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Removal of Atrazine from Water Using Covalent Sequestration

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Monochlorotriazines including atrazine and its major metabolites, deethylatrazine and deisopropylatrazine, are susceptible to nucleophilic aromatic substitution. Competitive reactions to rank the relative reactivity of nucleophiles with atrazine reveal that constrained secondary amines are the most reactive. When the nucleophile is attached to a solid support, atrazine can be sequestered from solution. As proof of concept, polystyrene resins displaying constrained secondary amines are shown to sequester atrazine, deethylatrazine, and deisopropylatrazine from water. Sequestration can be followed spectrophotometrically or using a liquid chromatography mass spectrometry protocol. The kinetics of sequestration are similar to that of granulated charcoal. Evidence for covalent bond formation comes from control experiments with unreactive herbicides and degradation analysis of the solid support. Using both ^{1}H NMR spectroscopy and mass spectrometry, covalent adducts are identified in ratios close to what is calculated theoretically. This method for sequestration is effective at removing atrazine from pond water.

KEYWORDS: Remediation; herbicide; atrazine; covalent; sequestration; polymer

INTRODUCTION

The broadleaf herbicide atrazine is one of the most widely used herbicides in the United States for weed control during the production of corn, sorghum, and other crops (*1*). Although many European countries have banned its use, over 60 million pounds of atrazine are used annually in the United States (*2*). As a result, atrazine is the most commonly detected herbicide in ground and standing water. The EPA has set the drinking water limit at 3 ppb, but atrazine levels often rise far above this level, especially after herbicide application and during spring runoff (*3*). In recent studies, atrazine was found to exceed its 3 ppb maximum contaminant level (MCL) up to 100 days after application (*4*, *5*). Furthermore, concentrations of dealkylated metabolites of atrazine, deethylatrazine and deisopropylatrazine, often exceed the atrazine MCL for up to 50 days after atrazine application. Deethylatrazine was found to be the most predominant metabolite detected with an average concentration of 2.5 ppb during the year following atrazine application. Atrazine has been linked to health risks in animals and humankind (*6*).

Currently, the best available technology for removal of atrazine from groundwater is activated charcoal (*7*, *8*). However, activated charcoal lacks selectivity for pollutants and absorbs innocuous organic compounds. Natural organic matter (NOM) competes with atrazine for adsorption on charcoal causing displacement of the adsorbed atrazine. As a consequence, the adsorption capacity of charcoal decreases with time or NOM throughput. The amount of atrazine displaced by NOM depends on the type of charcoal. The rate of displacement is a function of the type of charcoal and carbon dose. This atrazine displacement effect could influence the optimal operation conditions of the charcoal and result in desorption of trace organic pollutants into the environment. As a result, numerous groups are investigating alternatives to charcoal.

Alternative methods for the removal of atrazine from groundwater include engineered organoclays (*10*, *11*), dialysis (*12*), molecularly imprinted polymers (*13*, *14*), engineered microorganisms (*15*), oxidation strategies (*16*−*18*), photodegradation using ultraviolet light (*18*, *19*), nano- or ultrafiltration (*20*, *21*), and polar polymeric supports (*22*). The use of reactive solid supports for the selective removal of atrazine from water has not been reported. Reactive resins have been successfully applied to the efficient removal of electrophilic (*23*−*25*) and nucleophilic (*26*−*28*) compounds from solutions. However, applications have been limited mainly to organic and combinatorial synthesis. Here, we describe a strategy for the sequestration of atrazine and its major metabolites. The strategy is presumably generalizable across many of the triazine herbicides, as well as other select herbicides such as metolachlor (*vide infra*). The mechanistic basis for this strategy is the electrophilicity of monochlorotriazines (eq 1). Under ambient conditions, substitution of the chlorine atom occurs in water with select nucleophiles (*29*).

This paper describes the selection of nucleophilic groups for atrazine sequestration using solution phase competition reactions; the use of these groups on a solid support for the sequestration of atrazine, its metabolites, and another triazine herbicide; and the evidence for covalent attachment. The

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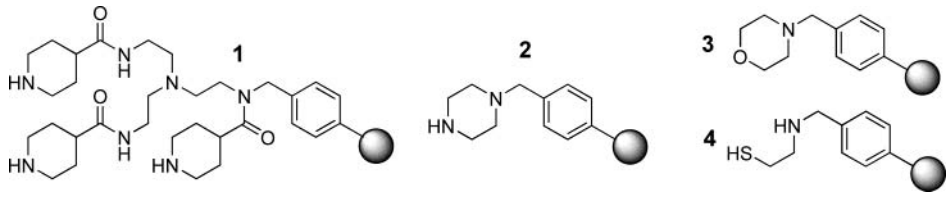
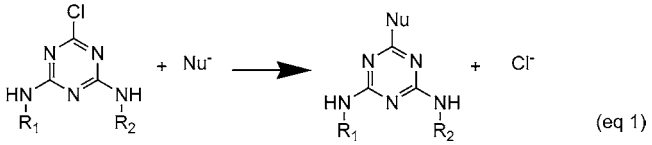
Chart 1. Resins Used in This Study



manuscript concludes with a discussion of our future efforts directed, in part, at turning this environmental science into useful technology.



(eq 1)

Atrazine	R_1 = ethyl;	R_2 = isopropyl
Deethylatrazine	R_1 = H;	R_2 = isopropyl
Deisopropylatrazine	R_1 = ethyl;	R_2 = H

MATERIALS AND METHODS

Concentrations Used for These Experiments. The experiments described were performed over a broad concentration regime. Low concentration experiments (<120 ppb) were performed by atmospheric pressure chemical ionization (APCI) using a Thermofinnigan LC Q Deca mass spectrometer. For more data intensive exercises including kinetic and mechanistic analyses, a higher concentration regime (12−30 ppm) was employed to allow for spectrophotometric monitoring of the process.

Competition Reactions of Piperidine, *N*-Methylpiperazine, *N*-Methylmorpholine, and Ethanethiol with Atrazine. To a 0.1 M solution of atrazine (0.2 g, 0.9 mmol) in tetrahydrofuran was added a solution containing a 3-fold excess of each of the following: piperidine (0.24 g, 2.8 mmol), *N*-methylpiperazine (0.28 g, 2.8 mmol), *N*-methylmorpholine (0.28 g, 2.8 mmol), and ethanethiol (0.21 mL, 2.8 mmol). The solution was stirred at room temperature for 24 h. Thin-layer chromatography (TLC) showed the absence of atrazine in the solution and the presence of two new UV active spots. The solvent was removed, and the crude mixture was analyzed by electrospray ionization mass spectrometry (ESI-MS) and showed ions corresponding to the addition of piperidine and *N*-methylpiperazine to atrazine. No ions corresponding to the mass of the other two possible adducts of atrazine with ethanethiol or *N*-methylmorpholine were observed. The two UV active spots were isolated and corresponded to the addition of piperidine and *N*-methylpiperazine to atrazine in a molar ratio of 1.5 to 1, respectively.

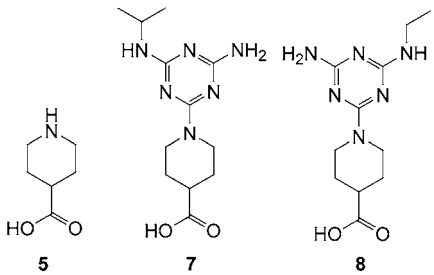
Sequestration of Atrazine from Aqueous Solution Containing 100 ppb Atrazine. Thirty milligrams of each resin (**1**−**4**) was added to individual 5 mL fritted syringes containing 3 mL of 100 ppb aqueous solutions of atrazine. The syringes were capped and placed on a wrist action shaker for 24 h. The solutions were collected, leaving the resin on the frit of the syringe, and analyzed by liquid chromatography (LC)-MS. To quantify the amount of atrazine remaining in solution, a 20 μL solution of analyte was injected onto a Waters XTerra MS C18 2.0 mm × 150 mm column and eluted with a 60:40 water:methanol gradient. Total ion count (area under the curve) was determined upon APCI using a Thermofinnigan LC Q Deca mass spectrometer. These areas were compared to those obtained from a predetermined calibration curve. Each study was reproduced in triplicate.

Analysis of the Kinetics of Sequestration. Ten 10 mL fritted syringes were loaded with 50 mg of resin **1**. Each syringe was filled with 5 mL of a 12 ppm solution of atrazine and placed on a wrist action shaker. One sample was removed and analyzed every half hour for the first 2 h. Subsequently, the samples were removed from the shaker every 2 h for the next 6 h. The last two samples were removed after 12 and 24 h of incubation, respectively. The amount of atrazine remaining in solution after removal of the resin was measured using UV−vis absorption at 221 nm and compared with a calibration curve.

Identification and Quantification of Covalent Herbicide Adducts by Acid Hydrolysis. After sequestration reactions with metribuzin, metolachlor, cyanazine, and atrazine (performed as above), the resins were isolated and washed with methanol and dichloromethane, and then subjected to acid hydrolysis. For the experiment, 40 mg of resin was heated at 100 °C with 1 mL of 1 N HCl for 6 h. The resin was separated from the solution by filtering through a fritted syringe, and the solution was collected in a 1.5 mL centrifuge tube. The solvent was removed by a centrifuge solvent evaporating system to give a white residue. The residue was resuspended in 1 mL of distilled water, and a 20 μL aliquot was diluted into 1 mL of water. After addition of the resulting solution to an autosample vial, an additional 0.8 mL of distilled water was added to the vial. Each sample was analyzed by LC-MS. The areas of the peaks corresponding to the 4-piperidinecarboxylic acid, **5**, and the covalent adduct with atrazine **6** were compared to a standard curve of various ratios of **5** and **6** (see Figure 5). Covalent adducts were identified from the residue obtained from acid hydrolysis of resin **1** incubated with cyanazine and metolachlor, but no peak corresponding to a covalent adduct of **5** with metribuzin could be identified.

NMR Identification and Quantification of Covalent Atrazine. To a 1 L aqueous solution of 30 ppm atrazine, 1 g of resin **1** was added. The plastic container was placed horizontal on a platform shaker for 72 h. The resin was separated from the solution by filtering through a sintered glass funnel. After the first ∼100 mL was filtered, three successive 100 mL fractions were collected to determine the concentration of atrazine left in solution. After the entire 1 L was filtered, the resin was washed with methanol and dichloromethane. The resulting organic solvent from the wash was removed, and the resulting residue was dried under vacuum. The residue was redissolved in 50 mL of water to determine the amount of atrazine extracted from the resin. The resin was dried and then subjected to acid hydrolysis with 1 N HCl for 5 h at 100 °C. The solvent was removed under vacuum, and the residue was dried under vacuum. An ESI-MS was obtained from the residue as well as ^{1}H NMR. The collected spectra were compared to the spectra of synthetic standards.

Hydrolysis of Resin Used in Sequestration of Deethyl and Deisopropyl Atrazine. Each 500 mg portion of resin used in the sequestration experiments of 50 mL of 12 ppm deethyl atrazine or deisopropyl atrazine was washed with several portions of MeOH, dichloromethane, and water and then individually subjected to acid hydrolysis with 10 mL of 1 N HCl for 5 h. The resin was separated from the solution by filtration, and the solvent was removed. The residue was dried under vacuum and then suspended in water for ESI-MS analysis. Peaks corresponding to 4-piperidine carboxylic acid, **5**, and adducts with deethyl atrazine, **7**, and deisopropyl atrazine, **8**, were observed by positive mode time of flight ESI-MS.



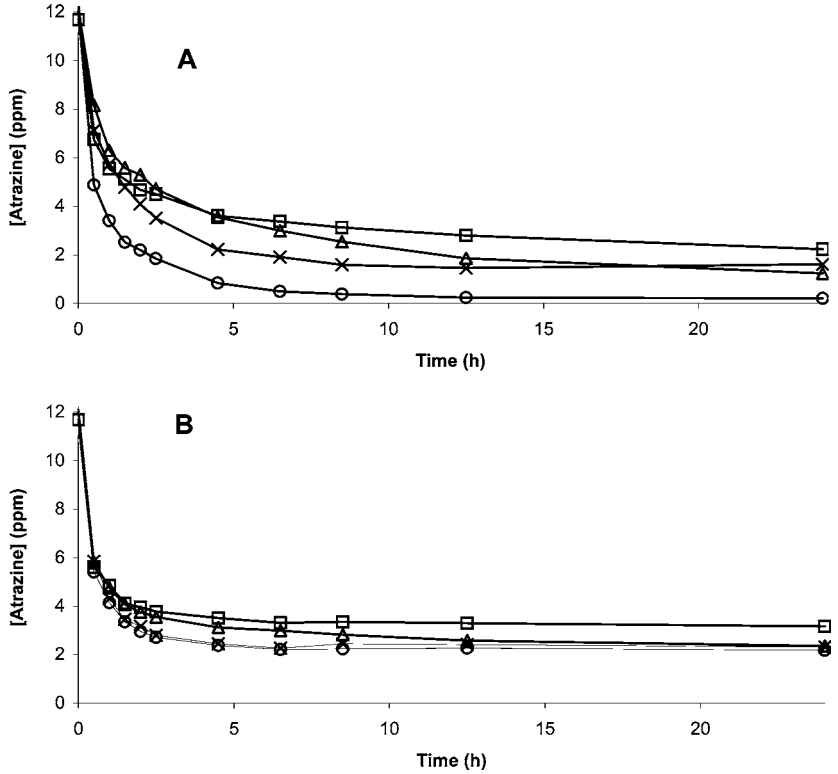
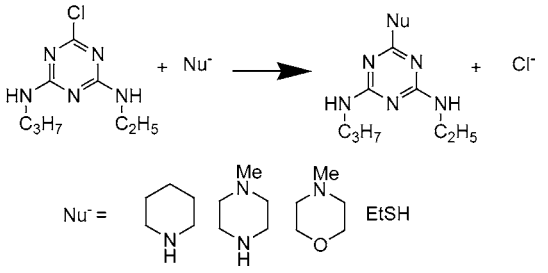


Figure 1. Kinetics of atrazine sequestration at pH 3 (□), 7 (△), and 11 (×) using (**A**) a 10 mM phosphate and (**B**) the same conditions with 0.5 M NaCl. Data at pH 7 (○) without buffer. The error bars are not shown for clarity. On the basis of triplicate analysis, errors are approximated by the size of the symbols used to represent the data.

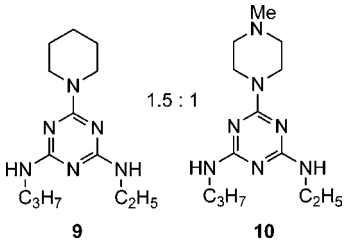
RESULTS AND DISCUSSIONS

Identification of the Nucleophile of Choice. Our experience with dendrimers based on melamine suggested that amine nucleophiles would react efficiently with atrazine and other triazine herbicides (*29*). To make the search for the appropriate nucleophile more efficient, a competition reaction wherein the nucleophiles were present in excess was performed in which four nucleophiles were allowed to react with atrazine (eq 2).



The reaction mixture was characterized by ESI-MS. Only two products were observed corresponding to reaction of piperidine and *N*-methylpiperazine. No products corresponding to substitution with *N*-methylmorpholine or ethanethiol were detected by MS or by TLC. To quantify the difference in nucleophilicity of the piperidine and piperazine groups, the products were isolated by conventional silica gel chromatography and weighed. The piperidine adduct, **9**, and the *N*-methylpiperazine adduct, **10**, appear in a 1.5:1 molar ratio. We attribute the difference in reactivity between the piperidine and the piperazine rings to the electron-withdrawing effect that the second nitrogen of the piperazine exerts.

Nucleophiles on Resins. Four polystyrene resins (**1**−**4**) displaying the same nucleophilic groups were evaluated for their ability to remove atrazine from aqueous solutions (**Chart 1**).[30]



9 **10**

Each resin (10 mg/mL) was incubated with a 100 ppb atrazine solution for 24 h. Resin **1** was significantly more efficient than **2**−**4** at sequestering atrazine by removing greater than 98% of the herbicide. Resin **2** removes only 12%. Resins **3** and **4**, lacking groups that will react with atrazine, removed less than 1% of the total atrazine in solution.

Sequestration using **1** is relatively independent of pH and ionic strength. **Figure 1A** shows that atrazine is removed from 12 ppm solutions buffered with 10 mmol of phosphate at pH 3, 7, and 11 (81, 90, and 87%, respectively) at similar rates. **Figure 1B** shows that at high salt concentration, atrazine sequestration is reduced by an order of magnitude as compared to distilled water but by only a nominal extent in buffered samples. The kinetics for removal of deethylatrazine and deisopropylatrazine from water buffered at pH 7 are identical to that of atrazine within experimental error based on triplicate analysis.

Selectivity. Resin **1** is selective for the triazine herbicides. Solutions containing 12 ppm of atrazine, cyanazine, metolachlor, and metribuzin were incubated with **1** (10 mg/mL) for 24 h in unbuffered water. Both atrazine and the triazine herbicide cyanazine were efficiently sequestered (>96%). Approximately 75% of metolachlor, a molecule that presents a weakly reactive α-chloroacetamide group, was sequestered by the resin. Metribuzin, a molecule devoid of electrophilic groups, was only partly sequestered (25%) from solution. After sequestration, the

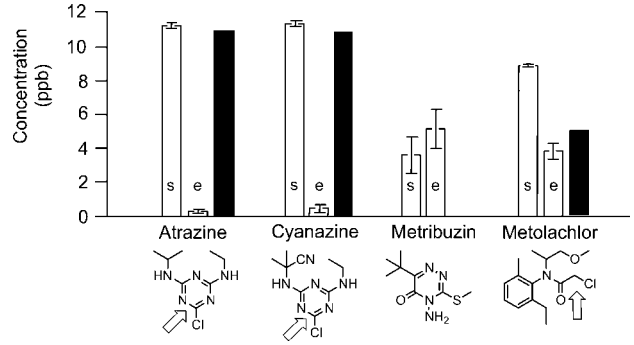


Figure 2. Amount of herbicide sequestered (s), extracted from the resin upon washing (e), and attributed to covalent attachment (black). Arrows indicate the reactive sites.

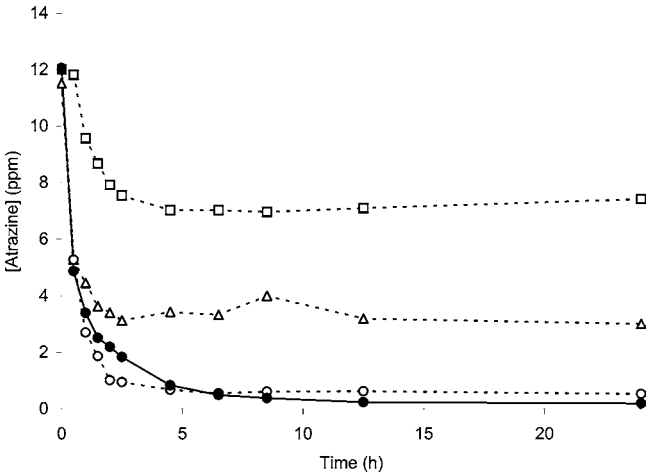


Figure 3. Kinetics of sequestration of metribuzin (□), metolachlor (△), cyanazine (○), and atrazine (●) by 50 mg of resin **1** using 5 mL solutions of the pesticides.

resins were rinsed with methanol and dichloromethane in order to extract any adsorbed herbicide (**Figure 2**). Only small amounts of atrazine and cyanazine eluted (~3%). Approximately 50% of the sequestered metalochlor was extracted, and all of the metribuzin was removed from the resin. In corroboration of covalent sequestration, adducts of cyanazine and metalochlor with 4-piperidinecarboxylic acid were observed by mass spectrometry. The kinetics of sequestration are shown in **Figure 3**. The two triazines are sequestered at similar rates, while metolachlor and metribuzin are sequestered more slowly. This trend is consistent with the relative solubilities and reactivities of the molecules.

In Field Use. In the field use of this strategy requires that the observed reactivity be maintained in standing water samples. To this end, **1** was evaluated for its ability to remove atrazine from pond water. After the pond water was passed through filter paper to remove particulates, the filtrate was spiked with 120 ppb of atrazine, a concentration observed in agricultural runoff following application. At both 10 and 5 mg/mL of **1** in pond water, the atrazine concentration was decreased to undetectable levels (<1 ppb) in 24 h. Using 1 mg/mL of **1**, the atrazine concentration is reduced to less than the limit of quantification, 1.2 ppb, in 24 h.

Direct comparison with charcoal supports this belief: at 5 days using equivalent weights (0.05 mg/mL), powdered charcoal and 400 mesh granulated charcoal sequester >10 ppm of a 12 ppm solution of atrazine, while resin **1** and 300 mesh charcoal (Calgon, used in plant settings) each sequester 4 and 5 ppm, respectively (**Figure 4**).

Evidence for Covalent Sequestration. Resin **1** forms a covalent bond with sequestered atrazine. After **1** (1 mg/mL) was incubated with a solution of 30 ppm atrazine and the resin was washed, acid hydrolysis releases 4-piperidinecarboxylic acid, **5**, and the corresponding covalent adduct with atrazine, **6** (**Figure 5**). Using synthetic standards to verify assignment, the relative amounts of **5**:**6** were quantified by ^{1}H NMR (40:1) and MS (70:1) after correcting for the relative ionization efficiencies of each compound. These values are consistent with the amount of atrazine sequestered from solution and the reactive equivalents per gram (18:1) of resin used. Covalent adducts of deethylatrazine and deisopropylatrazine, **7**, with piperidine 4-carboxylic acid, **8**, were also observed by MS.

CONCLUSIONS

Solid-supported nucleophiles (as represented by **1**) can remove atrazine, its metabolites, and other triazine herbicides from water. The superior performance of **1** over **2**−**4** is consistent with solution phase reactivity of the nucleophiles with atrazine. The sequestration of atrazine is practically unaltered by changes in pH and ionic strength. Additionally, the presence of NOM does not preclude the removal of atrazine from the pond water. Degradation analysis of the resin supports the hypothesis of covalent sequestration.

This strategy has potential for field applications. When compared with activated carbon, the proof of concept resin is competitive in terms of sequestration potential. This observation is more amazing upon consideration that there has been no optimization of the solid support to maximize the number of reactive groups. By increasing the density and accessibility of

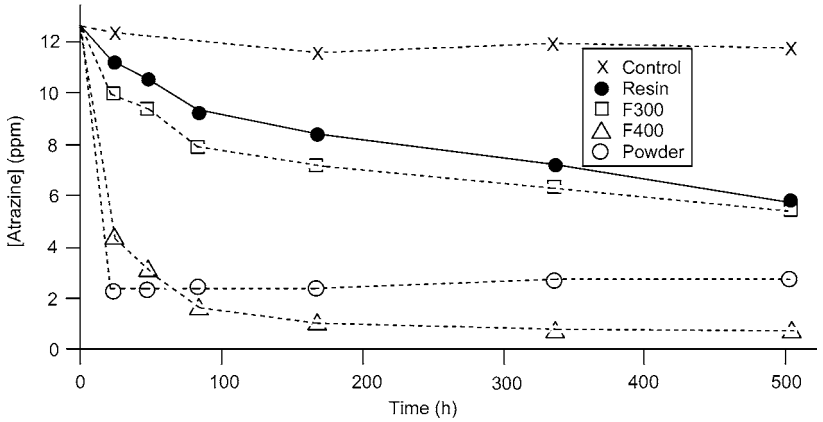


Figure 4. Comparison of **1** with charcoal from Calgon Corporation at three different mesh sizes (F300, F400, and powdered). Error bars from triplicate analysis are omitted for clarity as they rarely exceed the size of symbol used to designate the data point.

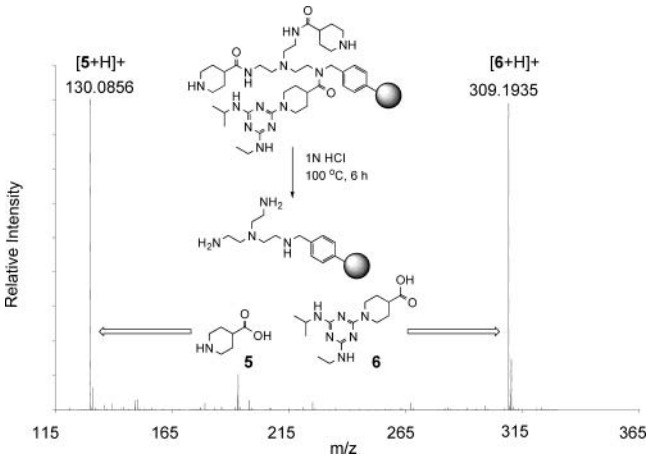


Figure 5. Mass spectrum of the hydrolysis cocktail.

piperazine groups, issues of scale can be addressed. That is, the current embodiment of the technology would allow 1 g of resin to sequester 0.5 g of atrazine (or all of the atrazine in 5000 L of water at 100 ppb levels) if all reactive groups are available.[31] Other supports with significantly higher surfaces areas derivatized with reactive secondary amines including clays, silica gels, and mesoporous silicas could further reduce the amount of material needed. Installing these nucleophilic groups on cotton fabrics or other natural fibers such as jute or burlap allows us to couple this inexpensive chemistry and technology to erosion control. Finding new materials is the object of current interest of many groups to support their proposed strategies (*32*−*34*). We recognize that regeneration of these supports is not possible in the current scheme. While incineration remains a possibility, the high selectivity and loading may not preclude this strategy from application given this limitation. The advantages of this strategy merit its continued investigation. The materials are relatively inexpensive, and covalent sequestration is selective for electrophilic herbicides including the triazines and to a lesser extent, metolachlor. In due course, we hope to establish this novel, environmentally aimed approach as useful technology.

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