FATE AND TRANSPORT OF HERBICIDES AND THEIR TRANSFORMATION PRODUCTS IN THE POTOMAC RIVER BASIN, VIRGINIA, USA

by

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DEDICATION

This is dedicated to my dear wife, Sophie who has been with me through every moment, my son Nathan who became a steely-eyed river man and my son Alex who has made every step of the way fun.

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I would like to thank my advisor, Dr. Gregory D. Foster for his guidance and inspiration, my family for their emotional and logistical support, Dr. Vikas Chandhoke, dean of the College of Science and my supervisor of 15 years, June Liu, for laboratory assistance and finally, my high school student interns and mentees, Sophia Youn, Ishan Baradan, Stella Kim, So-Jung Kim, Alex Streicher, Spencer Clark, Jeremy Weller and Andrea Lorico.

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LIST OF ABBREVIATIONS

ACE	acetochlor
ALA	alachlor
ATR	atrazine
DAR	desethylatrazine to atrazine ratio
DEA	desisopropyl atrazine
DEDIA	desethyl desisopropyl atrazine
DEHA	desethyl 2-hydroxy atrazine
DIA	desisopropyl atrazine
DIHA	desisopropyl 2-hydroxy atrazine
EMDL	estimated method detection limits
EMR	metolachlor ethane sulfonic acid to metolachlor ratio
ESA	ethane sulfonic acid moiety
ESI	electrospray ionization
HAR	2-hydroxyatrazine to atrazine ratio
IS	internal standard
IS-CID	in-source, collision-induced dissociation
LC-MS(Q)	liquid chromatography- single-quadrupole mass spectrometry
MET	metolachlor
NFSR	North Fork of the Shenandoah River
OA	oxanilic acid moiety
PROP	propazine
SIM	simazine
SPE	solid phase extraction
SS	surrogate standard
TP	transformation product

ABSTRACT

FATE AND TRANSPORT OF HERBICIDES AND THEIR TRANSFORMATION PRODUCTS IN THE POTOMAC RIVER BASIN, VIRGINIA, USA

Thomas B. Huff, PhD

George Mason University, 2011

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This study is comprised of three projects designed to characterize the fate and transport of S-triazine herbicides (e.g., atrazine), chloroacetanilide herbicides (e.g., metolachlor) and the transformation products (TPs) of those herbicides in surface waters of the Potomac River watershed in Virginia. The projects include instrumental-method development, field-method development and long-term implementation of those methods in a twelve month study on the North Fork of the Shenandoah River.

The goal of project 1 was to develop a robust method of analyzing surface water samples for the target analytes using solid-phase extraction (SPE) cartridges and a single quadrupole LC-MS system. Estimated method detection limits averaged 0.3 ± 0.1 ng L^{-1}. Spike recoveries ranged from 94.2% ± 4.8% for S-triazines and their TPs and 95.9% ± 19% for chloroacetanilides and their TPs, thus qualifying the method for instrumental analysis.

The goal of project 2 was to develop field sampling protocols by examining the temporal changes in the seasonally applied herbicides over a seven month period and relating those changes to spatial variables in the upper Shenandoah River basin and in Cedar Run, both tributaries to the Potomac River. TP concentrations increased rapidly following the application period. Substantial differences in TP to atrazine ratios distinguished the Shenandoah River from the Cedar Run basin. Principal component analysis showed that concentration did not correlate well with river flow (discharge).

The goal of the third project was to characterize the major biogeochemical processes in a river system located in the western section of Virginia. Surface water samples were obtained during 14 sampling trips over a 12 month period beginning on 29 March 2008 and culminating on 28 February 2009 from 4 sites along the North Fork of the Shenandoah River in Virginia. Detection frequencies were 100% for five of the target analytes. The desethylatrazine to atrazine concentration ratio (DAR) increased linearly over the study period with a value of ~0.4 at the spring flush period to ~2.5 at the end of the study. Transformation rates for S-triazines ranged from 0.025 to 0.031 day^{-1}. The removal rates for total herbicide concentrations ranged from 0.019 to 0.050 day^{-1}. Steady-state concentrations for 3 of the 4 sites were above 100 ng L^{-1}.

The instrumental and field methods developed in this study proved effective and a long term study using those methods successfully characterized the primary processes affecting the fate and transport of these herbicides in an important surface water system.

CHAPTER 1: A ROBUST METHOD FOR PARTS-PER-TRILLION ANALYSIS OF HERBICIDES AND TRANSFORMATION PRODUCTS IN SURFACE WATER

Introduction

Recent studies examining the concentrations of herbicides in surface waters have expanded target analyte lists to include transformation products along with the parent compounds (Kalkhoff et al., 2003; Krutz et al., 2004; McConnell et al., 2007; Panshin et al., 2000). These transformation products (TPs) are produced through abiotic and biotic processes in the soil, groundwater and surface waters near their point of application (Behki and Khan, 1994; De Souza et al., 1998; Vibber et al., 2007). When TPs have been included in surface water reconnaissance studies, the detection frequencies for total parent and TPs often increase from 60 to over 90% (Kolpin et al., 1998), which provides more detailed information on the fate and transport of pesticides in the aquatic environment.

The quantification of herbicides and associated TPs at low parts-per-trillion concentrations (ng/L) serves as a challenging problem in the study of the fate and transport of contaminants in surface waters. In the Chesapeake Bay region, for example, considerable effort has been devoted to developing methods to quantify accurate loadings of triazine herbicides to the mainstem Bay through the major tributaries, including

atrazine [2-chloro-4-ethylamino-6-isopropylamino-*S*-triazine], simazine [2-chloro-4,6-bis(ethylamino)-*S*-triazine] and propazine [2-chloro-4,6-bis(isopropylamino)-*S*-triazine], and the parent chloroacetanilide herbicides include metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-2-methoxy-1-methylethyl-acetamide], alachlor [2-chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl)-acetamide] and acetochlor [2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)-acetamide]. Atrazine is the single most widely used herbicide in the United States and can be found in parts-per-million (mg L^{-1}) concentrations in agricultural runoff (Bringolf et al., 2004) to low parts-per-trillion concentrations in Chesapeake Bay. The United States Environmental Protection Agency's Maximum Contaminant Level (MCL) for atrazine is 3.0 μg L^{-1} (US-EPA, 2004). Atrazine may cause serious health effects in humans exposed to concentrations greater than the MCL for relatively short periods of time. These effects include congestion of heart, lungs, and kidneys, low blood pressure, muscle spasms, weight loss and damage to adrenal glands (US-EPA, 2003). It has been shown to be an immune system disruptor in aquatic species, (Brodkin et al., 2007) and studies to date have debated whether atrazine is an endocrine—disrupting chemical that can effect reproduction and produce intersex anomalies (Bringolf et al., 2004; Carr et al., 2003; Hayes et al., 2003). At the time of this writing, atrazine is undergoing a re-evaluation by the EPA to consider additional toxicological research on atrazine and its chlorinated transformation products (US-EPA, 2009).

Atrazine (ATR) is transformed in soils predominantly to form desethyl atrazine (DEA) and desisopropyl atrazine (DIA) by non-specific cytochrome P450

monooxygenases (Devers et al., 2005) in microbes. These enzymes are found in ubiquitous soil bacteria such as *Pseudomonas sp.* (Khan and Behki, 1990; Seffernick et al., 2002) and *Rhodococcus spp*. strains TE1 (Behki et al., 1993) and B-30 (Behki and Khan, 1994). Simazine (SIM) and propazine (PROP) can also be transformed to mono- and diaminoatrazine by the same monooxygenases. The microbial enzymes necessary to transform atrazine or its dealkylated transformation products into hydroxyatrazine (HA), desisopropyl hydroxyatrazine (DIHA) and desethyl hydroxyatrazine (DEHA) are not naturally present in soils. However, repeated use of atrazine has resulted in enhanced degradation wherein bacteria or consortia of bacteria evolve the capacity to transform atrazine into hydroxyatrazine through atrazine chlorohydrolase enzymes, especially in carbon and/or nitrogen poor soil (Shaner and Henry, 2007). These atrazine or triazine adapted soils may increase atrazine total mineralization relative to non-adapted soils (Krutz et al., 2010). Alachlor, acetochlor, and metolachlor are biotransformed primarily by glutathione-S-transferase enzymes found in plants and microbes (Field and Thurman, 1996) forming ethane sulfonic acid (ESA) or an oxanilic acid (OA) chloroacetanilide derivatives. TPs tend to have lower organic carbon-normalized soil adsorption coefficients (K_{oc}) than the associated parent chemicals (Kaune et al., 1998). The desorption coefficients of the ESA and OA conjugates of chloroacetanilide herbicides are as much as 66% lower than those of metolachlor, indicating a higher tendency to desorb from soil and enter the subsurface water (Krutz et al., 2004). The changes in the molecular structure brought on by transformations impact the environmental fate and

transport of TPs as well as additional analytical considerations. The polar nature of TPs makes analysis more difficult in most cases.

The principal objective of our study was to show that reliable quantitation of triazine and chloroacetanilide herbicides in surface waters can be performed by taking full advantage of in-source collision induced dissociation (IS-CID) capabilities of LC-MS(Q), which is equipped with an orthogonal electrospray ionization (ESI) source that utilizes two ion-skimming cones. Voltage on the first cone can be set low to preserve the parent ion for each analyte in either $[M+H]^+$ positive ionization mode or $[M-H]^-$ negative ionization mode. When the voltage is increased, sufficient kinetic energy is imparted on the ion in order to induce fragmentation of the parent ion. This ionization mechanism produces the same fragmentation ions as those produced within collision cells in tandem mass spectrometry (QQQ) for certain compounds. The target analytes in this study are readily detectable at low ng L^{-1} concentrations using the IS-CID technique, even in surface waters where traditional LC-MS(Q) has displayed substantial matrix suppression effects that can occur through mobile phase additives, matrix effects as well as the ionization properties of the compounds being studied (Gosetti et al.). The secondary study objective was to apply the method to measure the surface water concentrations of herbicides and TPs in an agricultural region where they are extensively used. Finally, the tertiary study objective was to determine if the LC-MS method could facilitate the study of transformation of atrazine in microbiological isolates from agricultural soils in the Chesapeake Bay region. Such a study would determine if these soils and suspended

particulates have adapted the ability to transform parent herbicides into degradation products.

Materials and Methods

Materials

HPLC-grade solvents were purchased from Fisher Scientific (Pittsburg, PA). S-triazine and chloroacetanilide herbicide standards were purchased from Sigma-Aldrich (St. Louis, MO). Stable isotope standards were purchased from Cambridge Isotope Labs (Andover, MA) and from Toronto Research Chemicals (North York, Ontario, Canada). Solid-phase extraction (SPE) products were purchased from Waters Corporation (Milford, MA). LC-MS consumable supplies were purchased from Waters Corporation.

Lab grade water was produced in house using a recirculating deionization skid with UV sterilization treatment (HydroMax, Emmitsburg, MD) followed by polishing with an Elga Maxima ultra-purifier producing 18.2 MΩ water that is further treated for organics via a second UV lamp (Elga Labwater, Marlow, Buckinghamshire, UK).

Study Sites for Field Work

Field samples of surface water were taken from locations in the Cedar Run basin of northern Virginia (USA) in agricultural landscapes dominated by the use of atrazine, simazine, acetochlor and metolachlor. Cedar Run drains an area of relatively-intensive corn, hay, soybean and dairy cow operations in the Middle Potomac-Anacostia-Occoquan

region of the Atlantic Piedmont (Figure 1). Cedar Run joins Broad Run and Bull Run to form the Occoquan Reservoir, which serves as a drinking water source for over 1 million households in Fairfax County, VA. Little sampling has been conducted to date in the upper reaches of the Potomac River basin near the agricultural-urban boundary of the watershed in northern Virginia.

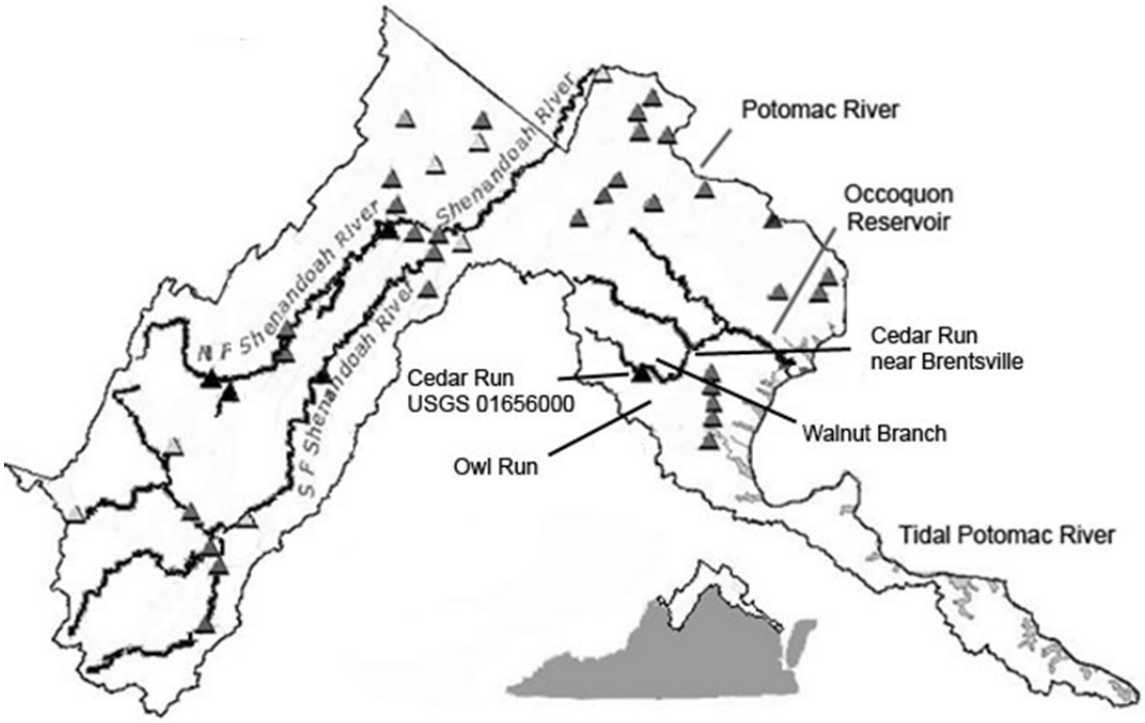


Figure 1: Map of the Potomac River Watershed in Virginia showing Cedar Run and its proximity to the Occoquan River (USGS, 2008).

Prior to springtime planting, pre-emergent herbicides are applied to control broadleaf plants, since those plants would contaminate the whole-plant feed and affect the quality of the milk produced. Runoff from crop fields enters Cedar Run through groundwater and small seasonal streams that form during storm events.

Four sites in the Cedar Run basin were sampled between August and November 2009. One site is located at the Route 28 bridge over Owl Run near Calverton, VA (38°37'57.35"N, 77°40'42.89"W). Owl Run is a standing pond created by a low-lying dam on a cattle farm. It is transformed into a fast running tributary to Cedar Run during storm events. Walnut Branch is primarily a dry stream bed with ponds formed in low lying sections (38°39'13.79" N, 77°37'11.13"W). It also becomes a swift running tributary to Cedar Run during storm events.

The primary sample site is on Cedar Run near Catlett, VA adjacent to a USGS gauging station (38°38'12" N, 77°37'31" W), which measures continuous data including discharge (m^3s^{-1}), gauge height, temperature and specific conductance. These parameters are broadcast in real time on the web (http://waterdata.usgs.gov/va/nwis/). The drainage area is 242 square kilometers.

The second site on Cedar Run was located near Brentsville, VA adjacent to a bridge along Prince William County Road 619 (38°41'14.24" N, 77°29'27.05" W). This site was near the confluence of Cedar Run and Broad Run, which combine to form the Occoquan River. The Occoquan River is the primary source for drinking water for Fairfax County, VA, an urban county with a population of over one million. The area between Catlett and Brentsville is primarily wooded with some agricultural land use.

Field sampling

The analytical method was field-tested using river water samples collected in the Cedar Run basin. Samples were collected on 7 August, 16 and 27 September and 12 November 2009. Samples were obtained using a Fultz submersible pump fitted with a 15.4 m Teflon® tube (Fultz Pumps, Inc., Lewistown, PA). Water samples were pumped into ~20 L stainless steel Cornelius kegs. The kegs were prewashed with warm soapy tap water, rinsed with tap water, and sequentially rinsed with de-ionized water, lab water and methanol using pressure from gaseous nitrogen to blow out the dip tube. The water sampling flow rate from the river into the kegs was approximately 1 L min^{-1}. When the kegs were filled, they were sealed with the gas-tight lid and transported to the lab and stored at 4 ^{o}C pending filtration and extraction.

Water samples were pressure filtered within 24 hr of collection. A high-purity nitrogen gas cylinder (Roberts Oxygen, Rockville, MD) was fitted with a two-stage regulator with a manually operated needle valve. The output pressure was adjusted to 100 psi. The regulator outlet was attached to the inlet ball valve on the Cornelius keg with 6.35 mm additive-free silicone tubing (Dow Corning, Midland, MI). The keg outlet ball valve was connected to a 142 mm stainless steel filter holder (Millipore Corporation, Billerica, MA) holding a 142-mm diameter Whatman Grade GF/F 0.7 μm nominal pore dia. glass microfiber filter overlaid with a 150 mm diameter grade GF/D 2.7 μm nominal pore dia. glass microfiber pre-filter (Whatman Inc., Florham Park, NJ), both of which were pre-rinsed under pressure with HPLC grade methanol followed by distilled water. The needle valve was pulsed to build sufficient head-space pressure in the keg to begin

water sample flow through the filters. The flow rate was kept at a level that allowed efficient filtration without damaging the filters. The needle valve was repeatedly pulsed when flow rate slowed. Sample filtrate was directed into 1 L glass media bottles, which had been pre-cleaned and solvent rinsed. Four 1-L aliquots per river water sample as well as several duplicates were stored at 4 ^{o}C awaiting subsequent solid phase extraction.

Sample extraction

Individual samples were comprised of four 1-L aliquots of the original ~20-L sample that were composited post extraction. Each 1-L bottle was spiked with 30 μL of surrogate recovery standards consisting of 0.3 ng μL^{-1} of simazine-d10 and 0.4 ng μL^{-1} of desethyl terbuthylazine for a total of 120 μL per sample. The 1-L sample filtrates were extracted using 250 mg Oasis HLB Plus cartridges (Waters Corporation, Milford, MA) that were fitted with empty 6-mL syringe barrels and placed on a Supelco Visiprep vacuum manifold (Sigma-Aldrich, St. Louis, MO). The cartridges were sequentially activated with 3 mL each of MTBE, methanol and reagent water, respectively. The samples were loaded on the HLB cartridges at a flow rate of ~5-10 mL min^{-1}. Following extraction, each HLB cartridge was subsequently washed with 3 mL of 5% (v/v) aq. methanol to remove inorganic interferences. The cartridges were desiccated for 30 minutes under vacuum and eluted with 2X 4 mL portions of 10% (v/v) methanol in MTBE. The eluents were combined into 12-mL silanized glass centrifuge tubes.

Sample extracts were concentrated using a Centrivap centrifugal vacuum concentrator (Labconco Corporation, Kansas City, MO). When sample extracts were

concentrated to approximately 1 mL, 1 mL of 0.1% acetic acid in reagent water was added to match the initial HPLC mobile phase conditions. The extracts were further reduced to 1 mL and quantitatively transferred to 2-mL amber auto-sampler vials (National Scientific Company, Rockwood, TN). The internal standards monocrotophos and terbuthylazine (2007) were added at 186.7 and 157.6 ng per sample, respectively. Sample extracts were stored at -20 ^{o}C pending analysis.

The particulate matter on the sample filters were not extracted since surface water samples as large as 10 L in other studies showed negligible concentrations of the target analytes (Liu et al., 2002; McConnell et al., 2007). Therefore, only the dissolved phase of river water was analyzed here as part of the methods development study.

Microbial transformations

During the 12 Nov. sampling trip, 1-L water samples were collected in sterile 1-L glass media bottles from the sites at Owl Run, Cedar Run and Walnut Branch. The samples were filtered in a level II biosafety cabinet using sterile Kontes® Ultra-Ware® filter flasks with a 47-mm filter holder with a stainless-steel support screen (Kimble Chase Life Science and Research Products LLC, Vineland, NJ). A filter set consisting of one 47-mmWhatman GF/F glass fiber filters with 0.7 μm nominal pore dia. under a Whatman GF/D glass fiber pre-filter, which had been previously autoclaved. Filtered particles were extracted into sterile river water from Cedar Run in capped glass tubes that were shaken overnight on a shaker table. Extracts were incubated in sterile river water in darkness at room temperature for 14 days until the media was visibly cloudy.

One set of 10 tubes of sterile river water from each sampling location contained dextrose and 10 μg mL^{-1} of atrazine. Each tube was inoculated with 500 μL of the bacterial culture. Tubes were incubated at room temperature in darkness for 12 days. One set of 10 tubes of sterile Cedar Run River water and dextrose without inoculum were also incubated for 14 days in darkness as a control for abiotic hydrolysis. A second set of 10 control tubes without inoculum were set in a window to control for photolysis and hydrolysis. On days 1, 2, 3, 5, 7, 10 and 12, 1-mL aliquots were taken from a tube from each set and placed in HPLC autosampler vials. The vials were stored frozen at -20 ^{o}C pending LC-MS analysis.

Instrumental analysis

Sample extracts were subsequently analyzed by liquid chromatography-mass spectrometry (LC-MS(Q)) for triazine and acetanilide herbicides and their transformation products. The LC system consisted of a Waters Alliance 2695 Separations Module (Waters Corporation, Milford, MA) with a quaternary pump, a refrigerated autosampler compartment, a heated column compartment and an inline vacuum degasser. The mass spectrometer was a Waters-Micromass ZQ2000 single quadrupole system with an orthogonal electrospray ionization (ESI) source (Waters Corporation).

Positive-ion electrospray ionization analysis

Because of their amine functional groups, the triazine herbicides and their transformation products as well as the parent chloroacetanilide herbicides were analyzed

using positive-ion electrospray ionization (Table 1). The HLB extracts were held at 4 ^{o}C in the auto-sampler chamber during analysis. The HPLC column used was a T3 Atlantis with an end-capped dC18 bonded phase in 3 μm particles and 100 Å pore size capable of operating in 100% aqueous conditions (Waters). The column measured 2.1 mm ID by 150 mm length, and it was held at 35 ^{o}C to maintain a constant temperature throughout the study period.

Table 1: Compounds evaluated using positive-mode electrospray ionization mass spectrometry

Analyte	SIM Group	RT (min)	Precursor Ion (m/z)	CV	Product Ion (m/z)	CV
DIHA	1	1.67	156	25	156 → 86	50
DEHA	1	1.73	170	25	170 → 128	40
DEDIA	1	2.35	146	25	148§	25
2-Hydroxy Atrazine	1	2.57	198	25	198 → 156	45
Desisopropyl Atrazine	2	4.83	174	25	174 → 146	40
Desethyl Atrazine	2	8.63	188	25	188 → 146	40
Simazine-d10†	3	14.90	212	27	212 → 134	40
Simazine	3	15.26	202	27	202 → 124	40
Atrazine	4	19.50	216	27	216 → 174	40
Propazine	4	23.00	230	27	230 → 188	40
Terbuthylazine‡	4	23.90	230	27	230 → 174	40
Metolachlor	5	27.50	252	27	252 → 176	40
Alachlor	5	27.70	238	27	238 → 162	40
Acetochlor	5	27.80	224	27	224 → 148	40

† Surrogate Spike Standard, ‡Internal Injection Standard, § ^{37}Cl isotope ion, SIM, Selected Ion Monitoring; RT, Retention Time; CV, cone voltage; DIHA, Desisopropyl 2-Hyroxy Atrazine; DEHA, Desethyl 2-Hydroxy Atrazine; DEDIA, Desethyl Desisopropyl Atrazine

Chromatography was performed using a binary gradient of an aqueous and an organic mobile phase. The aqueous mobile phase A consisted of 0.1% v/v acetic acid in reagent water, and organic mobile phase B consisted of 0.1% v/v acetic acid in acetonitrile. The flow rate was 0.2 mL per minute. Initial conditions began with 100% aqueous MP-A. Organic MP-B was ramped to 30% over 7 minutes, followed by ramping to 77% at 27 minutes into the run. All analytes eluted at this point, and the column was then flushed by ramping to 100% MP-B over an additional 3 minutes and held there for 5 minutes. The mobile phase composition was returned to initial conditions linearly over 3 minutes and held for 15 minutes in order to adequately re-equilibrate the column.

Mass spectrometer source parameters were determined experimentally using constant-flow syringe infusion of individual analytes. The ESI probe was operated in the positive ion mode with a capillary voltage of 4 kV. Optimal conditions included an extractor voltage of 5 V and an RF voltage of 0.5 V. The source and desolvation temperatures were 150 ^{o}C and 350 ^{o}C, respectively. The desolvation gas was nitrogen produced by a regulated head pressure above a liquid nitrogen dewar, and the flow rate was 250 L/hr. The cone guard flow was 50 L/hr. The electron multiplier voltage was 650 V. The mass spectrometer was operated in selected ion monitoring mode (SIM). Cone voltages were tested at several points from 25 V to 50 V to determine the ideal voltages needed to produce optimum in-source collision induced dissociation (IS-CID), thus providing at least two ion fragments for identification. Cone voltages, precursor and product ions and chromatographic retention times are provided in Table 1.

Negative-ion electrospray ionization analysis

Because of their carboxylic acid functional groups, the transformation products of chloroacetanilide herbicides metolachlor, acetochlor and alachlor were analyzed using negative ion electrospray ionization. The sample extracts were held at 4 ^{o}C in the sample chamber during analysis. The HPLC column, source and desolvation temperatures, gas flow rates and electron multiplier were described previously. The column temperature was held at 60 ^{o}C.

Table 2: Chloroacetanilide transformation products evaluated using negative-mode electrospray ionization.

Analyte	RT (min)	Quantifier Ion (m/z)	CV (V)	Qualifier Ion (m/z)	CV (V)
Alachlor OA	15.5	160	27	264	40
Acetochlor OA	15.9	146	27	264	40
Alachlor ESA	16.6	176	40	314	50
Acetochlor ESA	16.9	162	40	314	50
Metolachlor ESA	16.9	328	27	121	40
Metolachlor OA	17.7	206	27	278	40
Butachlor ESA†	23.4	356	27	121	40

OA, oxanilic acid; ESA, ethane sulfonic acid; †internal injection standard; RT, retention times; CV, cone voltages

Chromatography was performed using a binary gradient of an aqueous and an organic mobile phase as stated previously. Initial conditions began with 80% aqueous MP-A and 20% organic MP-B. MP-B was ramped to 70% over 30 minutes. Subsequent

to the completion of the run, the column was flushed and equilibrated as described previously. The total run time was 55 minutes.

The mass spectrometer source parameters were optimized experimentally using a constant-flow syringe injection of individual analytes directly into the probe. The ESI probe was operated in the negative ion mode with a capillary voltage of 3.2 kV. Optimal conditions included an extractor voltage of 5 V and an RF voltage of 0.5 V. In the case of the acidic transformation products, proper selection of cone voltages was essential for successful analysis. Ideal cone voltages were determined by experimentation. Lower cone voltages were applied to most quantitative precursor ions to prevent excessive fragmentation through in-source collision induced dissociation (IS-CID). Higher cone voltages impel ions into the source with greater kinetic energy, and produce a greater degree of fragmentation, thus allowing better spectral identification through qualifier product ions. The desired qualifier ions were obtained by assigning higher cone voltages to those ions in the selected ion method table during data acquisition (Table 2).

Quality control and quality assurance

Estimated instrument method detection limits (EMDLs) for this study were determined by 10 repeat injections of a low-concentration calibration standard (Table 3) according to reported methods (t x √s) (US-EPA, 2005a). Five-point calibration curves were used for quantitation by using the internal injection standard method. Actual method detection limits (MDLs) were determined by using either EMDLs or 3X system blank signals at matching retention times, whichever value was larger.

Table 3: Protocol quality control and quality assurance metrics

Analyte	EMDL (ng L^{-1})	% rsd	Blanks (ng L^{-1})	R^2	X coefficient
DIHA	0.1	0.9%	0.00	0.9991	1.30
DEHA	0.3	1.3%	0.00	0.9888	1.20
DEDIA	0.1	0.9%	0.10	0.9940	0.90
2-Hydroxy Atrazine	0.2	1.4%	0.00	0.9987	4.60
Desisopropyl Atrazine	0.3	1.9%	0.20	0.9932	1.80
Desethyl Atrazine	0.2	1.1%	0.00	0.9967	2.20
Simazine	0.5	2.3%	0.10	0.9961	6.10
Atrazine	0.1	0.8%	0.10	0.9975	3.40
Propazine	0.5	2.5%	0.20	0.9866	13.80
Metolachlor	0.4	2.5%	0.40	0.9924	2.10
Alachlor	0.2	1.2%	0.00	0.9917	1.10
Acetochlor	0.4	2.1%	0.30	0.9943	1.10
Alachlor OA	1.2	1.6%	0.02	0.9995	0.02
Acetochlor OA	1.4	1.8%	0.01	0.9995	0.02
Alachlor ESA	1.1	1.4%	0.01	0.9998	0.78
Acetochlor ESA	0.6	1.0%	0.01	0.9998	0.48
Metolachlor ESA	0.4	0.6%	0.02	0.9995	0.74
Metolachlor OA	0.6	0.8%	0.01	0.9998	0.65

DIHA, desisopropyl 2-hydroxy atrazine; DEHA, desethyl 2-hydroxy atrazine; DEDIA, desethyl desisopropyl atrazine; EMDL, estimated method detection limits.

Solid-phase extraction efficiency was assessed by performing reagent water and matrix spikes and determining recoveries (Table 4). Recoveries varied widely for DIHA, DEHA and DEDIA in both lab water and spiked river water tests. These compounds may be too polar for efficient solid phase extraction using HLB. However, these recoveries were similar to those reported in other studies that use Oasis HLB cartridges for s-triazine herbicides and their transformation products (McConnell et al., 2004). Because of their low recoveries, these compounds were omitted from this study.

Table 4: Spike recoveries for Oasis HLB Plus solid-phase extraction cartridges

	Lab Water Spike			Filtered River Water Spike		
Analyte	Level ($ng\ L^{-1}$)	Mean (N=4) Recovered ($ng\ L^{-1}$)	Recovery	Level ($ng\ L^{-1}$)	Mean (N=4) Recovered ($ng\ L^{-1}$)	Recovery
DIHA[†]	80.5	5.9 ± 60%	7.30%	80.5	7.1 ± 80%	8.80%
DEHA[‡]	79.5	15.8 ± 8.3%	19.7%	80.8	15.8 ± 59%	19.5%
DEDIA[§]	84.0	20.4 ± 4.8%	24.2%	95.9	33.9 ± 8.1%	35.2%
2-Hydroxy Atrazine	83.5	77.2 ± 10%	91.8%	99.2	96.3 ± 0.7%	96.6%
Desisopropyl Atrazine	75.6	70.5 ± 14%	92.8%	87.0	73.9 ± 1.1%	84.5%
Desethyl Atrazine	87.4	78.2 ± 14%	88.9%	118	114 ± 2.1%	96.2%
Simazine	88.3	79.5 ± 17%	89.6%	103	98.9 ± 2.3%	95.1%
Atrazine	81.6	97.7 ± 16%	119%	141	131 ± 1.7%	92.7%
Propazine	87.4	81.2 ± 16%	93.1%	87.4	88.3 ± 2.9%	100%
Metolachlor	87.7	57.4 ± 20%	65.1%	115	92.2 ± 2.9%	80.0%
Alachlor	93.1	58.0 ± 20%	61.9%	93.8	64.6 ± 4.5%	68.5%
Acetochlor	77.3	45.3 ± 20%	58.3%	77.6	51.9 ± 4.6%	66.5%
Alachlor OA	76.6	53.4 ± 7.8%	69.8%	88.7	103 ± 3.5%	116%
Acetochlor OA	78.6	55.6 ± 5.7%	70.7%	78.6	93.0 ± 3.8%	118%
Alachlor ESA	83.3	64.0 ± 8.6%	76.8%	95.0	115 ± 5.7%	121%
Acetochlor ESA	82.4	73.4 ± 6.0%	89.0%	222	220 ± 3.0%	98.8%
Metolachlor ESA	83.4	75.9 ± 9.6%	91.0%	112	107 ± 7.4%	95.4%
Metolachlor OA	86.9	90.0 ± 4.0%	104%	86.9	82.8 ± 13%	95.3%
Simazine-d10 SS	70.3	57.0 ± 17%	81.1%	70.3	57.1 ± 4.5%	81.2%
DETBA SS	69.0	71.0 ± 18%	103%	69.0	78.5 ± 3.0%	114%

DIHA, desisopropyl 2-hydroxy atrazine; DEHA, desethyl 2-hydroxy atrazine; DEDIA, desethyl desisopropyl atrazine; DETBA, desethyl terbuthylazine; OA, oxanilic acid moiety; ESA, ethane sulfonic acid moiety.

Simazine-d10 surrogate standard recoveries in the Shenandoah River samples averaged 80.6% ± 23%, while recoveries for desethyl-terbuthylazine averaged 69.3% ± 23%. Background detections of the target analytes were minimal and considered to be primarily the result of random noise. The reported sample concentrations were blank-corrected through a background subtraction procedure.

Duplicate samples were obtained on 27-September at Cedar Run and on 12 November at Walnut Branch, the seasonal stream feeding Cedar Run downstream from the sampling site. Relative percent differences (RPD) for individual analytes are included in the results tables in the subsequent section. The mean RPD for the Cedar Run site was 13.3% and ranged from 1.0% to 36.0%. The mean RPD for the Walnut Run site was 10.1% and ranged from 0.1% to 24.5%.

Results and Discussion

Instrumental analysis

The mass spectra of selected target analytes are illustrated in Figs. 2, where comparisons of full-scan spectra are shown between analytical reference standards and surface water detections at parts-per-trillion concentrations in the 7 Aug. Owl Run surface water samples. The full-scan mass spectra obtained for the analytes in the surface water HLB extracts provided unambiguous confirmation of the identifications relative to the standards and were of library searchable quality. In each full-scan spectrum, the cone

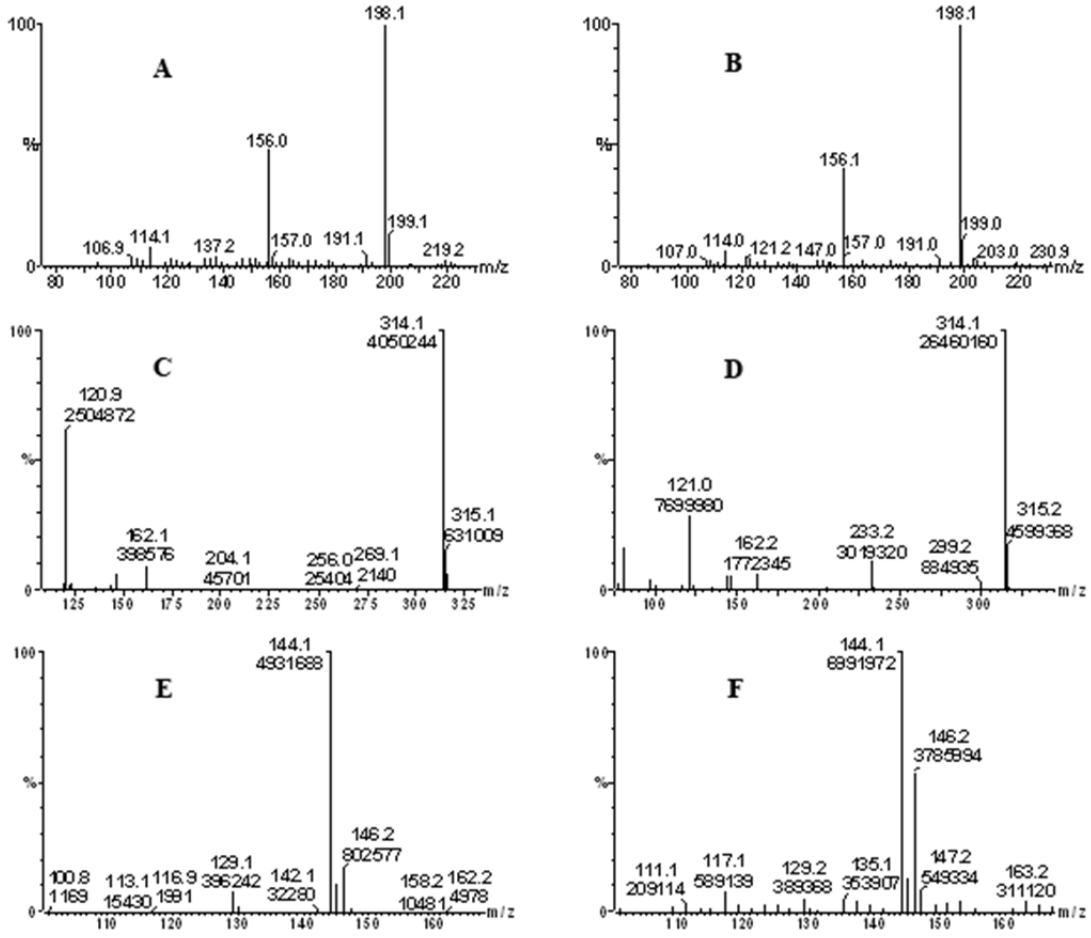


Figure 2: Full-scan LC-MS(Q) spectra of selected herbicide transformation products from authentic standards and 8-Jul-2010 Owl Creek samples. (A): 2-Hydroxy Atrazine Standard, (B): 2-Hydroxy Atrazine in sample, (C): Acetochlor ESA standard, (D): Acetochlor ESA in sample, (E): Acetochlor OA standard, (F): Acetochlor OA in sample.

voltage was maintained at a high level 50 V in order to increase IS-CID fragmentation of each precursor ion, thus producing a detailed spectrum that includes even lower abundance molecular fragments. Note that when quantitative analysis was performed in SIM mode, low CVs of 20-27 V were applied to the quantifier precursor ions to minimize

IS-CID and optimize signal-to-noise ratio, and higher cone voltages were applied to qualifier product ions to increase IS-CID and produce sufficient qualifier ions to increased selectivity for target analytes. The full spectra in these figures validate the quantification and identification capabilities of the IS-CID technique using actual samples. The in-source collision induced dissociation (IS-CID) technique showed minimal signal suppression in the surface water samples, and thus allowed reliable quantitation and confirmation using at least 3 ions for each analyte.

Recoveries

Solid-phase extraction efficiencies as measured by both reagent and filtered river water spike recoveries (Table 4) displayed a normalized distribution. A paired t-test shows that recoveries of the two methods were significantly different ($p < 0.005$). In 14 of the 17 analytes, the filtered river water recoveries were somewhat larger than those in the reagent grade water tests. This would imply that matrix effects were not a substantial factor in the instrumental analysis of the samples. This phenomenon was particularly true for recoveries of the oxanilic acid transformation products of the chloroacetanilide herbicides which were as much as 48% greater. The most notable exception is atrazine which had a recovery of 119% in reagent water and 92.7% in filtered river water.

Surface water samples

Analysis results of the triazine and acetanilide herbicides in the surface water samples from the Cedar Run Basin are presented in Tables 5, 6a and 6b. Due to low extraction efficiencies for desisopropyl-hydroxyl-atrazine, desethyl-hydroxyl-atrazine, and desethyl desisopropyl atrazine using the HLB cartridges in both reagent grade lab water (7.3%, 19.7, and 24.2%, respectively) and in filtered river water (8.8%, 19.5%, and 35.2, respectively), these three transformation products of triazine herbicides were not included in the results.

The most highly concentrated chloroacetanilide herbicides were the ethane sulfonic acid and oxanilic acid OA conjugates of acetochlor in the Owl Run samples. This compares with similar studies in regions with substantial corn and soybean crops (Battaglin et al., 2003; Hostetler and Thurman, 2000; Kolpin et al., 1998; McConnell et al., 2007). The highest concentrations of the parent acetochlor were found on 27 Sept. indicating there may have been a fall application of this herbicide.

Table 5: Triazine and Triazine Transformation Product Concentrations (ng L^{-1}).

Station	Date	Simazine	Atrazine	Propazine	HA	DIA	DEA
Cedar Run	7-Aug	22.3	89.1	0.740	17.4	7.47	20.7
Cedar Run	16-Sep	36.0	267	4.82	54.0	27.1	68.9
Cedar Run	27-Sep	11.1	42.3	<EMDL	16.4	7.79	16.6
Cedar Run 2X	27-Sep	10.7	55.3	<EMDL	19.0	8.35	19.7
Cedar Run	12-Nov	17.8	56.5	3.76	18.1	7.42	18.2
Owl Run	7-Aug	1080	1540	39.8	289	206	404
Owl Run	16-Sep	74.3	390	11.0	258	71.6	213
Owl Run	27-Sep	72.9	286	5.41	178	55.5	190
Owl Run	12-Nov	37.0	26.6	2.09	18.6	15.4	23.6
Brentsville	7-Aug	55.8	101	1.46	57.9	16.0	30.0
Brentsville	16-Sep	45.1	81.6	1.53	35.4	13.5	24.9
Brentsville	27-Sep	10.3	23.5	<EMDL	15.8	4.64	7.40
Walnut	12-Nov	10.6	58.3	<EMDL	32.8	4.25	11.7
Walnut 2X	12-Nov	11.9	63.1	<EMDL	38.9	4.05	14.1

<EMDL - concentration below estimated method detection limits; HA, 2-hydroxy atrazine; DIA, desisopropyl atrazine; DEA, desethyl atrazine,

In nearly all samples, the sum of the chloroacetanilide herbicide transformation product concentrations substantially exceeded the concentrations of their parent compounds. With the exception of 27 Sept., the sum of the acetochlor OA and ESA TPs relative to the parent acetochlor ranged from 72% to 100% with a mean of 82% over all sites and sample dates. The sum of the metolachlor OA and ESA TPs relative to metolachlor ranged from 78% to 99% with a mean of 90% across all sites and sampling dates, thus reinforcing the findings of other studies that included transformation products as target analytes (Battaglin et al., 2003; Kalkhoff et al., 1998; Kalkhoff et al., 2003; Kolpin et al., 1998).

The most highly concentrated triazine herbicides were atrazine and simazine which accumulated in the Owl Run site. The highest triazine TPs were desethyl atrazine (DEA) with an average concentration of 80.8 ng L^{-1}, ranging from a low of 7.4 ng L^{-1} to a high of 404 ng L^{-1}, and 2-hydroxy atrazine (HA) with a mean concentration of 77.9 ng L^{-1} ranging from a low of 15.8 ng L^{-1} to a high of 289 ng L^{-1}. The other dealkylated atrazine, desisopropyl atrazine (DIA) showed a mean concentration of 34.2 ng L^{-1}. This relative concentration may be attributable to the observation that DEA tends to be more mobile in the surface and sub-surface soils than DIA (Kruger et al., 1996).

Table 6: Chloroacetanilide and Chloroacetanilide Transformation Products Concentrations (ng L^{-1})

Station	Date	Met	Met-ESA	Met-OA	Ace	Ace-ESA	Ace-OA	Ala	Ala-ESA	Ala-OA
Cedar Run	7-Aug	36.3	93.2	48.1	2.56	<EMDL	6.62	<EMDL	4.15	<EMDL
Cedar Run	16-Sep	25.1	167	92.1	0.480	<EMDL	7.11	<EMDL	1.63	<EMDL
Cedar Run	27-Sep	17.6	39.8	17.0	147	<EMDL	1.74	<EMDL	9.60	<EMDL
Cedar Run 2X	27-Sep	18.4	41.9	17.2	212	<EMDL	1.55	<EMDL	11.3	<EMDL
Cedar Run	12-Nov	157	588	361	10.8	116	54.6	<EMDL	26.3	<EMDL
Owl Run	7-Aug	26.0	455	245	55.9	3780	3670	<EMDL	7.69	<EMDL
Owl Run	16-Sep	3.27	383	179	3.31	1860	1590	2.16	5.42	<EMDL
Owl Run	27-Sep	6.25	332	127	61.9	1560	1180	<EMDL	14.2	<EMDL
Owl Run	12-Nov	9.30	751	303	0.810	889	464	0.76	3.03	<EMDL
Brentsville	7-Aug	7.87	173	48.3	0.710	<EMDL	17.5	<EMDL	2.98	<EMDL
Brentsville	16-Sep	11.9	59.0	26.8	0.310	<EMDL	7.06	0.35	1.93	<EMDL
Brentsville	27-Sep	8.54	19.5	10.0	6.59	<EMDL	3.17	<EMDL	3.85	<EMDL
										<EMDL
Walnut	12-Nov	160	619	447	<EMDL	27.5	12.8	<EMDL	6.11	<EMDL
Walnut 2X	12-Nov	140	618	446	<EMDL	27.5	12.8	<EMDL	4.78	<EMDL

<EMDL, below estimated method detection limits; Met, metolachlor; Ace, acetochlor; Ala, alachlor; ESA, ethane sulfonic acid moiety; OA, oxanilic acid moiety

Seasonal Streams in Cedar Run Basin

The Cedar Run basin contains numerous small streams that are seasonably dry during baseflow conditions. Some of the streams tend to pond in low-lying sections. These seasonal streams may act as sinks for large concentrations of herbicides and their TPs in places where groundwater accumulates. When rainfall becomes sufficiently high, water retained in the ponds is flushed into Cedar Run creating a potential pulse or plume of high herbicide concentrations.

The Owl Run sampling was performed adjacent a state highway and several active agricultural fields planted alternately with hay, corn and soybeans. East of the site is a relatively small cattle operation that has partially obstructed the stream in order to create a farm pond for the livestock. During the dry season, the flow from this site is negligible and the pond is kept full by a high groundwater table. The stream flow of Owl Run on 7 Aug. was negligible. At that time, the concentration of herbicides and their TPs in the pond were high with combined triazine concentrations (parent + TPs) of nearly 3.7 $\mu g\ L^{-1}$ and the combined concentrations of chloroacetanilides (parent + TPs) of over 8.1 $\mu g\ L^{-1}$. The rain events that occurred on 16 and 27 Sept. either diluted or partially flushed the pond at the Owl Run site, and the major storm event of 12 Nov. appears to have almost completely flushed the pond.

Prior to the 12 Nov storm event, concentrations of acetochlor-ESA in both Cedar Run sites were below EMDLs. However, concentrations in the pond section of Owl Run were 3,780, 1,860, and 1,560 ng L^{-1} on 7 Aug, 16 Sep, and 27 Sep, respectively. Following the storm, acetochlor-ESA was detected in Cedar Run at 116 ng L^{-1}.

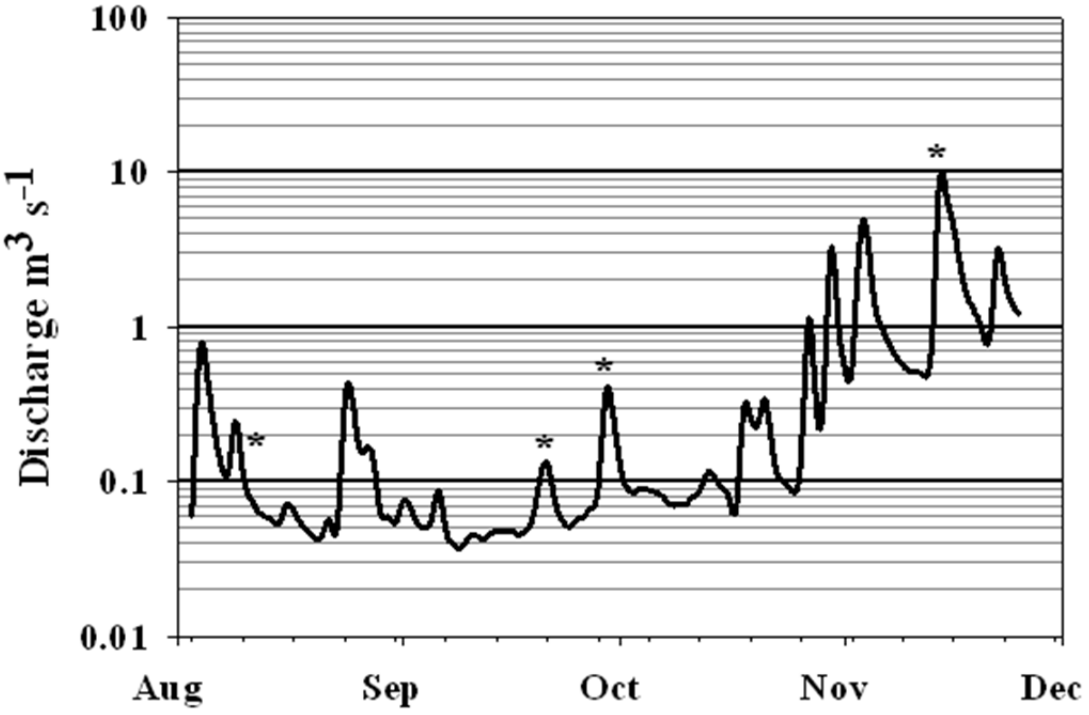


Figure 3: Daily Discharge Data (m^3 s^{-1}) from USGS Gauging Station #0165600 at Cedar Run near Catlett, VA.*Sampling Dates.

The major storm event of 12 Nov also filled the stream bed of Walnut Creek which had been ponding in several spots, but had not been flowing since the spring rainy season. Sampling in the high flow of the storm showed concentrations that were in the same range or higher than the much larger Cedar Run, particularly for 2-hydroxy atrazine which was 32.8 to 38.9 ng L^{-1} compared to 18.1 ng L^{-1} at Cedar Run. Walnut Creek and Cedar Run had metolachlor in the same range of ~150 ng L^{-1} and metolachlor ESA in the same range of ~ 600 ng L^{-1}. Metolachlor OA was higher in Walnut Creek at ~445 ng L^{-1} than at Cedar Run with ~360 ng L^{-1}.

Microbial transformation of atrazine

On 12 Nov 2009, 1-L water samples were taken from each site in sterile glass media bottles. These samples were filtered and native bacteria were extracted from the filters, pre-incubated and inoculated into sterile, amended river water from each site. Figure 4 shows the increase in production of 2-hydroxy atrazine over the 12-day study period for the samples that were inoculated with native bacterial communities. This differs substantially from the production of 2-hydroxy atrazine in the controls that were not inoculated but were subject to the potential for hydrolysis and photolysis. No transformation products other than 2-hydroxy atrazine were observed in the bacterial cultures. The atrazine transformation experiment establishes the validity of the LC-MS(Q) method in applications involving surface water measurements and laboratory experiments in herbicide transformations.

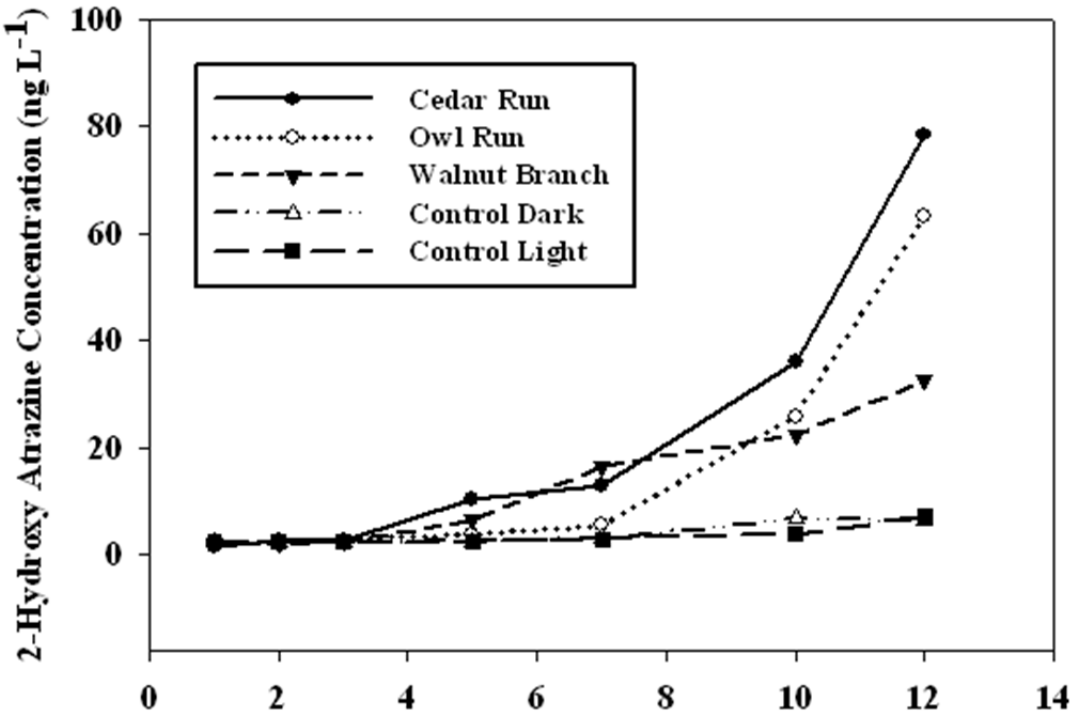


Figure 4: Growth Curves Showing Production of 2-Hydroxy Atrazine over the 12 Day Microbial Experiment

Conclusions

The method described in this study proved to be a highly effective means of determining the presence and concentrations of both triazine and chloroacetanilide herbicides and their primary transformation products in surface water at parts-per-trillion concentrations. The single-quadrupole mass spectrometer proved to be ideally suited for measuring concentrations of these target analytes in surface water extracts. The in-source collision induced dissociation (IS-CID) technique enabled by changing the cone voltage

in the electrospray ionization probe produced full-scan spectra of analytes in complex environmental sample extracts that matched those in authentic standards. This technique was able to overcome the limitations of single quadrupole mass spectrometers for this list of common environmental pollutants.

Field testing the method presented here was able to document fate and transport of widely used herbicides in an important tributary that feeds a source of drinking water for nearly 1 million households. The method also identified local phenomena wherein high concentrations of herbicides and their TPs in pond sections of seasonal streams ultimately flowed into a drinking water source during a major storm event. Furthermore, microbial analysis indicates that this tributary's basin encompasses triazine-degradation adapted soils that may be capable of moderating the impacts of atrazine applications.

LC-MS(Q) operating in the IS-CID mode proved capable of providing the necessary analytical requirements for field studies that combine the chemical and microbial data. Therefore, further work quantifying changes in atrazine and its transformation products' concentrations over an extended time period following application is possible and worthwhile.

CHAPTER 2: TEMPORAL AND SPATIAL DISTRIBUTIONS OF TRIAZINE HERBICIDES AND THEIR TRANSFORMATION PRODUCTS IN SMALLER POTOMAC-RIVER TRIBUTARIES

Introduction

Herbicides and Transformation Products

U.S. farmers were expected to harvest nearly 79 million acres of corn and more than 72 million acres of soybeans in 2008 (NASS, 2008). Both corn and soybeans are principal crops in the Potomac River Basin in Virginia. Herbicides are used on over 91% of crops in Virginia with atrazine (2-chloro-4-ethylamino-6-isopropylamino-*S*-triazine), metolachlor [2-Chloro-N-(2-ethyl-6-methyl-phenyl)-N-(1-methoxypropan-2-yl)acetamide], simazine [2-chloro-4,6-bis(ethylamino)-*S*-triazine], alachlor [2-chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl)-acetamide] and 2,4-D [(2,4-dichlorophenoxy)acetic acid] comprising the most abundantly used herbicides on corn and soybeans. Atrazine is applied to over 78% of corn crops in Virginia (USDA, 2003a). Research in surface and groundwater concentrations of these herbicides remains important for assessing the health of Potomac River Basin watersheds.

Atrazine is the one of the most widely used herbicide in the world. It has been found in mg/L concentrations in runoff from heavily agricultural regions (Bringolf et al., 2004). The United States Environmental Protection Agency's MCL for atrazine is 3.0

μg/L. The European Commission has banned the use of atrazine in 7 countries and prior to that the maximum level was considered to be 0.1 μg/L (European-Commission, 2004).

Although the toxicology of atrazine has been controversial, it has been shown to be an immune system disruptor in aquatic species (Brodkin et al., 2007), and studies to date have debated whether atrazine is an endocrine-disrupting chemical that can effect reproduction and produce intersex animals (Bringolf et al., 2004; Carr et al., 2003; Hayes et al., 2003). Atrazine has also been shown to induce oxidative stress and DNA damage within cells (Yan et al., 2009).

Atrazine (ATR) can transform into six major products. The chlorinated transformation products include dealkylated desethyl atrazine (DEA), desisopropyl atrazine (DIA), and desethyl-desisopropyl atrazine (DEDIA). Simazine (SIM) can also transform into DEA and the less-used herbicide propazine [2-chloro-4,6-bis(isopropylamino)-*S*-triazine], (PROP) can transform into DIA. De-alkylization occurs abiotically or by non-specific cytochrome P450 monooxygenases (Seffernick et al., 2002). The monooxygenated enzymes are present in common soil bacteria such as *Pseudomonas sp.* (Khan and Behki, 1990) and *Rhodococcus spp*. (Behki and Khan, 1994) have evolved genes capable of encoding the monooxygenase enzymes.

The de-chlorinated transformation products include 2-hydroxyatrazine (HA), desethyl-2-hydroxy atrazine (DEHA), and desisopropyl-2-hydroxy atrazine (DIHA). De-chlorination and hydroxylation can occur either abiotically or biologically by the enzyme atrazine chlorohydrolase found in soil bacteria such as *Pseudomonas sp.* (Seffernick et al., 2002).

Current studies are attempting to determine the toxicological effects of triazine herbicide transformation products. Both DEA and DIA have been listed as priority pesticide transformation products (Sinclair et al., 2006). Work has shown that DEA and DIA metabolites can increase aromatase activity in human cell lines to the same extent as simazine, propazine, atrazine (Sanderson et al., 2001). Aromatase converts androgens into estrogens and can convert testosterone into estradiol (Carr et al., 2003). Increased estradiol levels in males has been associated with intersex morphology leading to oocytes formation in the testes of several species (Milnes et al., 2006).

Previous studies have shown that herbicide transformation products comprise a significant percentage of the total herbicide concentration in an aquatic system (Kolpin et al., 1998). Parent herbicide concentrations are highest during spring and summer applications periods (Foster et al., 2000) However, their transformation products can be more persistent throughout the year and predominate the herbicide burden well beyond the application period (Kalkhoff et al., 2003).

The changes in the molecular structure brought on by the transformation process impacts the environmental fate and transport of TPs. This is evidenced by changes in soil adsorption coefficients (K_{oc}) where de-alkylated s-triazine herbicides have lower Koc values (Kaune et al., 1998). The occurrence and distribution of parent herbicides and their TPs can vary from basin to basin (Rebich et al., 2004). Even relatively small differences in local environmental factors such as soil types and climatic conditions can have a significant impact on the composition of herbicides and TPs in a particular basin associated with corn and soy bean agriculture (Kalkhoff et al., 2003).

Hydroxy atrazine can exist in either a keto form or an enol form. Experimentally, the log K_{oc} values for the keto form of hydroxy atrazine were found to be 1.2 log units higher than the enol form (Kaune et al., 1998). The EPA EPI Suite software determines log K_{oc} values 1.0 log units higher for keto versus enol forms of hydroxyl atrazine (US-EPA, 2007). With a log Koc of 3.36, hydroxy atrazine has been found to be nearly

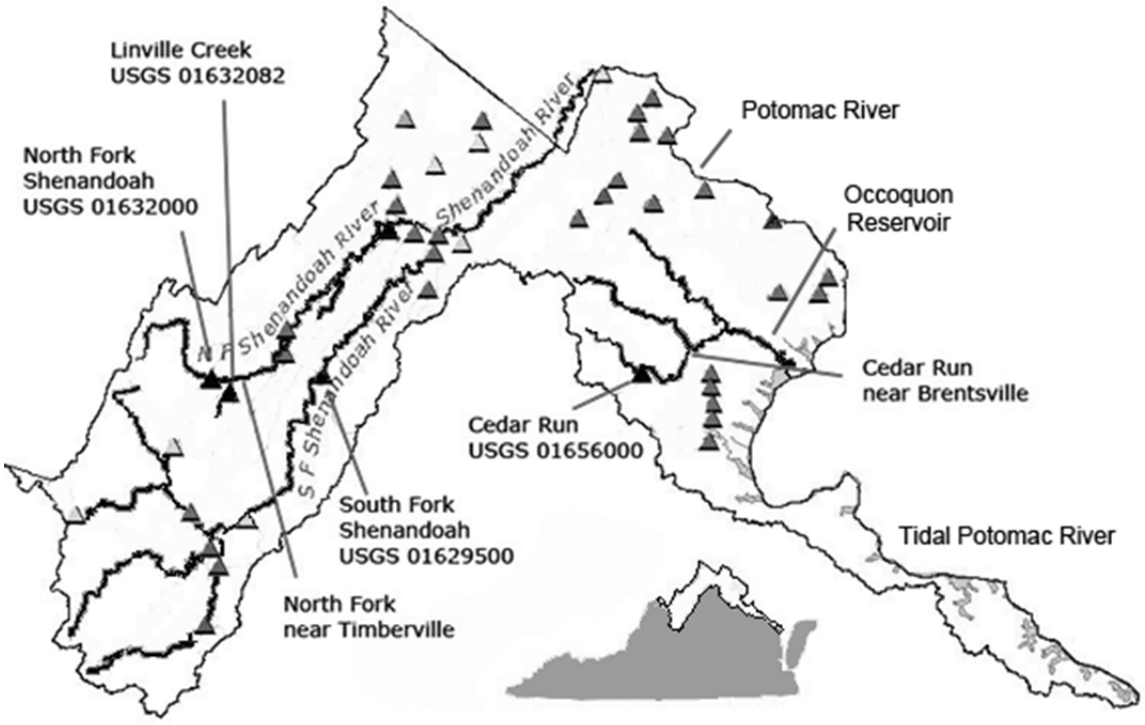


Figure 5: Map of the Potomac River Basin in Virginia showing the 6 sampling sites. ▲USGS Gauging Stations which monitor stream flow, water temperature and specific conductivity (USGS, 2008a).

immobile compared with atrazine, DEA and DIA in some soil types (Kruger et al., 1996; Krutz et al., 2003a; Krutz et al., 2003b). This may be explained by its hydrogen bonding potential with silicates.

Study Area

In 2009, the US Environmental Protection Agency released data from an atrazine ecological watershed monitoring program. Data from 2004-2008 include the monitoring results from Midwestern streams, as well as sugarcane crop production watersheds. However, tributaries such as the Potomac and Shenandoah Rivers which lie in the Chesapeake Bay watershed, the nation's largest estuary, were not included in the Atrazine Monitoring Program.

Herbicide and their transformation product concentrations in the Chesapeake Bay have been documented as recently as 2008 (Alvarez et al., 2008; McConnell et al., 2007). The bay's watershed is comprised of 166,000 km^2 and is drained by over 150 major rivers and streams. Numerous studies of toxic pollutants have been conducted in the watershed tributaries (Alvarez et al., 2008).

The Potomac River basin comprises 38,000 km^2 or roughly 23% of the watershed land area (ICPRB, 2008). Evidence of estrogenic endocrine disruption and the presence of testicular oocytes in fish species have been noted in the Potomac River basin (Alvarez et al., 2009; Iwanowicz et al., 2009). The need exists for further documentation of Chesapeake Bay and Potomac River Basin concentrations of herbicides and their transformation products.

Table 7: Geographical and geological site descriptions for 2007 Virginia sampling locations.

	Cedar Run	Cedar Run	North Fork Shenandoah	Linville Creek	North Fork Shenandoah	South Fork Shenandoah
Site Acronym	CED	BRS	ATV	LIN	BTV	LUR
Latitude	38°38'12"	38°36'53"	38°38'13"	38°36'24"	38°38'08"	38°38'46"
Longitude	77°37'31"	77°33'11"	78°51'11"	78°48'13"	78°46'44"	78°32'06"
Location	Catlett	Brentsville	Cootes Store	Broadway	Timberville	Luray
USGS Unit	02070010	02070010	02070006	02070007	N/A	02070005
Drainage (km^2)	241.9	N/A	543.9	118.4	N/A	3,553.5
Altitude (m)	60.7	58.0	320.6	313.9	301.4	220.0
Reference Site	Source	Catlett	Source	Source	Cootes	Source
Ref Alt (m)	136	60.7	638.3	394.4	320.6	340
Alt Drop (m)	75.3	2.7	317.7	80.5	19.2	120.0
Distance (km)	23.3	26	32.0	15.4	9.5	44.0
Drop (m/km)	3.2	0.1	9.9	5.2	2.0	2.7
Geology	G-S	G-S	SS	LS	LS	SS & LS
% Agriculture	41	N/A	8.0	74.4	22.6	40.2
% Forested	52	N/A	91.1	23.1	76.1	55.7
% Urbanized	7	N/A	0.3	2.1	0.8	3.5

G-S: Gneiss-Schist
SS: Sandstone
LS: Limestone

This study examines several tributaries to the Potomac River located further upstream near intensive agricultural activities. The first region comprises smaller sections of the North Fork of the Shenandoah River and Linville Creek, a small tributary. The larger South Fork of the Shenandoah River is included as a reference point. The second region is Cedar Run, a smaller tributary of the Potomac River. The map (Figure 5) shows the Virginia basin of the Potomac River watershed and includes the study locations. Table 7 lists the spatial descriptions for each of the sites.

Shenandoah River

The Shenandoah River is supports significant historical, social and recreational activities. The Shenandoah Valley receives over 250,000 visitors per year. The river's fishery has been impacted by pollution. Several sections have been designated as impaired for fecal coliform, nutrients, PCBs and mercury. Lesions, fish kills and the discovery of testicular oocytes associated with intersex phenomenon have been reported along sections of the river (Blazer et al., 2007; Garman and Orth, 2007).

The North Fork of the Shenandoah River lies in Rockingham County, Virginia. Five sections of the North Fork have been listed as impaired by the US Environmental Protection Agency. The upstream sections lie within an agricultural area that raises poultry and grows corn and soybeans. Poultry manure is used to fertilize crops and the primary source of impairment is fecal coliform bacteria. Herbicides are applied to crop fields prior to spring planting as a pre-emergent treatment for broad-leaf plants. The map (Figure 1) shows the four sampling sites in the Great Valley sub-province of the Basin

and Ridge geological province within the Shenandoah River basin. Sites include the USGS gauging station on the North Fork of the Shenandoah at Cootes Store, Virginia which is located approximately 30 km downstream from the West Virginia border. The USGS gauging station on Linville Creek near Broadway, Virginia, is a tributary to the North Fork located approximately 1.7 km upstream from the mouth. The furthest site downstream on the North Fork is in Timberville, Virginia below a poultry processing facility. The fourth site in this region is on the larger South Fork Shenandoah River near Luray, Virginia.

Cedar Run

Cedar Run is a stream located in central Fauquier and Prince William Counties in Virginia in the Outer Piedmont geological subprovince (Figure 1). As part of the Middle Potomac-Anacostia-Occoquan Subbasin, it drains an area of relatively-intensive corn, hay, soybean and dairy cow operations. The corn, hay and soybean crops comprise the primary food source for the dairy operations and the resulting manure is used to fertilize the crop fields. Prior to planting, pre-emergent herbicides are applied to control broadleaf plants that would contaminate the whole-plant feed and affect the quality of the milk produced. Runoff from crop fields enters Cedar Run through groundwater and small surface streams that form during storm events. The stream is a tributary to the Occoquan River and the Occoquan Reservoir that provides drinking water to one million households in the heavily-urbanized Fairfax County.

Two sites on Cedar Run were sampled monthly from April to October 2007. The first site is located near Catlett, Virginia adjacent to the US Geological Survey gauging station number 01656000 which measures continuous data including discharge (ft^3/s), gauge height, temperature and specific conductance. These parameters are broadcast in real time to the web at http://waterdata.usgs.gov/.

The second site on Cedar Run is located near Brentsville, Virginia adjacent to a bridge along Prince William County Road 619. This site is located near the confluence of Cedar Run and Broad Run which combine to form the Occoquan River. The Occoquan River is the primary source for drinking water for Fairfax County, Virginia, an urban county with a population of over one million. The area between Catlett and Brentsville is primarily wooded with some agricultural operations.

A supplemental site was sampled on 3 occasions during the spring rainy season. Walnut Creek is a small stream that reaches significant discharge during storm events. Like other seasonal streams that feed into Cedar Run, it reverts to a dry stream bed during the summer dry season.

Objectives and Scope

The primary objective of this research was to determine temporal changes of triazine herbicide concentrations in surface waters in upstream sub-basins of the Potomac River basin over an extended period of time beyond the traditional spring time application period. The secondary objective was to determine the concentrations of

triazine herbicide transformation products over the same period. The third objective of this research was to examine the spatial differences among the two sub-basins and correlate those changes to basin size, land use, and hydrological factors.

To accomplish these objectives, base flow and storm flow water samples were collected monthly from two sites on Cedar Run in Fauquier County, Virginia and four sites on the Shenandoah River in Rockingham and Page Counties, Virginia, during the period from April through October, 2007. The total number of samples was 58 including 10 duplicate samples. Surface-water samples were extracted using solid-phase extraction (SPE) and analyzed by liquid chromatography-mass spectrometry (LC-MS) for triazine herbicides, and their transformation products.

Materials and Methods

Supplies

HPLC-grade solvents were purchased from Fisher Scientific, Pittsburg, PA. Herbicide chemical standards for atrazine, simazine, propazine, terbuthylazine, desethyl atrazine, desisopropyl atrazine and hydroxyl atrazine were purchased from Sigma-Aldrich, St. Louis, MO. The stable isotope standard simazine-d10 was purchased from Cambridge Isotope Labs, Andover, MA. Oasis HLB solid-phase extraction products were purchased from Waters Corporation, Milford, MA. LC-MS consumable supplies were purchased from Waters Corporation.

Lab grade water was produced in house using a recirculating deionization skid with UV sterilization treatment (HydroMax, Emmitsburg, MD) followed by polishing with an Elga Maxima ultrapurifier producing 18.2 MΩ water that is further treated for organics via a second UV lamp (Elga Labwater, Marlow, Buckinghamshire, UK).

Sample Preparation

River water samples were obtained using a Masterflex Environmental Sampler portable peristaltic pump loaded with plasticizer-free Masterflex C-Flex L/S-24 tubing (Cole Parmer Instrument Company, Vernon Hills, IL). The 25-foot section of tubing was fitted with a stainless steel flow-through tubing weight. Water samples were pumped into ~20 L stainless steel Cornelius kegs that were washed with warm soapy tap water and rinsed with tap water. The kegs were then sequentially rinsed with de-ionized water, lab water and methanol using pressure from gaseous nitrogen to blow out the dip tube. Sampling flow rate was approximately 1 liter per minute. When the kegs were filled, they were sealed with the gas-tight lid and transported to the lab.

Water samples were pressure filtered within 24 hours of the day they were sampled. A high-purity nitrogen gas cylinder (Roberts Oxygen, Rockville, MD) was fitted with a two-stage regulator with a manually operated needle valve. Output pressure was set at 100 psi. The regulator outlet was attached to the Cornelius keg' stainless steel inlet ball valve with ¼" Pharma-grade reinforced tubing containing no phthalates, no plasticizers, and no latex (Dow Corning, Midland, MI). The keg's outlet ball valve was connected to a 142 mm stainless steel filter holder (Millipore Corporation, Billerica, MA)

holding a 142-mm diameter Whatman Grade GF/F 0.7 μm glass microfiber filter overlaid with a 150 mm diameter grade GF/D 2.7 μm glass microfiber pre-filter (Whatman Inc., Florham Park, NJ), both rinsed under pressure with HPLC grade methanol. The needle valve was pulsed to build sufficient head space pressure in the keg to begin water sample flow through the filters. The flow rate was kept at a level that allowed efficient filtration without damaging the filters. The needle valve was repeatedly pulsed when flow rate slowed. Sample filtrate was directed into 1 L glass media bottles which had been cleaned and solvent rinsed. One-liter and 500-mL aliquots were stored at 4 ^{o}C for subsequent solid phase extraction.

The 1-L sample filtrate aliquots were extracted using a general solid-phase extraction (SPE) procedure (Waters, 2002). The Oasis HLB plus cartridges containing 250 mg of hydrophilic-lipophilic balanced sorbent were fitted with an empty 6-mL syringe barrel and placed on a Supelco Visiprep vacuum manifold (Sigma-Aldrich, St. Louis, MO). The cartridges were sequentially activated with 3 mL of MTBE, 3 mL of methanol and 3 mL lab water. One-liter samples were loaded using Supelco Visiprep large volume samplers (Sigma-Aldrich) at a flow rate of ~5-10 mL per minute. The cartridge was subsequently washed with 3 mL of 5% (v/v) methanol in reagent water to remove inorganic interferences. The cartridges were eluted with two 4-mL aliquots of 10% (v/v) methanol in MTBE and collected into 12-mL deactivated glass centrifuge tubes.

Sample extracts were concentrated using a Centrivap centrifugal vacuum concentrator (Labconco Corporation, Kansas City, MO). Four of the 1-L SPE extracts

from were combined to form a single composite sample and further concentrated on the Centrivap. When sample extracts were concentrated to approximately 1 mL, 1 mL of 0.1% acetic acid in reagent water was added, and the extracts were further reduced to 1 mL so that the sample matrix was equivalent to the initial liquid chromatography mobile phase conditions. Extracts were quantitatively transferred to 2-mL 12 x 32 mm Target DP amber silanized glass autosampler vials with screw-cap lids and Teflon® septa (National Scientific Company, Rockwood, TN). The internal standards monocrotophos and terbuthylazine were added per a previous study (McConnell et al., 2007), and samples were stored at -20 ^{o}C pending analysis.

Instrumental Analysis

To avoid the need for derivatization of 2-hydroxy atrazine, sample extracts were subsequently analyzed by liquid chromatography-mass spectrometry (LC-MS) using a Waters Alliance 2695 Separations Module and a Waters-Micromass ZQ2000 with electrospray ionization (ESI) (Waters Corporation, Milford, MA). The HPLC column used was a 2.1 x 150 mm Waters T3 Atlantis C18aq held at 35 ^{o}C to maintain a constant temperature throughout the study period.

A binary gradient of an aqueous mobile phase A consisting of 0.1% v/v acetic acid in lab water and mobile phase B consisted of 0.1% v/v acetic acid in acetonitrile. The flow rate was 0.2 mL per minute. Initial conditions began with 100% aqueous MP-A. Organic MP-B was ramped to 30% over 7 minutes, followed by ramping to 77% at 27

minutes into the run. All analytes eluted at this point, and the column was then flushed by ramping to 100% MP-B over an additional 3 minutes and held there for 5 minutes.

Mass spectrometer source parameters were determined experimentally using a constant flow syringe injection of individual analytes. The ESI probe was operated in the positive ion mode with a capillary voltage of 4 kV, extractor voltage of 5 V and an RF voltage of 0.5 V. The cone voltage was set to 27 V, source temperature was set to 150 ^{o}C and desolvation temperature was set to 350 ^{o}C. The desolvation gas and cone-guard flow was 250 L/hr and 50 L/hr, respectively. The electron multiplier was set at 650 V. The mass spectrometer was operated in selected ion monitoring mode (SIM).

Principal Component Analysis

The sampling stations at Cedar Run near Catlett, the North Fork of the Shenandoah River near Cootes Store, Linville Creek near Broadway and the South Fork of the Shenandoah River near Luray each have a USGS gauging station that collects a variety of temporal and spatial parameters for the basin upstream from those sites. The spatial variables includes drainage area (km^2), percent of drainage area that is agricultural, agricultural area (km^2), discharge (m^3/s), specific conductance ($\mu S\ s^{-1}$), temperature (^{o}C), and concentrations (ng/L) for each herbicide and transformation product.

A correlation matrix of this data was created for multivariate statistics using principal component analysis (PCA) to reduce the variables into components that describe the data in more-useful terms. In cases where compounds weren't detected or

were below estimated method detection levels, values were entered conservatively as equal to half of the EMDL in order to facilitate PCA (Skrbic and Đurisic-Mladenovic, 2007).

Quality Control, Quality Assurance

Estimated method detection limits (EMDLs) for this study were determined by 10 repeat injections of a low-concentration calibration standard. The standard deviation for the replicates was multiplied by the student t-test value at the 95% confidence level (1-α) with 9 degrees of freedom (N-1) (US-EPA, 2005b). EMDLs were 0.13, 0.50, 0.53, 0.22, 0.29, and 0.21 ng L^{-1} for atrazine, simazine, propazine, HA, DIA and DEA, respectively.

Precision was measured using the percent relative standard deviation (% rsd) calculated by dividing the standard deviation by the mean and expressing the result as a percentage. The % rsd was 0.8, 2.4, 2.6, 1.4, 2.0, and 1.2 percent for atrazine, simazine, propazine, HA, DIA and DEA, respectively.

Five-point calibration curve linearity is reported as the regression coefficient of determination (R^2) values. The R2 values were 0.998, 0.996, 0.987, 0.999, 0.993, and 0.997 for atrazine, simazine, propazine, HA, DIA and DEA, respectively.

Solid-phase extraction efficiency was measured by calculating percent recoveries for four replicates of filtered South Fork Shenandoah River water spiked with ~ 85 ng L^{-1} of analytes. Recoveries were 93(± 2)%, 96(± 2)%, 101(± 3)%, 97(± 1)%, 85(± 1)%, and 97(± 2)% for atrazine, simazine, propazine, HA, DIA and DEA, respectively.

Simazine-d10 surrogate standard recoveries for the Shenandoah River sites averaged 78.8(± 16)%, while recoveries for Cedar Run samples averaged 125(±30)%. The data from Cedar Run samples may be somewhat over-reported based on surrogate recovery. Background levels in lab and field blanks were below EMDLs for this study and consisted primarily of chromatographic noise.

During the study period, seven duplicate samples were taken during both storm and base-flow conditions. The relative percent difference (RPD) was calculated for each set of duplicates. The mean RPDs were 12(±14)%, 10(±10)%, 14(±11)%, 36(±23)%, 10(±10)%, and 8(±14)% for atrazine, simazine, propazine, HA, DIA and DEA, respectively.

Results and Discussion

With one or two exceptions, atrazine, simazine, DEA, DIA, and HA were found in every sample. Atrazine concentrations were much higher than simazine. Propazine use appears to be very limited. Concentration data by site and date are listed in Supplemental Materials Tables 1 and 2. As noted in previous studies, there is usually a significant increase in herbicide concentrations during spring seasonal application period (Foster et al., 2000; Thurman et al., 1991). This is clearly apparent in these study sites. However, as the parent-compound (PC) concentrations decrease over the time period following application, atrazine was ever-present, and the transformation products (TPs) persisted with only relatively small changes in concentrations.

Despite the seasonal decreasing trend in atrazine concentrations, the surface water concentrations increased substantially during late season storm events that followed weeks of dry weather, including an August storm which affected the South Fork of the Shenandoah basin and an October storm event measured at the Cedar Run site near Brentsville. This would seem to indicate a sink of the parent atrazine exists in the basins where it cannot be easily transformed.

North Fork Shenandoah River near Cootes Store, VA

The Cootes Store site on the North Fork of the Shenandoah site drains about 543 km^2 of land that is 91% forested and 8% agricultural (Table 7). As would be expected, herbicide and TP concentrations are low compared with other sites. The highest concentrations of PCs and TPs at this site occur in June after the spring application period. The overall concentrations decrease as the season progresses. The overall stream burden for the S-triazine cumulative assessment group (CAG) prior to application ranged from a high of 61.4 in June to 12.2 ng L^{-1} in October. Unlike the other sites, S-triazine TPs dominate the total concentration throughout the study period, even during the peak application (Figure 2A).

North Fork Shenandoah River at Timberville, VA

The Timberville site on the North Fork is about 9 km downstream from Cootes Store. It drains an area that is 76% forested and 23% agricultural. Water concentrations for atrazine, simazine, DEA, DIA and HA during an April 29, 2007 storm event were

6.38, 2.23, 13.7, 6.39 and 0.903 ng L^{-1} respectively. The overall stream burden for the S-triazine CAG prior to application was 29.6 ng L^{-1}.

The April 29, 2007 Timberville numbers can be compared with concentrations from 2 POCIS devices deployed by the USGS and the Friends of the North Fork of the Shenandoah River about 25 km downstream of the sampling site in Mount Jackson, VA from March 10, through April 29, 2007 (Alvarez et al., 2008). The POCIS estimated water concentrations were 110 and 170 ng L^{-1} for atrazine, 5.5 and 8.2 ng L^{-1} for simazine, 8.5 for DEA, and DIA was below the method detection level. Hydroxy atrazine was not analyzed by the USGS GC-MS method.

Following springtime application, the highest concentrations of atrazine, simazine, DEA, DIA and HA at the Timberville site occurred in May and were 230, 45.9, 27.6, 14.3 and 32.5 ng L^{-1} respectively. The overall stream burden for S-triazine CAG was 352 ng L^{-1}. The overall stream burden for the chloroacetanilide CAG was 5.19 ng L^{-1}. This compared with estimated water concentrations for atrazine, simazine, DEA and DIA for two POCIS devices deployed by the USGS at Mt. Jackson from April 29 through June 9, 2007. They were 490 and 650, 24 and 18, 21 and 16 ng L-1 respectively. DIA was below their method detection levels (Alvarez et al., 2008).

The concentrations of atrazine and simazine decreased noticeably following the May application, but then remained essentially stable from July through October. Concentrations of DEA, DIA and HA remained relatively stable around 15 – 20 ng L^{-1} following application.

Linville Creek in Broadway, VA

The differential between Cootes Store and Timberville overall concentrations underscored the need to include the contribution of Linville Creek which drains approximately 118 square kilometers of land that is 23% forested and nearly 75% agricultural into the North Fork. At 2%, it is the most urbanized of the Shenandoah sites, but like the other sites in the Shenandoah Valley, urban land use is small. The Linville Creek contribution to the North Fork was monitored during the August, September and October sampling trips. The overall stream burden for S-triazine CAG was 121, 83.9 and 50.5 ng L^{-1} respectively. The overall stream burden for the chloroacetanilide CAG was 3.81, 1.55 and 1.64 ng L^{-1} respectively.

South Fork Shenandoah River near Luray, VA

The South Fork of the Shenandoah site near Luray, Virginia represents a marked contrast to the smaller sites on the North Fork and on Linville Creek. Here, the basin drains 3500 km^2 of land which is approximately 56% forested and 40% agricultural. The concentrations are generally in the same range as the Timberville site with the exception of a basin wide storm event in August. This storm event occurred further south that the North Fork which was not impacted by an increase in runoff and groundwater. It is notable that the highest contributions to the total concentrations were from atrazine and simazine despite the time span between the sampling date and the spring application period.

In comparison to the study sites, 8,776 samples were collected from the EPA's Atrazine Monitoring Program streams. Atrazine was detected in 84% of the samples with a mean concentration of 0.650 ug L-1 and a maximum concentration of 25.8 ug L-1. Simazine had a detection frequency, mean and maximum concentration of 36%, 0.187 and 24.6 ug L-1 , respectively. DEA had a detection frequency, mean and maximum concentration of 68%, 0.155 and 5.45 ug L-1 , respectively, and DIA had a detection frequency, mean and maximum concentration of 47%, 0.11 and 4.53 ug L^{-1} , respectively (US-EPA, 2007b).

Cedar Run near Catlett, VA

As noted, the Cedar Run site near Catlett drains an area that is 41% agricultural and 52% forested (Tab 7). It was an effective site that provided substantial hydrological data from its USGS gauging station. The mean sampling-period concentrations of atrazine, simazine, HA, DIA and DEA were 107, 12.5, 37.6, 18.4 and 30.2 ng L^{-1}, respectively with higher concentrations in May and June near the traditional application periods. The maximum atrazine concentration of 480 ng L^{-1} was measured on June 13 in a spring storm event. The concentrations of HA, DIA and DEA remained relatively constant with percent relative standard deviations around 45% over the study period.

During the spring rainy season, samples were taken from Walnut Creek over the three dates, April 15, May 14 and June 3. This seasonal stream directly drains several corn fields, and it had the highest concentration of atrazine (1,730 ng L^{-1} on April 15) than any other study site. On June 3, it measured the highest level of any transformation

product, HA at 2,310 ng L-1, than any other site. The high HA concentration may be indicative of the ability of soil to hold and transform atrazine through de-chlorination and hydroxylation through adapted soil microorganisms (Shaner and Henry, 2007).

Cedar Run near Brentsville, VA

Cedar Run merges with Broad Run, a stream that drains a mostly-urbanized basin. Together they form the Occoquon River and with Bull Run, feed the Occoquan Reservoir. Although there are no spatial data available, it is obvious that the basin size is much larger as is the area of agricultural input. The sampling location demonstrated a lag in receiving atrazine following application. Furthermore, the atrazine concentration decreased more slowly than the other locations.

Relative S-Triazine Parent and Transformation Product Concentrations

Figures 6 and 7 depict the overall S-triazine stream concentrations as well as the relative amounts that each TP contributes. As has been noted elsewhere, that herbicide TPs comprise a significant percentage of the total herbicide load in surface and ground waters (Kalkhoff et al., 2003; Kolpin et al., 1998). Clearly, there is a strong seasonal effect as atrazine and simazine concentrations drop rapidly after application. But the TP concentrations remain relatively constant and remain present throughout the study period.

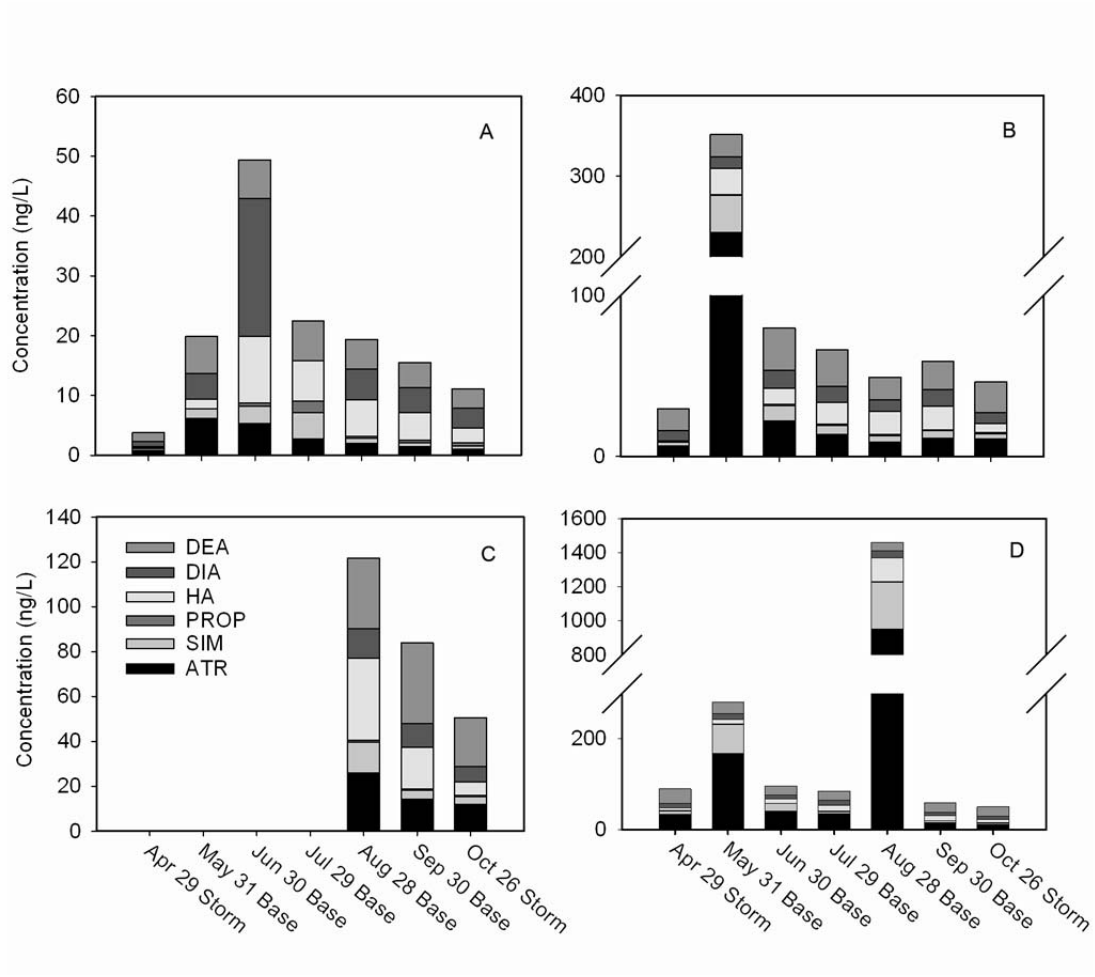


Figure 6: Total effective concentration of triazines and transformation products in the Shenandoah River Basin, 2007. (A): North Fork Shenandoah near Cootes Store. (B): North Fork Shenandoah near Timberville. (C): Linville Creek in Broadway. (D): South Fork Shenandoah near Luray. Stacked bars show the individual contributions of atrazine (ATR), simazine (SIM), propazine (PROP), 2-hydroxy atrazine (HA), desisopropyl atrazine (DIA), and desethyl atrazine (DEA) to the overall herbicide burden.

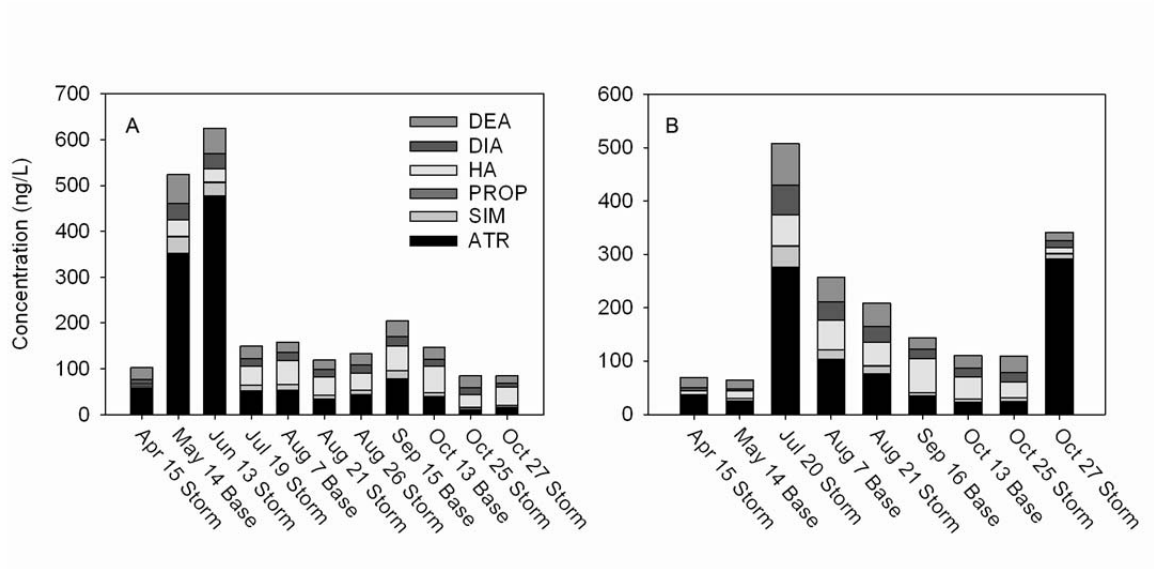


Figure 7: Total effective concentration of triazines and transformation products in Cedar Run Basin, 2007. (A): Cedar Run near Catlett. (B): Cedar Run near Brentsville. Stacked bars show the individual contributions of atrazine (ATR), simazine (SIM), propazine (PROP), 2-hydroxy atrazine (HA), desisopropyl atrazine (DIA), and desethyl atrazine (DEA) to the overall herbicide burden.

Frequently, the ratio of DEA to atrazine (DAR) has been used as an indication of surface runoff (Thurman and Fallon, 1996). The DAR in water typically decreases upon application of atrazine and after the first major storm runoff event, then increases as the post-application period progresses. (Scribner et al., 2000). This is apparent in the graphs (Figures 2 and 3). Lower DAR scores have been associated with groundwater influx into surface waters as PCs actively degrade to more-soluble TPs in the soil. The DAR for the two Cedar Run sites was consistently below one with a mean of 0.71 at the Catlett site and 0.57 at the Brentsville site. The South Fork averaged somewhat higher at 0.94. In all three sites, DEA concentrations spiked during the October storm event. It is likely that

ground water was the primary source of discharge. Higher specific conductance also coincided with the drier summer season which is frequently correlated with groundwater discharge.

Those sites were markedly different from the North Fork of the Shenandoah sites where the DAR averaged 2.2 at Cootes Store and 1.5 at Timberville. The lowest values occurred during the spring application period. The Valley and Ridge geological province may be more suited to surface runoff in this area, although the factors influencing DAR can be complex.

The ratio of DIA to DEA, referred to as D_2R, can be used in conjunction with the concentrations of the parent compounds to differentiate DIA derived from atrazine from that originating from the breakdown of other triazine herbicides (Shipitalo and Owens, 2003). The ratio of DEA to DIA was 1.3 at Cedar Run Catlett whereas the ratio at the North Fork was 2.6. This implies that DIA may be more mobile in Cedar Run than in the North Fork and consequently, DEA may be more persistent. DEA is also a transformation product of simazine. Samples from Cedar Run showed higher concentrations and more frequent occurrences for simazine. This may also contribute to the higher ratio of DEA to DIA in Cedar Run compared to the Shenandoah River sites where fewer detects of simazine were found.

Principal Component Analysis

Principal component analysis (PCA) was performed on the correlation matrix using SPSS statistical software (SPSS Statistics, Version 17.0, IBM Corporation, Somers,

NY). Loading factors were extracted and rotated using Kaiser normalization and varimax rotation in order to reduce the dimensions of the data to four principal components that account for 82.6 % of the variance (Table 8). Components beyond these four had variances less than 1.0, and therefore, had little contribution to the model. The bold-text factors in the table have a greater magnitude than 60% of the highest magnitude factor within that component group.

Figure 4 shows the first three principal components which account for 72.7% of the modeled data. All S-triazine and transformation product variables were described well by the first principal component (PC1) with the exception of 2-hydroxy atrazine. Concentrations of the parent herbicides and the two dealkylated transformation products DEA and DIA increase in the PC1 dimension. Interestingly, the relative order of the parent compounds corresponds to the order in which these herbicides are used. There is a Figure 8 shows the first three principal components which account for 72.7% of the modeled data. All S-triazine and transformation product variables were described well by the first principal component (PC1) with the exception of 2-hydroxy atrazine. Concentrations of the parent herbicides and the two dealkylated transformation products DEA and DIA increase in the PC1 dimension. Interestingly, the relative order of the parent compounds corresponds to the order in which these herbicides are used. There is a clear correlation between concentrations of atrazine and DEA. However, none of the

Table 8: Varimax-rotated factor loadings from principal component analysis of all samples at sites where temporal and spatial data is available. Bold loadings indicate a magnitude greater than 60% of the maximum absolute value for each principal component.

	PC 1	PC 2	PC 3	PC 4
Julian Date	-0.1244	-0.3238	**0.6029**	0.2746
% Agro in Basin	0.2212	0.0039	**0.8496**	-0.3123
Drainage (km^2)	0.1687	**0.9136**	0.1062	0.2278
Agro Area (km^2)	0.2087	**0.8964**	0.2004	0.1778
Discharge (m^3/s)	0.1050	**0.7856**	-0.0972	**-0.4979**
Conductance (μS/cm)	-0.0184	0.4242	**0.8350**	0.0919
Temp (^oC)	0.2680	0.1334	-0.0418	**0.7763**
Atrazine (ng/L)	**0.9624**	0.0971	-0.0622	0.0469
Simazine	**0.9048**	0.2012	-0.0203	0.1687
Propazine	**0.8016**	0.1512	0.0124	0.3483
2-Hydroxy Atrazine	*0.4964*	*-0.5206*	0.1603	0.0178
Desisopropyl Atrazine	**0.7298**	0.2675	**0.5193**	-0.1007
Desethyl Atrazine	**0.9686**	-0.0704	0.0796	-0.0177
Total	4.324	2.978	2.151	1.278
% of Variance	33.3	22.9	16.5	9.8
Cumulative %	33.3	56.2	72.7	82.6

spatial or temporal variables show a significant increase in this dimension. This seems to indicate that the amount of applied herbicide--for which data is not available--could have the greatest impact on herbicide concentrations in the surface.

HA is less clearly described by all the components. It contributes to a small portion of PC1 which would seem to make it less dependent on concentrations of atrazine than on other factors not modeled here. HA does have a slight inverse correlation with other variables in PC2 which is made up principally of the spatial variables drainage size,

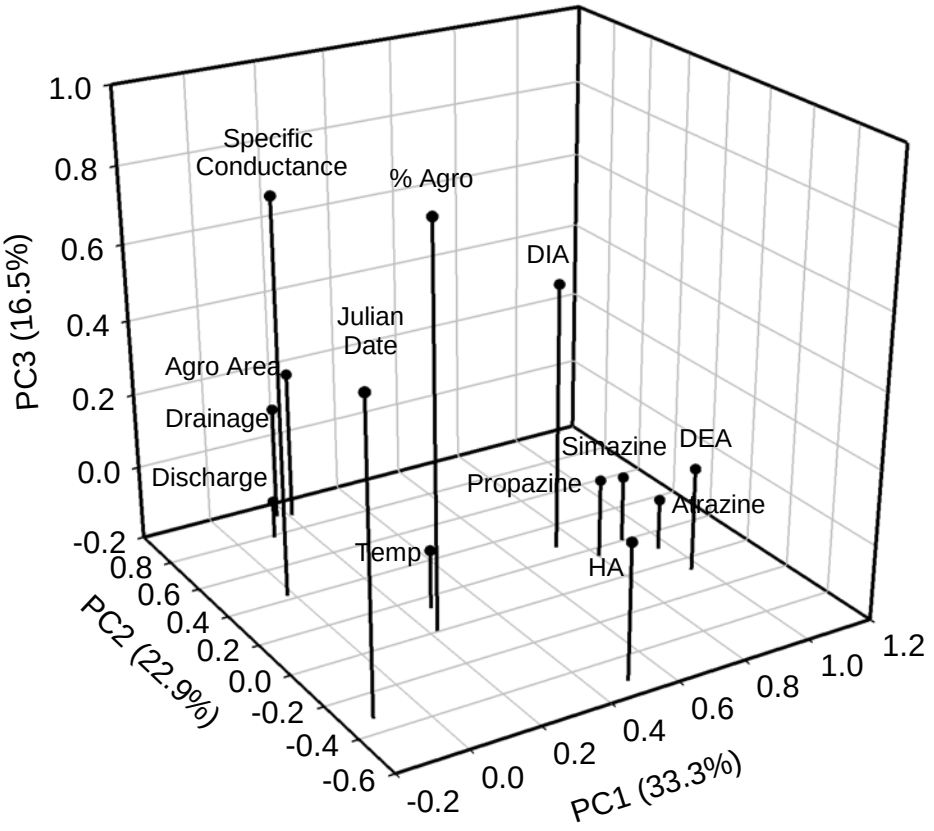


Figure 8: First three components from principal component analysis of temporal and spatial variables plotted in a 3D loading factor plot. Loading factors underwent Kaiser normalization and varimax rotation.

agricultural area and discharge. This negative contribution seems to support the notion that hydroxy atrazine may nearly immobile compared with atrazine, DEA and DIA in some soil types (Kruger et al., 1996; Krutz et al., 2003a; Krutz et al., 2003b; Mersie and

Seybold, 1996) and that most HA may be transformed from atrazine *in situ* by abiotic processes or from bacteria in the surface waters.

Desisopropyl atrazine also makes a notable contribution to PC3. PC3 is the only component with a significant contribution from Julian date thus making it the only transformation product that appears to be influenced by temporal factors. DIA also seems to be influenced by % agricultural area and specific conductance. Throughout the study area, specific conductance generally decreases as discharge increases, particularly during storm events where runoff is high. Since both DIA and conductance increase in the PC3 dimension, it would seem that DIA may be more closely correlated with groundwater discharge into the surface waters.

Conclusions

The primary objective of this research was to determine the concentrations of triazine herbicides in the study area over an extended period of time. The data presented showed a temporal pattern of atrazine and simazine concentrations related to the traditional spring time application period as well as storm events. However, the presence of these herbicides was noted in low concentrations throughout the study period. The need for the ecosystem-wide impacts of S-triazines remains important for assessing overall aquatic community health as well as the impact on drinking water sources.

The secondary objective was to determine the concentrations of triazine herbicide transformation products (TPs) over the same period to determine. As is clear in Figures 2 and 3, these TPs substantially increase the overall herbicide burden in surface water

systems. For further toxicological studies to be meaningful, it is imperative that concentrations of TPs must be considered in addition to the parent S-triazines.

The third objective of this research was to examine the similarities and differences between several sub-basins based on spatial variables such as basin size, land use, meteorological phenomena, and geological factors. Differences between the Shenandoah and the Cedar Run basins are clearly noted by the desethyl atrazine to atrazine ratios. Furthermore, the inverse of this relationship may aid clearer understanding of overall basin hydrology by using the DAR as a marker for seasonal changes in the primary source of these herbicides and transformation products.

CHAPTER 3: CHARACTERIZING THE BIOGEOCHEMICAL PROCESSES FOR S-TRIAZINE AND CHLOROACETANILIDE HERBICIDES AND THEIR TRANSFORMATION PRODUCTS IN THE NORTH FORK OF THE SHENANDOAH RIVER BASIN

Introduction

There has been a renewed interest in the fate and transport of herbicides in surface waters in regions of intensive agricultural use because of recent evidence demonstrating endocrine disruptive effects (Kalkhoff et al., 2003; Krutz et al., 2004; McConnell et al., 2007; Panshin et al., 2000). Newer studies track transformation products (TPs) that are produced through abiotic and biotic processes in the soil, groundwater and surface waters near their point of application (Behki and Khan, 1994; De Souza et al., 1998; Vibber et al., 2007). Characterizing and understanding how these toxic compounds transform and move through the environment is essential so that effective remedial measures can be developed to minimize their impact on valued aquatic systems.

The parent triazine herbicides analyzed in this study include atrazine [2-chloro-4-ethylamino-6-isopropylamino-*S*-triazine], simazine [2-chloro-4,6-bis(ethylamino)-*S*-triazine] and propazine [2-chloro-4,6-bis(isopropylamino)-*S*-triazine], and the parent chloroacetanilide herbicides include metolachlor [2-chloro-N-(2-ethyl-6- methylphenyl)-N-2-methoxy-1-methylethyl-acetamide], alachlor [2-chloro-N-(2,6-diethylphenyl)-N-

(methoxymethyl)-acetamide] and acetochlor [2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)-acetamide].

Atrazine (ATR) can form six major transformation products (TP) through abiotic and biotic processes including three TPs studied here—desethyl atrazine (DEA), desisopropyl atrazine (DIA) and 2-hydroxy atrazine (HA). Simazine can form DIA, and propazine can form DEA as well. These TPs can be more or less mobile and more or less toxic than their parent herbicides (Battaglin et al., 2003).

Metolachlor, alachlor and acetochlor are transformed through biological processes by removal of a chlorine atom and the subsequent addition of an ionic functional group, particularly an ethane sulfonic acid (ESA) or an oxanilic acid (OA) group. These transformation products are formed through a glutathione conjugate intermediate through an enzymatic pathway catalyzed by glutathione-S-transferase enzymes found in plants and microbes (Field and Thurman, 1996).

The addition of these functional groups increases the water solubility of these herbicides and therefore, the transformation products are more likely to permeate the pore water in the vadose (unsaturated) zone and enter surface waters through groundwater discharge (Thurman et al., 1996). The resulting ESA and OA transformation products are most-often detected in greater concentrations than their parent compounds (Kalkhoff et al., 1998) and can persist in soils up to 4 years following application (Rebich et al., 2004).

This study examines the input of S-triazine and chloroacetanilide herbicides into a surface water system in close proximity to extensive agricultural activity. It does so by

examining their concentrations over time and in relationship to river flow. It then characterizes how those concentrations change with respect to transformative and dispersive processes.

Discharge into surface waters occurs through surface runoff from the drainage basin and from subsurface and groundwater input. This discharge is laden with parent and TP herbicides, particularly in the late spring and early summer when the herbicides are applied to crop fields prior to the emergence of undesired broadleaf plants. That pre-emergent application coincides with heavier spring rains and produces a spring flush where the herbicide concentrations are significantly higher (Thurman et al., 1992). The timing of this spring flush is vital to characterizing the persistence and dispersal of the herbicides throughout an annual cycle.

The ratios of transformation product concentrations to their parent compound concentrations are considered to be important markers for understanding the input processes in a watershed (Battaglin et al., 2003). They have been previously correlated to nonpoint-source groundwater pollution (Adams and Thurman, 1991). The desethyl atrazine (DEA) to atrazine ration (DAR) has been used as an indicator of atrazine-containing groundwater discharge into surface water when values exceed unity (Thurman et al., 1992). DEA is highly mobile in many soil types (Kruger et al., 1996). Furthermore, the DAR indicates atrazine residence time in soil during transport to the groundwater (Thurman and Fallon, 1996).

A DAR less than 1 has been used as a marker for determining the occurrence of the spring flush. DAR levels that are greater than 1 indicate a slow moving, diffuse flow

of groundwater through the soil leading to greater atrazine removal through abiotic processes such as adsorption and filtration (Bayless, 2001).

The 2-hydroxy atrazine (HA) to atrazine ratio (HAR) is less frequently used since HA moves slowly through soil, and HA is not typically analyzed in studies that use GC-MS instruments. The desorption rate of HA can be half that of DEA (Mersie and Seybold, 1996). The TP to parent ratios for chloroacetanilide herbicide are as much as 3 orders of magnitude higher that the S-triazines indicating a high level of mobility in surface and ground water (Battaglin et al., 2003).

Project Goals

The biogeochemical factors that influence the concentration and distribution of herbicides in rivers are made up of system-specific input and output processes. Input processes that increase herbicide concentrations in rivers include surface runoff and groundwater discharge of water laden with parent herbicides following their application to crop lands. The predominant output processes that lower concentrations include biotic and abiotic transformations, and advection via air-water exchange, settling of particles, diffusion into pore spaces of river sediments, longitudinal dispersion and dilution (Schwarzenbach et al., 2003). The input and output processes that predominate in one season may have lesser impacts during another season. Characterizing those processes over an extended sampling period provides a more-detailed understanding of the fate and transport of herbicides in surface water systems which in turn helps design preventative and remedial measures.

The central hypothesis of this project is that the principal input and output processes affecting pre-emergent herbicide concentrations can best be quantified by sampling, extracting and analyzing surface water over an annual cycle. This hypothesis will be tested by a study of the upper basin of the North Fork of the Shenandoah River near an area of heavy agricultural land use. The study will determine the concentration of herbicides and their transformation product (TP) concentrations over a twelve month sample period beginning prior to the spring flush event.

The following goals of this study will addressed:

- Characterize input processes
    - Determine changes in concentration with respect to time.
    - Determine TP to parent herbicide concentration ratios with respect to time.
- Characterize output processes
    - Determine the overall transformation rates.
    - Determine the overall dispersion rates.
- Determine steady state concentrations.

Study Area

The Shenandoah River supports significant historical, social and recreational activities. The Shenandoah Valley receives over 250,000 visitors per year. Several species of game fish have been severely impacted instances of severe lesions and large fish kills thought to originate from suppressed immune systems resulting from chemical

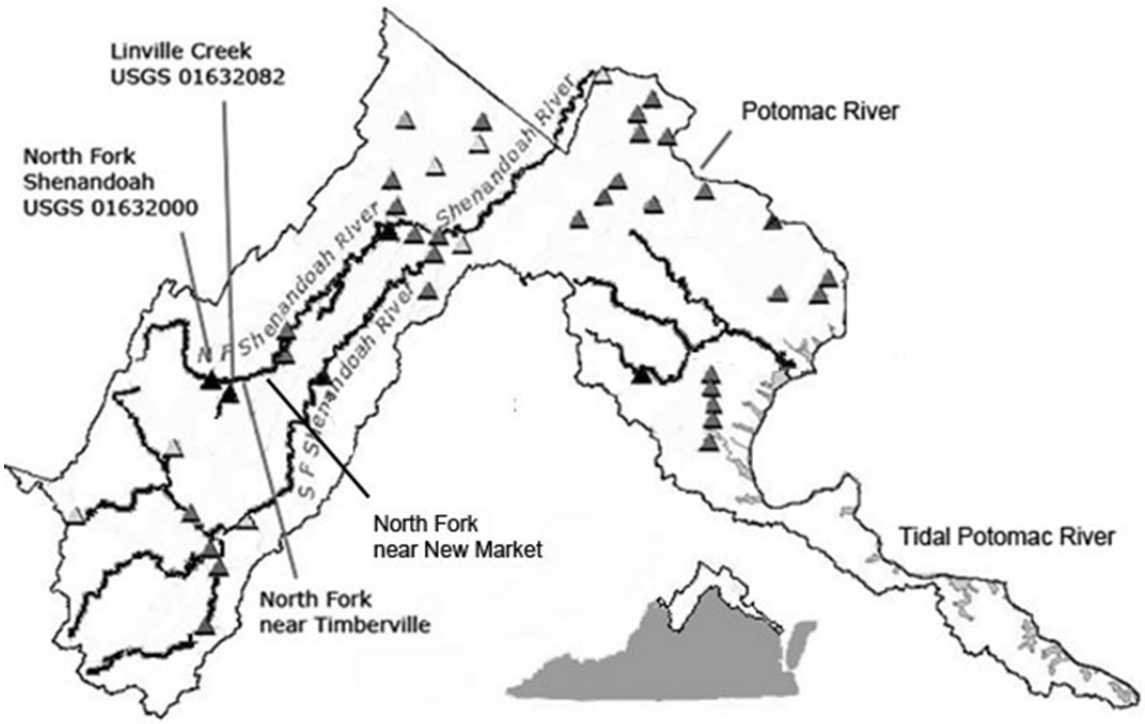


Figure 9: Map of the Potomac River Basin in Virginia showing the 6 sampling sites. ▲USGS Gauging Stations which monitor stream flow, water temperature and specific conductivity (USGS, 2008a).

pollution (Blazer et al., 2010; Robertson et al., 2009). The discovery of testicular oocytes associated with intersex phenomenon have been reported along sections of the river (Blazer et al., 2007; Garman and Orth, 2007). Several sections have been designated as impaired for fecal coliform, nutrients, PCBs and mercury (US-EPA, 2008).

The North Fork of the Shenandoah River (NFSR) lies in Rockingham County, VA (USA), which has heavy agricultural land use. Five sections of the North Fork have been listed as impaired by the Virginia Department of Environmental Quality (VA-DEQ, 2008). The upstream sections lie within an agricultural area that raises poultry and grows

corn and soybeans. Poultry manure is used to fertilize crops and the primary source of impairment is fecal coliform bacteria. Herbicides are applied to crop fields prior to

Table 9: Geographical and geological site descriptions for 2008 Shenandoah River Basin, Virginia sampling locations.

	NFSR	Linville Creek	NFSR	NFSR
Site Acronym	ATV	LIN	BTV	PMB
Latitude	38°38'13"	38°36'24"	38°38'08"	38°39'06"
Longitude	78°51'11"	78°48'13"	78°46'44"	78°41'53"
Location	Cootes Store	Broadway	Timberville	New Market
USGS Unit	02070006	02070007	N/A	N/A
Drainage (km^2)	543.9	118.4	N/A	N/A
Altitude (m)	320.6	313.9	301.4	289.3
Reference Site	Source	Source	Cootes	Timberville
Ref Alt (m)	638.3	394.4	320.6	301.4
Alt Drop (m)	317.7	80.5	19.2	12.1
Distance (km)	32.0	15.4	9.5	8.8
Drop (m/km)	9.9	5.2	2.0	1.4
Geology	SS	LS	LS	LS
% Agriculture	8.0	74.4	22.6	29.9
% Forested	91.1	23.1	76.1	68.3
% Urbanized	0.3	2.1	0.8	1.2

SS: Sandstone
LS: Limestone

spring planting as a pre-emergent treatment for broad-leaf plants. The map (Fig. 9) shows the five sampling sites in the Great Valley Sub-Province of the Valley and Ridge Physiographic Province within the Shenandoah River basin. Latitude and longitude coordinates as well as other spatial and geological parameters are listed in Table 9.

The sampling sites include the USGS gauging station on the NFSR at Cootes Store, VA, which is located approximately 30 km downstream from the West Virginia

border. The USGS gauging station on Linville Creek near Broadway, VA, a tributary to the NFSR, is located approximately 1.7 km upstream from the confluence. The third site is on the NFSR is located approximately 9.5 km below the confluence. The furthest site downstream is located below an area of high density corn and soybean crops near New Market, VA.

Materials and Methods

Supplies and Chemicals

HPLC-grade solvents were purchased from Fisher Scientific (Pittsburg, PA). Herbicide chemical standards for atrazine, simazine, propazine, terbuthylazine, desethyl atrazine, desisopropyl atrazine, hydroxy atrazine, metolachlor, metolachlor, metolachlor ethane sulfonic acid (ESA), metolachlor oxanilic acid (OA), alachlor, alachlor ESA, alachlor OA, acetochlor, acetochlor ESA and acetochlor OA were purchased from Sigma-Aldrich (St. Louis, MO). The stable isotope standard simazine-d10 was purchased from Cambridge Isotope Labs (Andover, MA). Ultra-high purity nitrogen was provided by Roberts Oxygen (Rockville, MD). Glass fiber filters were manufactured by Whatman Inc. (Florham Park, NJ). Oasis HLB Plus solid-phase extraction products were purchased from Waters Corporation (Milford, MA). LC-MS consumable supplies were purchased from Waters Corporation.

Lab grade water was produced in house using a recirculating deionization skid with UV sterilization treatment (HydroMax, Emmitsburg, MD) followed by polishing

with an Elga Maxima ultrapurifier producing 18.2 MΩ water that is further treated for organics via a second UV lamp (Elga Labwater, Marlow, Buckinghamshire, UK).

Sample Collection

Samples were obtained using a Fultz submersible pump fitted with a 15.4 m rubber-jacketed Teflon® hose (Fultz Pumps, Inc., Lewistown, PA). Water samples were pumped into pre-washed, solvent-rinsed ~20 L stainless-steel Cornelius kegs. The filled kegs were sealed, transported to the lab and stored at 4 ^{o}C pending filtration and extraction.

Sample Filtration

Water samples were pressure filtered with high-purity nitrogen within 24 hours of collection through a 142 mm stainless steel filter holder (Millipore Corporation, Billerica, MA) holding a 142-mm diameter Whatman Grade GF/F (nominal 0.7 μm pore diameter) filter overlaid by a 150-mm diameter GF/D (nominal 2.7 μm pore diameter) pre-filter (Whatman Inc., Florham Park, NJ). Sample filtrate was directed into 1 L glass media bottles which had been cleaned and solvent rinsed. Filtrate was stored at 4 ^{o}C for subsequent solid phase extraction.

Solid-Phase Extraction

Individual samples were comprised of four 1-L aliquots of the original ~20-L sample that were composited post extraction. Each 1-L bottle was spiked with 30 μL of

surrogate recovery standards consisting of 0.3 ng μL^{-1} of simazine-d10 and 0.4 ng μL^{-1} of desethyl terbuthylazine for a total of 120 μL per sample. The 1-L sample filtrates were extracted using Oasis HLB Plus cartridges (Waters Corporation, Milford, MA) containing 250 mg of hydrophilic-lipophilic balanced sorbent that were fitted with empty 6-mL syringe barrels and placed on a Supelco Visiprep vacuum manifold (Sigma-Aldrich, St. Louis, MO). The cartridges were sequentially activated with 3 mL each of MTBE, methanol and reagent water, respectively. The samples were loaded on the HLB cartridges at a flow rate of ~5-10 mL min^{-1}. Following extraction, each HLB cartridge was subsequently washed with 3 mL of 5% (v/v) methanol in reagent water to remove inorganic interferences. The cartridges were aspirated for 30 minutes under vacuum to purge water, and eluted with 2X 4-mL portions of 10% (v/v) methanol in MTBE. The eluents were combined into 12-mL deactivated glass centrifuge tubes.

Sample extracts were concentrated using a Centrivap centrifugal vacuum concentrator (Labconco Corporation, Kansas City, MO) and reconstituted in 1 mL of mobile phase and quantitatively transferred to 2-mL amber auto-sampler vials (National Scientific Company, Rockwood, TN). The internal standards monocrotophos and terbuthylazine were added per a previous study by McConnell, Rice, et al. (2007) at 186.7 and 157.6 ng per sample, respectively. Sample extracts were stored at -20 ^{o}C pending analysis.

The particulate matter on the sample filters were not extracted since surface water samples as large as 10 L in other studies showed negligible concentrations of the target

analytes (Liu et al., 2002; McConnell et al., 2007). Therefore, only the dissolved phase of river water was analyzed here as part of the methods development study.

Instrumental Techniques

Sample extracts were subsequently analyzed by liquid chromatography-mass spectrometry (LC-MS(Q)) as described previously (Huff and Foster, 2011a). The LC system consisted of a Waters Alliance 2695 Separations Module (Waters Corporation, Milford, MA) with a quaternary pump, a refrigerated autosampler compartment, a heated column compartment and an inline vacuum degasser. The analytical column was a T3 Atlantis that measured 2.1 mm ID by 150 mm length. The mass spectrometer was a Waters-Micromass ZQ2000 single quadrupole system with an orthogonal electrospray ionization (ESI) source (Waters Corporation).

S-triazine herbicides and their transformation products as well as the parent chloroacetanilide herbicides were analyzed using positive-ion electrospray ionization. The transformation products of chloroacetanilide herbicides metolachlor, acetochlor and alachlor were analyzed using negative ion electrospray ionization. Chromatography was performed using a binary gradient of an aqueous 0.1% v/v acetic acid and 0.1% v/v acetic acid in acetonitrile. The mass spectrometer was operated in selected ion monitoring mode (SIM). Cone voltages ranged from 25 V to 50 V in order to produce in-source collision induced dissociation (IS-CID) to provide at least two ion fragments for identification.

Quality control and quality assurance

Estimated method detection limits (EMDLs) for this study were determined by 10 repeat injections of a low-concentration calibration standard (Table 3) according to reported methods (US-EPA, 2005a). All analytical data were screened for EMDLs. Five-point calibration curves were used for quantitation by using the internal standard method.

Solid-phase extraction efficiency was assessed by performing reagent water and matrix spikes and determining recoveries as reported in Chapter 1. Simazine-d10 surrogate standard recoveries in the Shenandoah River samples averaged 80.6% ± 23%, while recoveries for desethyl-terbuthylazine averaged 69.3% ± 23%. Background detections of the target analytes were minimal and considered to be primarily the result of random noise. The reported sample concentrations were blank-corrected through a background subtraction procedure.

Data Analysis

S-triazine herbicide transformation rates (k) were determined by plotting surface water concentrations of parent compounds with respect to time. Best-fit curves were generated using Graphpad Software Prism 5 curve-fitting software (La Jolla, CA, USA) and the exponential decay formula (Eqn. 1) where C is the concentration in ng L^{-1}, C_0 is the initial concentration and t is the time in days.

$$C = C_0\, e^{-kt} \qquad \text{Equation 1}$$

In order to minimize the effects of non-transformative changes in the parent compound concentrations (i.e., dispersion and advection), the concentration is expressed as a fraction (C_f) of parent S-triazine concentrations [P] relative to the sum of the S-triazine and their transformation product concentrations [TP] (Eqn. 2). This assumes that the parents and TPs disperse at similar rates.

$$C_f = \frac{[P]}{[P]+[TP]} \qquad \text{Equation 2}$$

It is also assumed that surface water concentrations are influenced by a steady, year-round input of herbicides and transformation products from groundwater discharge and surface water runoff. Therefore, the transformation-rate model will likely have a non-zero asymptote below which C_f does not fall. The model here determines that plateau relative concentration ($C_{f,p}$) and calculates a span from $C_{f,p}$ to the highest initial relative concentration at the spring flush ($C_{f,0}$). This term is substituted for the initial concentration (C_0) in Equation 1 (Eqn, 3).

$$C_f = (C_{f,0} - C_{f,p})\, e^{-kt} \qquad \text{Equation 3}$$

Overall output rates (k in d^{-1}) of herbicides and their TPs are determined by combining all dispersive processes (i.e., transformation, diffusion into pore water, longitudinal dispersion) and plotting a total concentration (C_{tot}) with respect to time. The

model uses a modified exponential-decay equation (Eqn. 1) where C_{tot} is substituted for C_f in Equation 3 as it is assumed that a non-zero plateau exists from steady, year-round input. C_{tot} is calculated as the sum of parent compound concentrations [P], and transformation product concentrations [TP] (Eqn. 4)

$$C_{tot} = (C_{tot,0} - C_{tot,p})\, e^{-kt} \qquad \text{Equation 4}$$

The steady-state concentration expressed by the asymptotic plateau concentration $C_{tot,p}$ (ng L^{-1}) is an important ecological parameter as it represents the long-term exposure experienced by aquatic flora and fauna from the herbicides applied to crop lands.

Results and Discussion

Concentration with Respect to Time and Flow

A summary of all the concentrations analyzed in this project is presented in Table 2 for the 339 day study period beginning on 29 March 2008 and culminating on 28 Feb 2009. During this period, 14 sampling trips were made to 4 locations for a total of 58 samples plus 15 duplicate samples. Of the 73 total samples, 61 were taken during base flow conditions and 12 were taken during storm events. Atrazine, 2-hydroxy atrazine, desisopropyl atrazine, desethyl atrazine and metolachlor ethane sulfonic acid were found in every sample on every sample date indicating a year-round persistence. Simazine was

Table 10: Summary of river water concentrations (ng L^{-1}) over the course of the study period ranging from 29 March 2008 through 28 February 2009.

	ATR	SIM	PROP	HA	DIA	DEA	MET	MET-ESA	MET-OA	ALA	ALA-ESA	ALA-OA
	North Fork of Shenandoah River at Cootes Store (N=14, 3 Storm Samples)											
Mean	8.2	3.3	0.7	5.8	5.9	7.3	1.7	69.3	7.5	0.4	22.0	31.4
StdDev	11.4	4.2	0.3	6.0	6.3	7.3	2.2	66.3	7.7	0.1	15.8	68.8
MIN	1.1	0.3	0.3	0.4	0.8	1.9	0.1	10.7	1.3	0.4	10.4	1.6
MAX	36.2	11.4	1.0	22.9	19.0	22.3	6.2	216.0	27.0	0.5	40.0	228.6
Frequency	100%	79%	36%	100%	100%	100%	79%	100%	79%	21%	21%	79%
	Linville Creek at Broadway (N=17, 3 Storm Samples)											
Mean	52.6	16.3	1.2	24.1	19.1	43.3	6.2	206.6	35.6	0.5	19.2	37.1
StdDev	56.7	19.9	0.6	13.3	14.9	22.2	8.0	122.6	35.4	0.3	22.7	70.5
MIN	9.1	1.9	0.4	9.6	6.7	14.0	0.3	59.1	0.9	0.3	1.9	0.9
MAX	207.8	66.5	2.1	53.1	50.8	89.2	25.2	529.1	131.3	0.9	63.6	220.0
Frequency	100%	100%	35%	100%	100%	100%	100%	100%	100%	29%	94%	88%
	North Fork of Shenandoah River at Timberville (N=15, 3 Storm Samples)											
Mean	31.5	11.2	1.0	14.3	22.0	26.1	5.5	125.8	22.2	0.6	37.1	45.6
StdDev	34.2	12.8	0.6	8.7	27.4	17.4	6.0	82.2	19.3	0.2	56.5	98.0
MIN	5.1	1.2	0.3	3.3	4.0	8.1	0.3	51.3	2.4	0.5	1.4	0.9
MAX	104.0	38.8	1.8	28.3	106.7	60.3	16.9	265.5	62.3	0.8	217.8	326.9
Frequency	100%	100%	53%	100%	100%	100%	87%	100%	93%	13%	93%	80%

	North Fork of Shenandoah River at New Market (N=25, 3 Storm Samples)											
Mean	37.9	13.0	0.9	17.5	19.1	34.7	5.1	182.4	25.7	0.5	28.3	12.5
StdDev	30.6	11.9	0.5	10.3	14.3	18.9	5.3	112.7	20.1	0.2	24.2	23.4
MIN	3.1	1.0	0.3	5.0	3.4	7.2	0.1	33.1	1.3	0.2	5.1	2.4
MAX	102.4	41.8	1.7	32.8	52.8	75.4	18.8	377.4	71.6	0.8	119.2	100.6
Frequency	100%	100%	40%	100%	100%	100%	100%	100%	96%	24%	92%	76%

ATR, atrazine; SIM, simazine; PROP, propazine; HA, 2-hydroxy atrazine; DIA, desisopropyl atrazine; DEA, desethyl atrazine; MET, metolachlor; MET-ESA, metolachlor ethane sulfonic acid; MET-OA, metolachlor oxanilic acid; ALA, alachlor; ALA-ESA, alachlor ethane sulfonic acid; ALA-OA, alachlor oxanilic acid.

found in all samples with the exception of those from the Cootes Store, VA site, a site that is only 8% agricultural upstream land use.

The highest concentrations of herbicides and transformation products were found in Linville Creek in Broadway, VA, a stream that drains 118 km^2 of land that is 74% agricultural (Table 1). The lowest concentrations were found in the North Fork of the Shenandoah River (NSFR) site at Cootes Store, VA where the drainage basin is made up of only 8% agricultural lands.

The herbicide with the highest measured concentration throughout the study area was the ethane-sulfonic acid transformation product of metolachlor. The metolachlor and alachlor parent herbicides were found in much smaller concentrations than their transformation products. Acetochlor and its transformation products were not found in any of the study samples.

Figure 10 plots the total concentration of S-triazine herbicides and the total concentration of chloroacetanilide herbicides plotted on a two y-axis chart along with river discharge over the course of the study period. Figure 2a plots those values from the NFSR at Cootes Store with historical daily discharge data (m^3 s^{-1}) obtained from USGS gauging station 01632000. Figure 2b shows the same plot from Linville Creek at Broadway from USGS gauging station 01632082.

The plots clearly show the spring flush with the highest concentrations of S-triazine herbicides occurring in the late spring and early summer following the rains. The highest total concentrations of chloroacetanilide herbicides are made up by as much as 90% of the metolachlor ESA which peaks during the summer dry season. Most notable

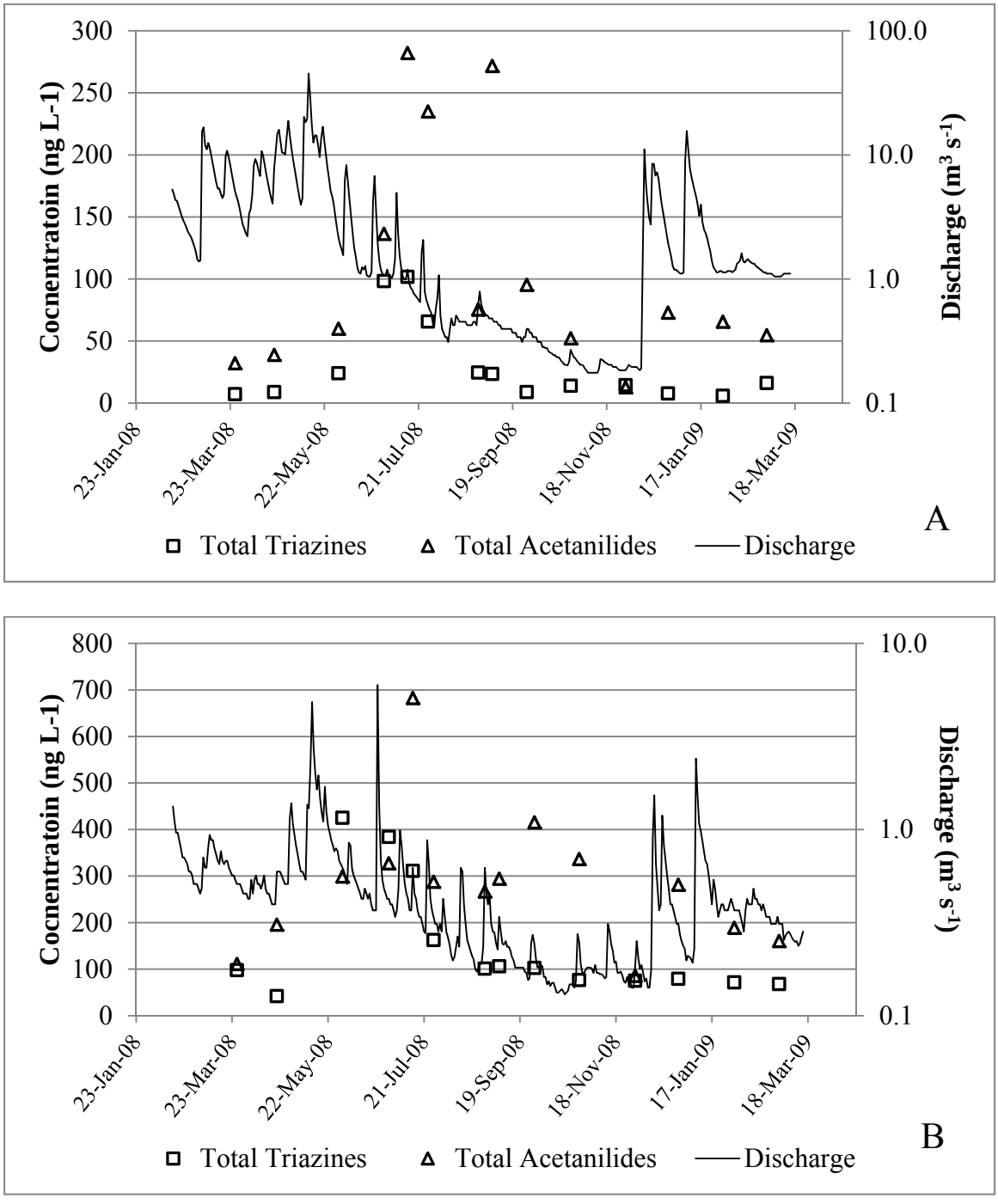


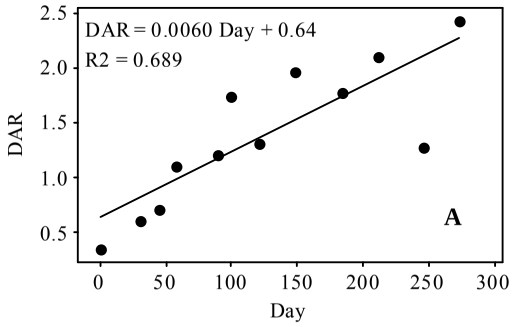
Figure 10: Plots of concentration (ng L^{-1}) and discharge (m^3s^{-1}) versus date over the study period for (A): North Fork of the Shenandoah River at Cootes Store and (B): Linville Creek at Broadway.

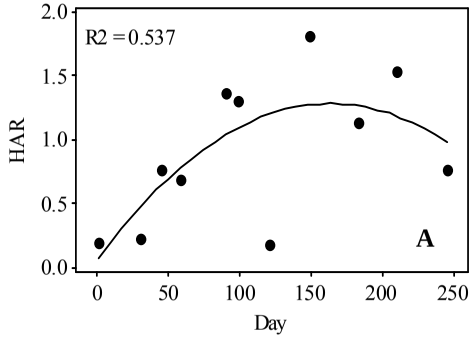
in the plots is the lack of correlation to the winter wet season as total concentrations show no appreciable increase in response to higher discharge.

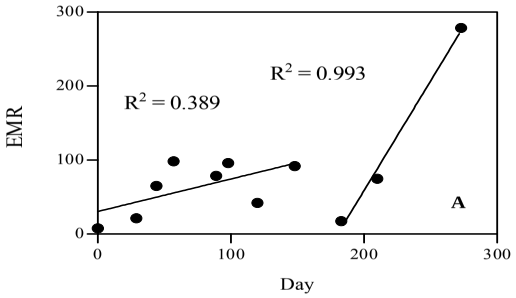
Transformation Product to Parent Ratios

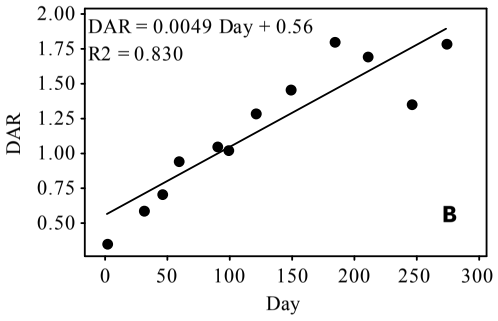
Figure 11 shows plots of desethyl atrazine to atrazine ratios (DAR), 2-hydroxy atrazine to atrazine ratios (HAR) and metolachlor ESA to metolachlor ratios (EMR) verses time (d). The initial ratio value was determined for 31 May 2008 when the ratio was lowest due to the seasonal atrazine application period. As the plots of DAR show, the ratios do not remain constant with respect to geochemical factors, but correlates to time as the transformation processes convert atrazine into desethyl atrazine while at the same time, the surface runoff concentration of atrazine is at its lowest levels.

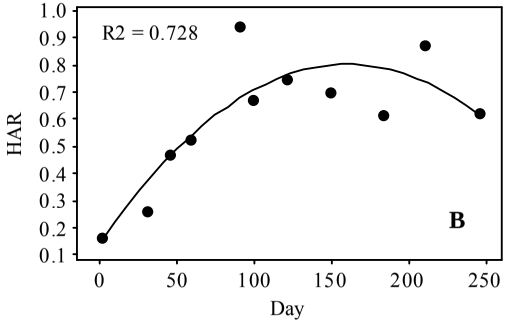
Somewhat surprisingly, the values of DAR increased linearly from the spring flush value of ~0.4 to a high of ~2.4 during the later months of the study. This model is particularly evident as the NFSR at Timberville and near New Market. The seasonal change compares with reported DAR values in Mid-West soils that were 0.3 pre-planting, 0.1 post planting, and 0.4 during the harvest season (Thurman et al., 1992). The linearity appears to indicate that the source of desethyl atrazine changes constantly from surface runoff to groundwater discharge and that the concentrations in the former decrease as the concentrations in the latter increase.

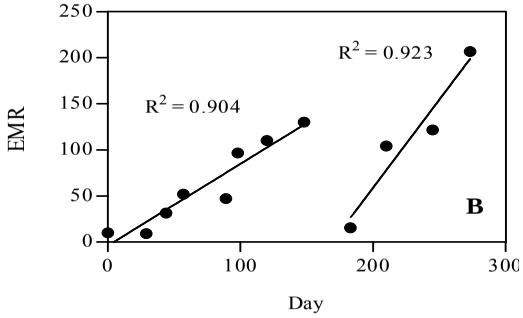

DAR = 0.0060 Day + 0.64
R2 = 0.689
DAR
Day
A


R2 = 0.537
HAR
Day
A


R² = 0.389
R² = 0.993
EMR
Day
A


DAR = 0.0049 Day + 0.56
R2 = 0.830
DAR
Day
B


R2 = 0.728
HAR
Day
B


R² = 0.904
R² = 0.923
EMR
Day
B

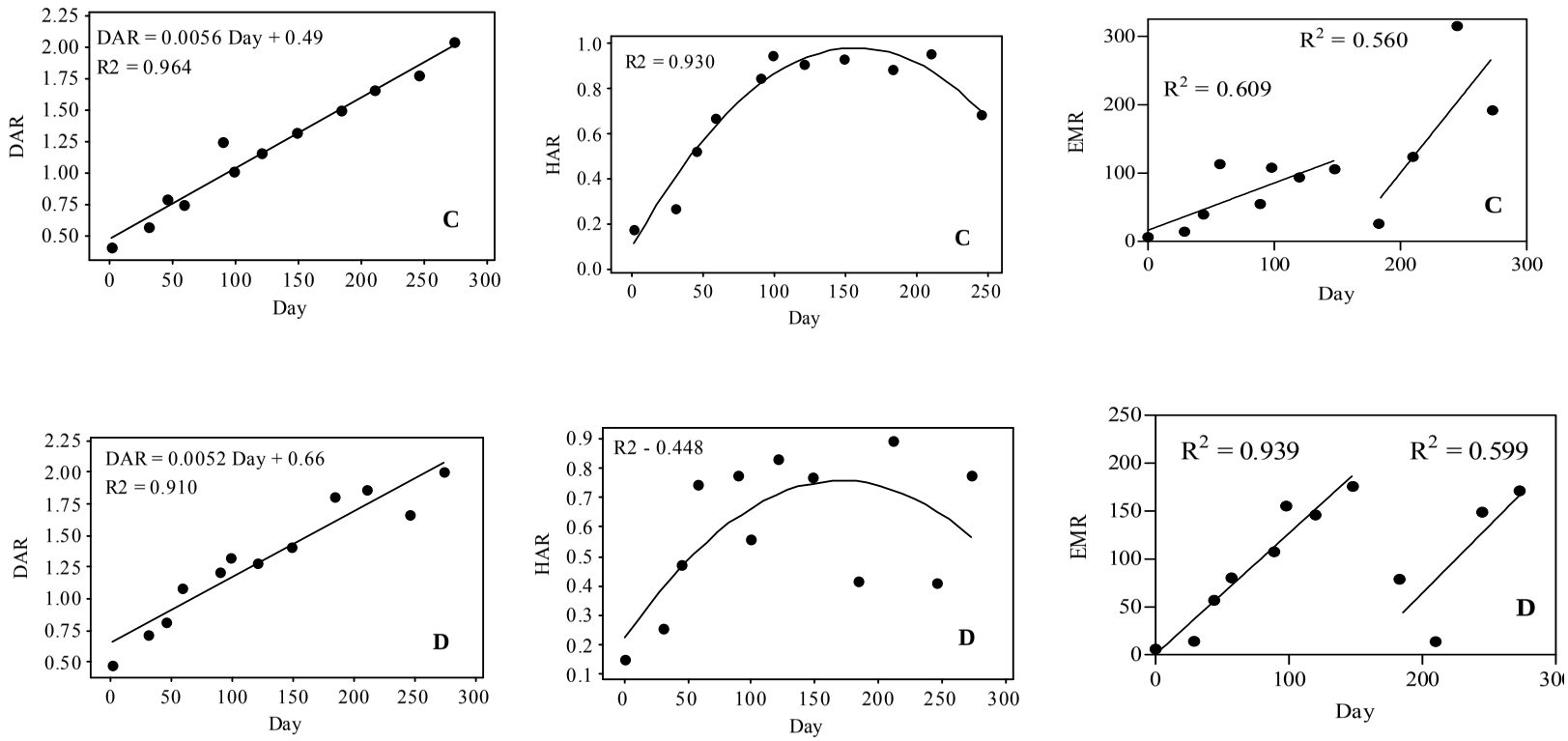


Figure 11: Plots of desethyl atrazine to atrazine (DAR), hydroxy atrazine to atrazine (HAR) and metolachlor-ESA to metolachlor (EMR) ratios. (A): North Fork of the Shenandoah River (NFSR) near Cootes Store, VA., (B): Linville Creek at Broadway, VA, (C): NFSR at Timberville, VA., (D): NFSR near New Market, VA.

HAR concentrations followed a quadratic time-series function with the lowest concentrations also detected during spring similar to DAR but with the maximum concentrations occurring between 150 to 175 days after the springtime lows and then tapering between that period and the last sampling dates. Many factors may account for this occurrence. Microbial transformation of atrazine to 2-hydroxy atrazine may be limited in the colder water as water temperatures in the study area range from a summertime high of 25 ^{o}C to a wintertime low of less than 1 ^{o}C (USGS, 2008b). The physical properties of 2-hydroxy atrazine may also play a role as its water solubility is 4.0 mg L^{-1} at 25 ^{o}C and much lower in cold winter water. The water solubility of DEA is higher at 280 mg L^{-1} at 25 ^{o}C. The reported soil adsorption coefficient (log k_{oc}) for HA is 3.4 L kg^{-1} compared with 1.9 L kg-1 for DEA also at 25 ^{o}C (US-EPA, 2007). When examined independently, the changes in the ratio of 2-hydroxyatrazine to atrazine with respect to time shows that the factors that influence HAR are substantially different than those that influence DAR.

Generally, the EMR increases substantially over time as run-off of metolachlor lessens following the planting season. EMR values reach as high as 300, a value much higher than those of the DAR and HAR. However, the plots show a break in the ratios between the 26 October and 30 November 2008 sampling dates. The initial period shown in the graphs shows an increase in EMR beginning soon after the spring flush and extending to 26 October. In each of the sample sites, the EMR then drops to near the low point measured for the spring season when runoff of metolachlor is high. The plots then reach maxima in the subsequent months. For this reason, two separate regression lines

are calculated, one for each period. The R^2 values for each of the linear fits are shown on the graph. The heavily-agricultural Linville Creek data shows good correlation for this split with R^2 values of 0.904 and 0.923 for the two periods.

It would seem from this data that a second flush or discharge of the parent metolachlor was produced either from an additional application throughout the region or some other phenomenon. Neither the DAR nor the HAR demonstrated this phenomenon. Increases in the surface water discharges were not observed during this period. The mean discharge for the preceding 90 day period for the Linville Creek site was 0.19 $m^3 s^{-1}$ with a standard deviation of 0.06 $m^3 s^{-1}$ while the discharge on the 30 November date was 0.18 $m^3 s^{-1}$. Late fall application seems likely and this practice has precedent with the use of metolachlor (Parker et al., 2005).

Transformation Rates

Transformation of the parent S-triazine herbicides atrazine, simazine and propazine into desethyl-, desisopropyl- and 2-hydroxy atrazine is evident in the transformation product to parent ratios above. The transformation rates from parent to product over an extended period of time are a critical perspective in understanding this phenomenon. Transformation rates were calculated by plotting the fraction of remaining parent compounds per the total concentration of parents and transformation products (Eqn. 2). The sum of atrazine, simazine and propazine concentrations was used since all three parent herbicides can transform into one or more of the products.

In this case, concentration data showed that the highest measured concentrations of parent compounds occurred on 29 June 2008 following the springtime pre-emergent application of herbicides. Therefore, the actual value of this initial concentration ($C_{f,0}$) was used as t_0. The sampling period subsequent to that date extended until 28 February 2009 and included 11 sampling dates for a total of 244 days. The XY scatterplots with connected data points of the C_f values are shown in Figure 4. The best-fit exponential decay curve is also plotted. Table 3 shows the best-fit model data for the curves. The calculated transformation rates ranged from 0.025 to 0.045 d^{-1} across the Potomac River sub-basins for the triazine herbicides. These rates compare well with the rates of 0.025 to 0.035 d^{-1} for measured and simulated transformation rates in soil as reported in Bayless (2001). The soil transformation rates are remarkably consistent.

Table 11: One-phase decay model for the sum of S-Triazine parent compounds relative to the total concentration of the parents and transformation products.

	NFSR Cootes Store	**Linville Cr Broadway**	**NFSR Timberville**	**NFSR New Market**
Best-fit values				
C_0 (ng L^{-1})	0.50 ± 0.06	0.53 ± 0.01	0.51 ± 0.01	0.49 ± 0.02
Plateau (ng L^{-1})	0.25 ± 0.03	0.31± 0.008	0.30 ± 0.008	0.31 ± 0.009
k (d^{-1})	0.038 ± 0.02	0.025 ± 0.004	0.027 ± 0.005	0.036 ± 0.009
Half Life (d)	18	28	26	19
R^2	0.64	0.97	0.96	0.92

C_0, initial concentration at t_0 on 29 June 2008
k, first-order decay rate constant
R^2, linear regression coefficient
NFSR, North Fork of the Shenandoah River.

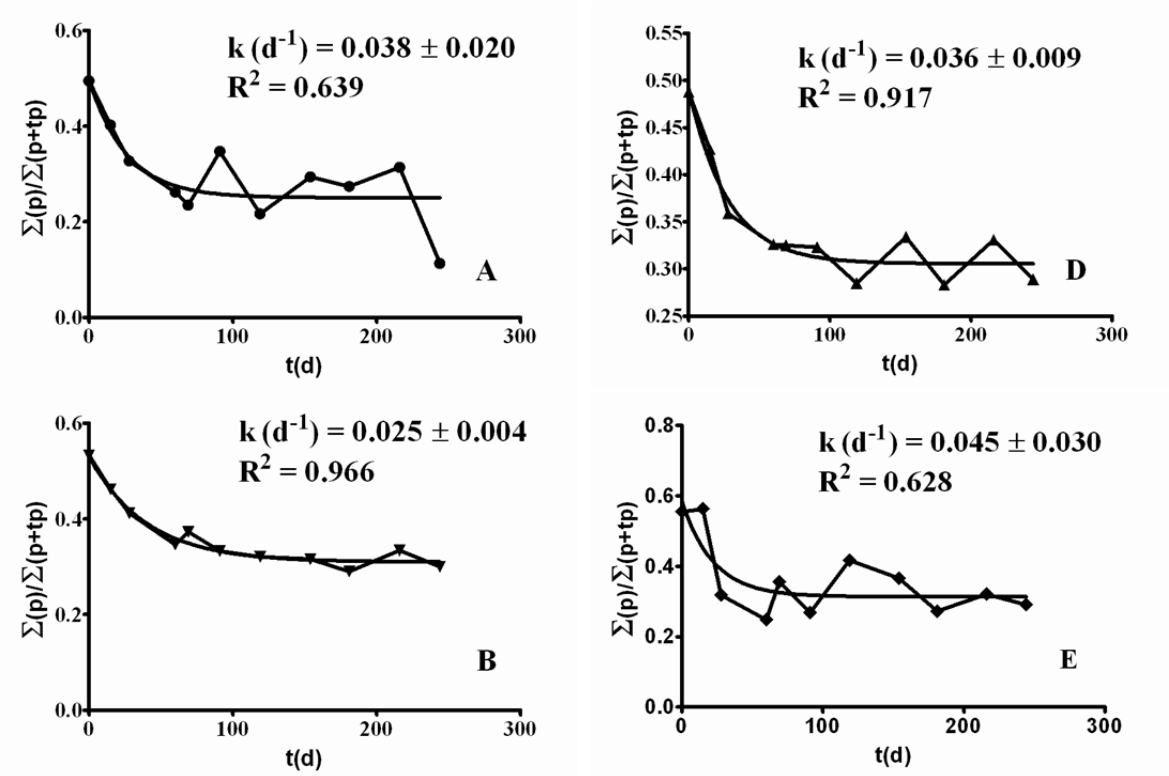


Figure 12: Transformation rates of S-triazine parent compounds (p) relative to the total concentration of parent compounds and transformation products (p + tp) are plotted against time. t_0 is the sample date (June 29, 2008) with the highest relative concentrations. Graph A is from the North Fork of the Shenandoah at Cootes Store, VA, USA, B is from Linville Creek at Broadway, C is from the North Fork of the Shenandoah River at Timberville, and D is from the North Fork of the Shenandoah at New Market

In addition to the transformation rates, the model also calculated an asymptotic plateau below which the fraction of parent concentrations (C_f) does not fall. The lowest C_f is 25% for the Cootes Store site where agricultural land use is only 8% of the drainage basin (Tab. 11). The asymptotic period during this study ran approximately 100 days. Given the length of time in days since the highest observed concentrations, it can be inferred by using this model that there is a continuous input source of non-transformed

atrazine, simazine and propazine, perhaps from groundwater discharge from soils zones where microbial transformation is metabolically limited.

Table 12: Removal rates and steady-state concentrations beginning at C_0 the initial concentration at t_0.

Best-fit values	NFSR Cootes Store	Linville Cr Broadway	NFSR Timberville	NFSR New Market
	S-Triazines and Transformation Products			
t_0	29 Jun 08	31 May 08	29 Jun 08	29 Jun 08
C_0 (ng L^{-1})	111 ±10	464 ±41	270 ± 19	285 ±18
Plateau (ng L^{-1})	6.9 ±5.9	51 ±27	40 ± 9	48 ±18
k (d^{-1})	0.023 ±0.006	0.016 ±0.004	0.036 ± 0.007	0.024 ±0.005
Half Life (d)	30.0	42.3	19.1	28.9
R^2	0.929	0.910	0.947	0.952
	Metolachlor and OA and ESA Transformation Products			
t_0	14 Jul 08	14 Jul 08	14 Jul 08	14 Jul 08
C_0 (ng L^{-1})	266 ±33	676 ±56	332 ±31	401 ±19
Plateau (ng L^{-1})	36 ±16	175 ±20	81 ±17	109 ±12
k (d^{-1})	0.039 ±0.015	0.22 ±0.16	0.030 ±0.010	0.026 ±0.005
Half Life (d)	18.0	6.09	23.0	26.2
R^2	0.866	0.910	0.893	0.966
	Alachlor and OA and ESA Transformation Products			
t_0	28 Sep 08	28 Sep 08	28 Sep 08	28 Sep 08
C_0 (ng L^{-1})	74 ± 2	252 ± 40	546 ±10	220 ± 5.8
Plateau (ng L^{-1})	2.0 ± 1.2	0.00	4.3 ± 5.8	13 ±3
k (d^{-1})	0.065 ±0.007	0.025 ± 0.014	0.053 ± 0.003	0.061 ± 0.005
Half Life (d)	10.7	27.6	13.1	11.4
R^2	0.996	0.905	0.999	0.997

k, first-order decay rate constant; NFSR, the North Fork of the Shenandoah River.

Dispersion Modeling and Steady-State Concentrations

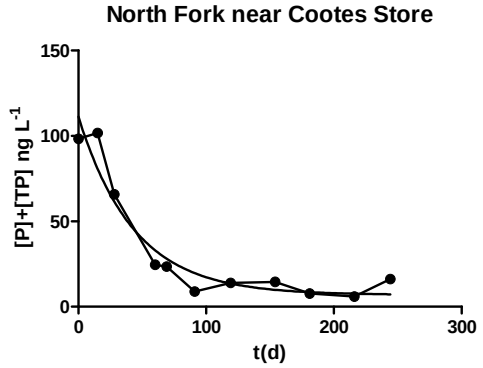
The sum of the parent herbicide and TP concentration decreased with respect to time over the sampling period. The highest concentrations were measured in the 28 June 2008 samples during the spring flush from the pre-emergence herbicide application. The best fit curve for this data was constructed plotting total concentration using a first-order exponential decay model generated by Prism 5 Curve-fitting software (Graphpad,Inc, La Jolla, CA, USA) (Fig. 13). The best fit data is represented in Table 4 for all S-triazines, metolachlor and alachlor. With the exception of one or two low-concentration occasions, acetochlor was not found in this study.

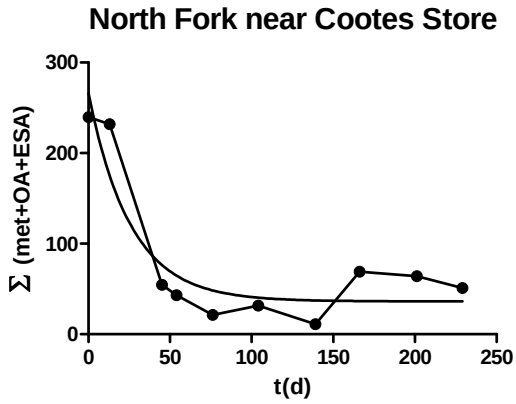
Since changes to the sum of the parent and TP concentrations account for all output processes, and both parents and TPs have relatively high water solubilities, the primary factor influencing the overall decrease in total concentration with respect to time arises through advection-dispersion associated with river flow. As the table shows, advection rates for the S-triazines averaged 0.032 ± 0.007 d^{-1} across the 5 study sites over the period. In the case of metolachlor, its TP dispersion rates ranged from a low of 0.026 to a high of 0.31 d^{-1}. The alachlor rates ranged from 0.19 to 0.050 d^{-1}.

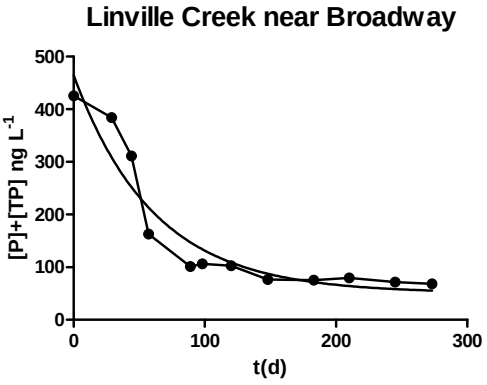
The air-water interface dispersion is minimal for these compounds having very low dimensionless Henry's law constants, generally less than $\sim 10^{-7}$. The high water solubilities of all the compounds studied with the possible exception of HA minimizes the impact of removal by adsorption and the settling of suspended particles. Pore water diffusion measurements were beyond the scope of this project. Therefore, it could be

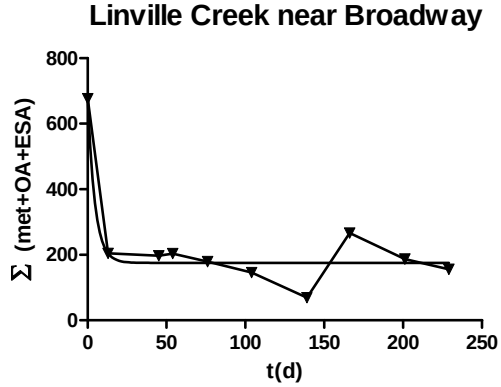
stated that for these compounds in this system, the primary removal process is longitudinal dispersion by river flow.

As was the case for transformation rates, there was an asymptotic plateau and the span from the plateau to the highest concentration was used in place of the initial









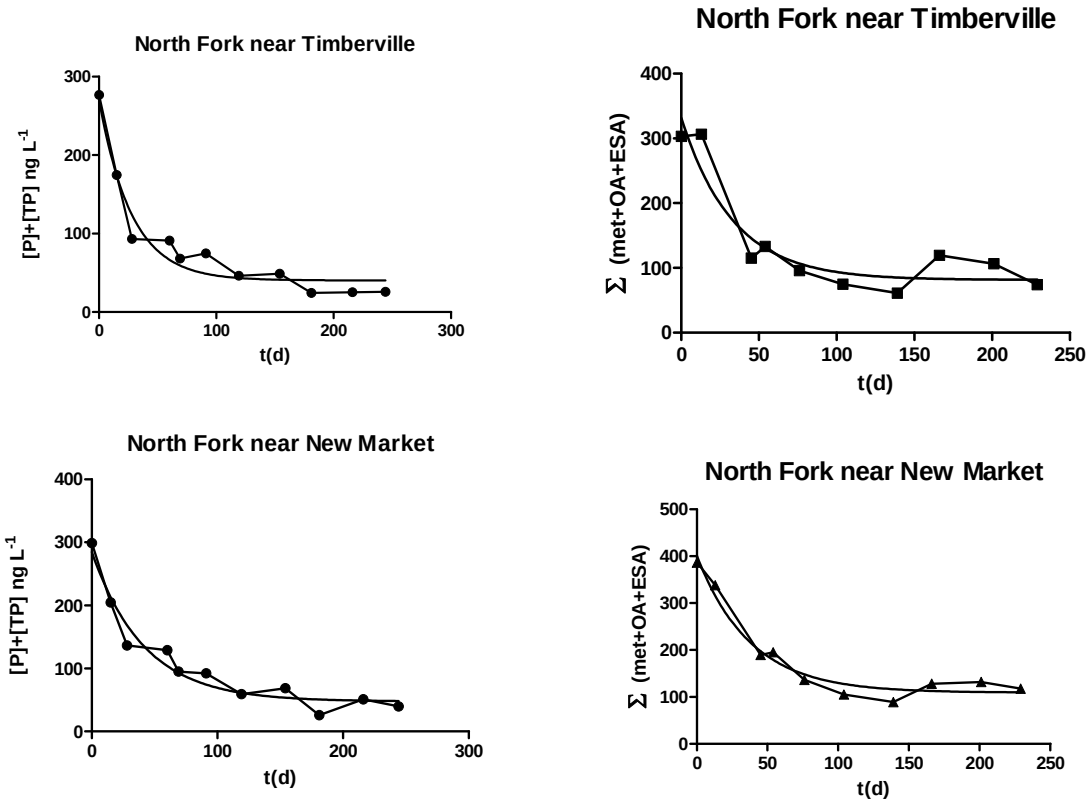


Figure 13: Removal rate plots for total parent compound [P] and transformation product (tp) concentrations versus time in days from t0. First order exponential decay for S-triazines are in the left column and metolachlor plots are in the right column.

concentration (C_0) in Equation 1. These plateau concentrations clearly indicate a steady-state condition where the concentration being lost to advection is equal to the input through surface water runoff and groundwater discharge into the river. The TP to parent concentration ratios in the previous section show that the surface runoff is a minor component of the steady-state concentration. As Table 12 shows, the steady-state concentration for S-triazines does not exceed 100 ng L^{-1} the maximum permissible contaminant levels for the European Commission countries (EU, 2003). However, the

steady state plateau levels do exceed this criterion in the Linville Creek and New Market sampling sites.

Conclusions

Plotting the concentrations of herbicides and their transformation products versus time clearly identified the spring flush for S-triazine herbicides and chloroacetanilides as occurring in late June and early July. The linear change in the desethylatrazine to atrazine ratio (DAR) from well below 1 to well above 2 clearly indicates that the input source of desethyl atrazine changes progressively from surface runoff to groundwater discharge. The predominant input source of these herbicides can be ascertained at any point throughout the year.

The transformation rates for atrazine compare well with those found in the Midwest region where atrazine is used extensively. This seems to indicate that the transformation rates are more dependent common processes such as the ubiquitous microbial communities that can evolve the ability to utilize carbon from atrazine after historical exposure.

The dispersion rates showed a rapid output of parent and transformation products which subsequently reached a steady-state concentration that was maintained for the remainder of the annual cycle. Clearly, the parent herbicides were depleted from the surface of the crop fields shortly after application, leaving a more stable groundwater

input. The asymptote of the curve drawn to the y-axis intercept represents a constant groundwater input. What's above is the surface runoff signal.

The central hypothesis of this study was that it is possible to characterize the major biogeochemical processes that affect the fate and transport of pre-emergent herbicides in the upper basin of the North Fork of the Shenandoah River by sampling surface water over a twelve-month period. This intensive sampling regimen provided a more-complete data set for calculating steady-state concentrations, transformation rates and transformation product-to-parent herbicide ratios. The yearly-cycle data elucidated the principle processes in this basin in a way that single or biannual sampling protocols could not.

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CURRICULUM VITAE

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