Analytical Methodology to Monitor the Environmental Fate of Atrazine and Cyromazine

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Introduction

Atrazine is an herbicide used globally. There is compelling evidence that atrazine is linked to deleterious effects in amphibians. One study demonstrated that upon exposure to atrazine, genetically male frogs became hermaphrodites. Male tadpoles lost the ability to develop masculine characteristics and feminization was observed to the point of developing viable ovaries. The catabolism of atrazine by microbial flora yields cyanuric acid which, if in the presence of melamine, yields insoluble and highly toxic melamine cyanurate. Cyromazine, a common insecticide, is metabolized by mammals to melamine. It is not unreasonable to consider coeval application of atrazine and cyromazine in farming communities. Herein is presented a sensitive and selective method to monitor these compounds and their metabolites in soil extract.

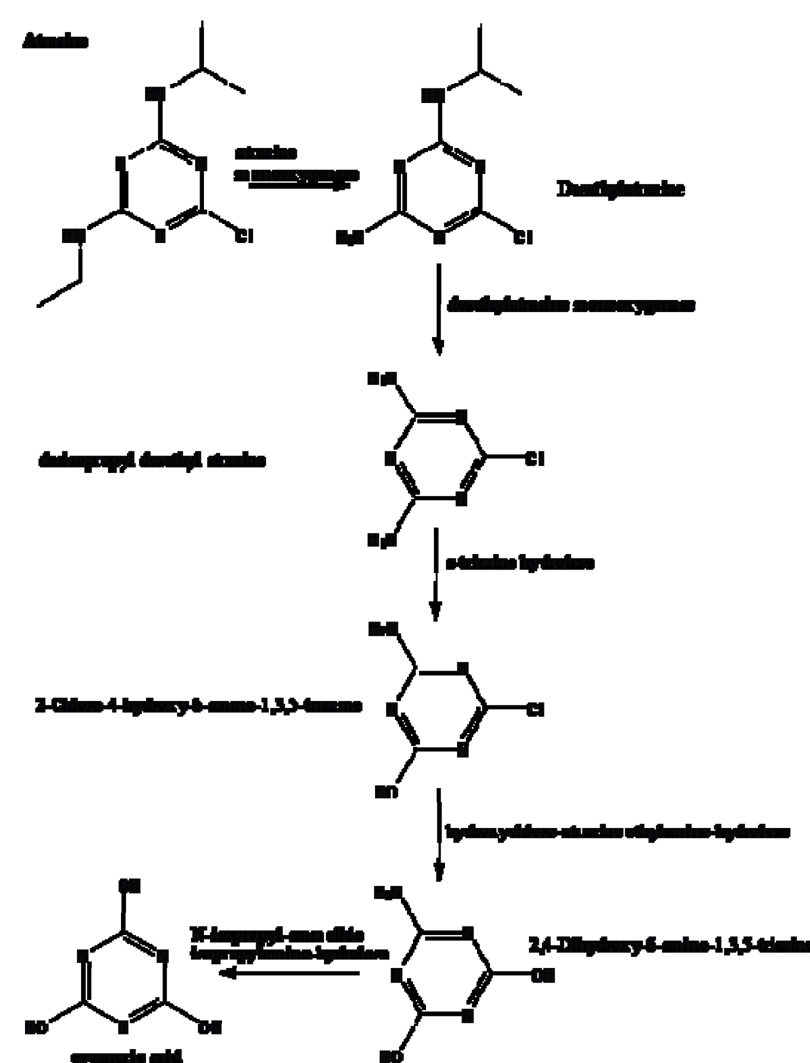
Atrazine Pathway

Microbial catabolism of atrazine has been found to occur via several pathways. Monitoring the first metabolite in the pathway may offer insight into the particular species involved. We have chosen to monitor the pathway with desethylatrazine as the first metabolite.

Microbes and first metabolites

Compound	Microbes	First Metabolic Product
Atrazine	Pseudomonas sp. ADP, Ralstonia sp. M91-3, Clavibacter sp., Agrobacterium sp. J14a	Hydroxyatrazine
Atrazine	Rhodococcus spp., NI86/21, TE1, Pseudomonas spp. 192/194, Streptomyces sp. PS1/5	Desisopropylatrazine
Atrazine	Rhodococcus spp. NI86/21, TE1, Nocardia sp., Alcaligenes sp. SG1, Streptomycessp. PS1/5	Desethylatrazine

Atrazine microbial metabolic pathway



Experimental

Atrazine, desethylatrazine, cyanuric acid, cyromazine, and melamine were prepared at a stock concentration of 1 mg/mL and spiked into extracted soil at 2 pbb through 100 pbb for analysis. Derivatization of the analytes was performed by adding 100 µl 49:49:1 BSTFA/1% TMCS:anhydrous ethyl acetate:anhydrous pyridine to the dried spiked matrix extract and heating at 70 C for 30 minutes. The GC was configured in a sequential, two-column fashion using a purged union to connect them. The purpose of this column configuration is to facilitate backflush of high boilers and heavy matrix and provide clean, reproducible chromatography and spectra.

Experimental

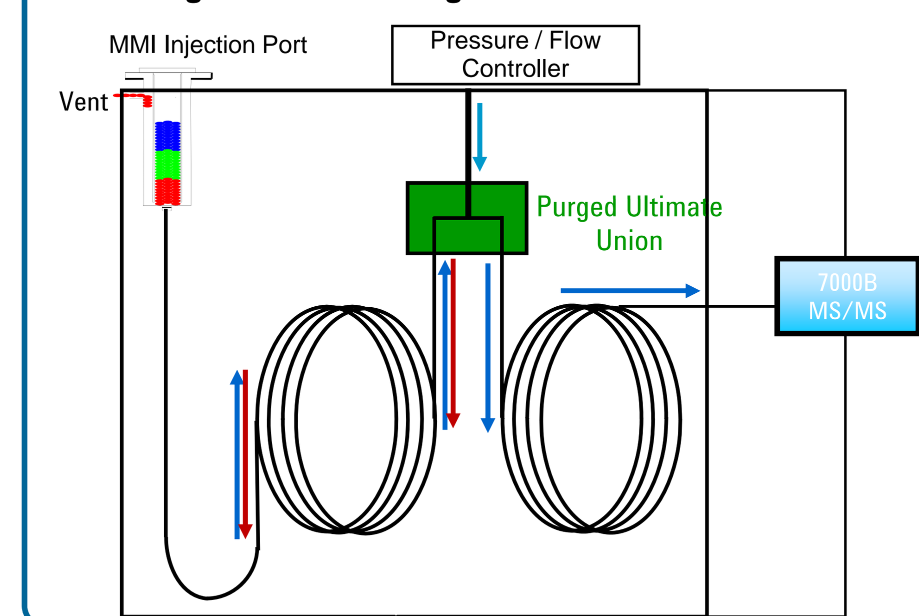
GC-MS/MS Conditions

Oven Program
- 65 °C for 1 min
- then 10 °C/min to 230 °C for 0 min
- then 30 °C/min to 300 °C for 2 min

Injection Volume 1 µL, pulsed splitless
Thermal Aux 280 °C
Column flow 1.2 mL/min
Source 280 °C
EI MS/MS Mode: MRM Transitions

Name	Precursor	Product	Collision Energy
Cyanuric Acid	345	330	10
Cyanuric Acid	345	215	8
Cyanuric Acid	345	188	12
Desethylatrazine	259	244	10
Desethylatrazine	259	217	10
Atrazine	215	200	10
Atrazine	200	104	20
Melamine	342	327	10
Melamine	342	285	20
Melamine	327	171	17
Cyromazine	310	309	10
Cyromazine	310	295	10

GC configuration showing back flush flows

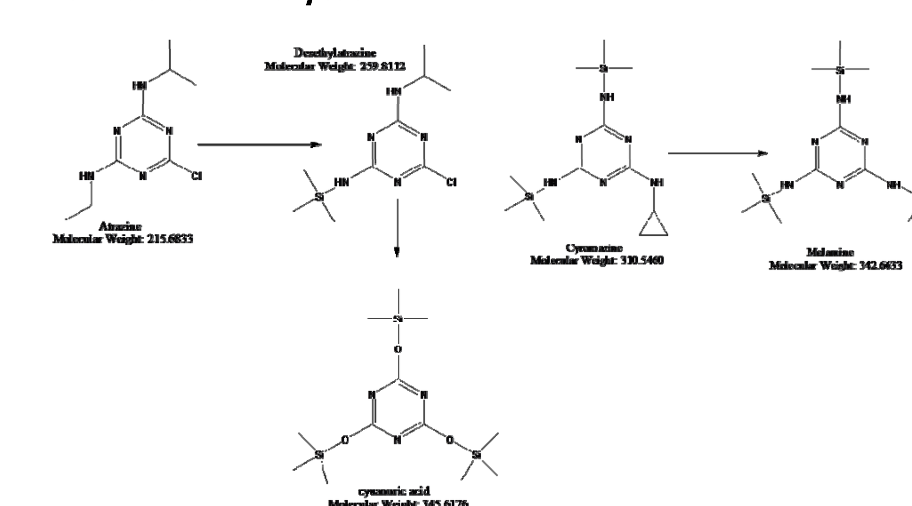


Blue arrows = analysis mode

Red arrows = backflush mode

Experimental

Derivatized Analytes

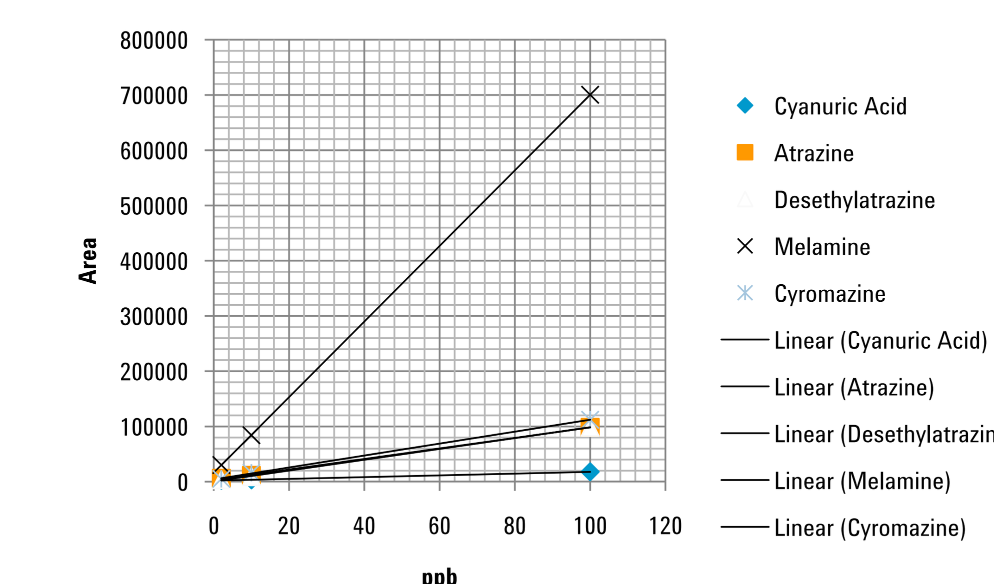


Results and Discussion

Calibration from 2.0 ppb to 100.0 ppb

Excellent signal to noise for all compounds at 2.0 ppb

Analyte	Retention Order	Peak to Peak S/N at 2 pb in Matrix	r^2 for cal curve
Cyanuric Acid	1	42:1	0.994
Atrazine	2	70:1	0.996
Desethylatrazine	3	4:1	0.999
Melamine	4	>1000:1	0.995
Cyromazine	5	39:1	0.999



Calibration Curves show for all analytes.

Results and Discussion

In 2009, soils from the Pacific Northwestern part of the United States was extracted for the purposes of pesticide analysis via GC tandem mass spectrometry. The soil was spiked with fifteen common pesticides. MS scan with AMDIS deconvolution and NIST08 library searching elicited the presence of 20 other possible compounds.

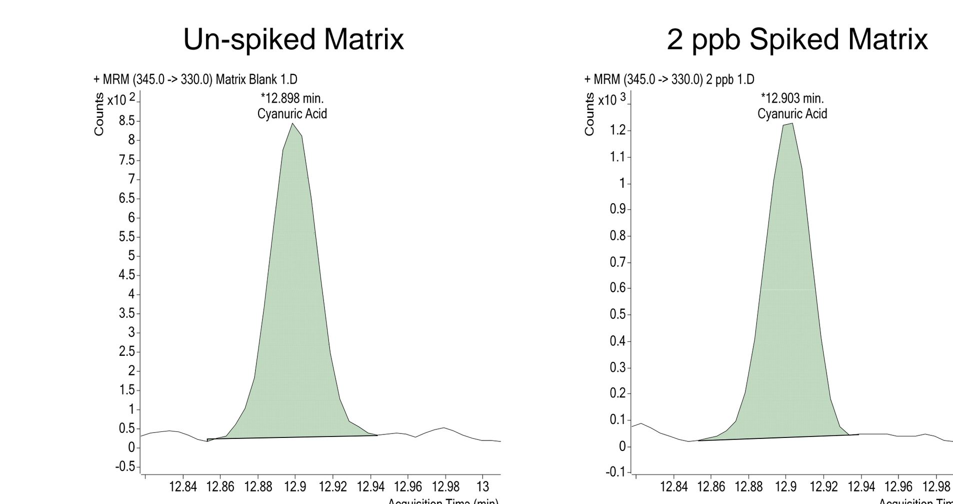
Unknowns and Targeted analysis of soil matrix

Azinphos methyl	>90		p,p DDD	>90	Malathion		Imiprothrin
Benzophenone	76		Permethrin	>90	Chlorpyrifos		p,p'-DDT
Chlorthalonil	>90		Phenanthrene-D10	76	Allethrin		Piperonyl butoxide
Cyhalothrin	>90		Phenol, 2-methyl-	72	o,p'-DDE		Cypermethrin I
Eugenol	70		phorate	>90	Dieldrin		Cypermethrin II
folpet	>90		squalene	83	o,p'-DDD		Cypermethrin III
gamma-tocopherol	55		Terbucarb	>90	Endosulfan II		Cypermethrin IV
hexachlorobenzene	>90		triphenyl phosphate	>90	o,p'-DDT		Deltamethrin
Metalaxyl	>90		Vitamin E	93			

Since none of the analytes in this study were expected to be present in the extracted soild matrix, the authors used it for this study. The data shown below gives a comparison of an un-spiked soil matrix blank which unexpectedly elicited the presence of cyanuric acid to a 2pp matrix spiked calibrator. Ion ratios were well within expected values and the corrected concentration of cyanuric acid in the un-spiked soil blank is approximately 2 ppb.

Matrix Blank actually contained Cyanuric Acid!

Sample			Method		Cyanuric Acid			(345.0 -> 188.0)	(345.0 -> 215.0)
Name	Type	Level	Exp. Conc.	Units	RT	Resp.	Final Conc.	Ratio	Ratio
Blank 1	Blank			ppb					
Matrix Blank 1	Sample			ppb	12.898	1487	1.25	35.61	45.43
2 ppb 1	Cal	1	2	ppb	12.903	2110	1.92	34.41	51.34



With the exception of cyanuric acid, no interference from the other compounds present in the matrix was observed and and all of the monitored analytes gave a linear curve fit coefficient (R2) greater than 0.994. This simple but elegant method demonstrates the feasibility for the detection of toxic atrazine metabolites in the environment. This method further shows the ability to monitor other metabolites in the environment, especially near irrigation and water run off areas that can feed back into the food chain.

Conclusions

The bioavailability of these compounds and their deleterious effects on various species are unknown. However, the propensity of atrazine and cyromazine and their microbial metabolic end products being present in the environment, especially in or near rural watersheds is high. As we know, cyanuric acid and melamine, the metabolites of atrazine and cyromazine, respectively form toxic, insoluble melamine cyanurate under aqueous conditions. The method design presented herein provides a tool for monitoring these compounds and possibly providing answers to the effects of aquatic species exposure to these compounds. The application lends itself to the analysis of fish and amphibian or any water based fauna tissue or bio-fluid. Detection limits of 2 ppb or less were determined for all analytes spiked into the soil matrix. Mid-column backflush also permits excellent reproducibility in terms of retention times and spectral accuracy.

Bibliography

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