hydroxyl group at the 2 position of the triazine ring. Therefore, under the same chromatography conditions used for HA, DEHA was in the keto form (Figure 8).

Further work was needed to confirm DEHA and to validate the electrospray HPLC/MS/MS spectra because the $[M + 2H]^+$ parent ion was observed and abundant diagnostic daughter ions were lacking. Therefore, direct probe MS was used to obtain full-scan mass spectra of DEHA (Figure 8). Direct probe MS of all DEHA samples exhibited the expected $[M]^+$ parent ion at m/z 169 with high relative abundance (Table 2). In addition, abundant diagnostic daughter ions at m/z 97, representing the 2-hydroxy-substituted triazine ring, and at m/z 81, representing the unsubstituted triazine ring, were observed. Major abundant daughter ions were also observed at m/z 69, formed by triazine ring breakage by loss of a HCN group, and at m/z 43 $[C_3H_7]^+$. Other higher mass daughter ions of DEHA may have been masked due to interactions with dibutyl phthalate (base peak at m/z 149) and unidentified contaminants that resulted in ions at m/z 119 and 147. A distinct disadvantage of direct probe MS was that temperature separation was incapable of separating contaminants from DEHA, producing spectra with many extraneous ions. Nonetheless, the DEHA spectra obtained by direct probe MS supported the conclusions that DEHA was present in the samples and that the electrospray HPLC/MS/MS spectra for DEHA was valid. Based on the assignment of fragments in the direct probe spectra of DEHA and its closer correspondence to the electrospray HPLC/MS/MS spectra of HA, DEHA was apparently in the enol form under conditions used for direct probe MS (Figure 8). The enol tautomer of DEHA apparently predominated in methanol for the direct probe samples whereas the pH 3 buffer used in the mobile phase of the electrospray HPLC/MS/MS analyses favored formation of the keto tautomer. Therefore, the unexpected differences in mass spectra between HA and DEHA using electrospray HPLC/MS/MS likely resulted from fragmentation of different tautomers.

Summary and Conclusions

More than 2 years of monitoring Goodwater Creek established that HADPs are surface water contaminants. HA was the most significant HADP with greater concentrations and frequency of detection than the N-dealkylated HADPs. Changes in HA and DEHA concentrations in the stream were inversely related to streamflow, indicating dilution during surface runoff events and a source of HADPs or formation of HADPs under baseflow conditions. HA mass flux was directly related to streamflow, demonstrating that HA was transported in solution by surface runoff events. HA fluctuated less in the stream than atrazine and DEA, resulting in lower average concentrations but similar median concentrations. HA persistence in the stream was

greater in late summer and fall than atrazine or DEA. The presence of all HADPs was confirmed by methods employing two different HPLC separation modes and detection using UV and various MS modes. Verification of the stream monitoring data represented the first direct proof of HADPs as surface water contaminants and established HA contamination of surface waters as an important fate pathway for atrazine in the Goodwater Creek watershed. On the basis of these results, the most likely mechanisms responsible for HADP contamination of surface water were as follows: (1) transport of HADPs in solution by surface runoff events, soluble HADPs most likely occur via desorption of HADPs from soil; (2) desorption of HADPs from stream sediment deposited by surface runoff events; and (3) chemical, microbial, and aqueous photolytic hydrolysis of atrazine and its chlorinated degradation products in the stream. Further studies to establish which of these processes are most important are currently under way.

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