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Determination of Atrazine Residues in Water and Soil by Enzyme Immunoassay

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Atrazine, a triazine herbicide, is the second most widely used pesticide in the United States with approximately 79 million pounds of active ingredient being applied each year (Anonymous 1987) as a pre- or post-emergent weed-control agent in the U.S. for corn, sorghum, sugarcane and pineapple. Because of its extensive use, possible seasonal stability and inadequate toxicological data (Wilson et al. 1987), there is a need for monitoring atrazine in water and soil. Municipal and private water sources should be monitored periodically in areas of frequent atrazine use. In soil, atrazine carry over may be injurious to certain rotational crops and thus the soil should be analyzed before planting (Ferris and Haigh 1987).

Methods presently available to measure atrazine residues in water and/or soil are colorimetry (Vickery et al. 1980), gas chromatography (Sirons et al. 1973; Lee and Chau 1983), thin layer chromatography (Sherma 1986), high-performance liquid chromatography (HPLC) (Vickery et al. 1980; Vermeulen et al. 1982; Ferris and Haigh 1987; DiCorcia et al. 1987) and immunoassay (Dunbar et al. 1985; Huber 1985). Of these methods, immunoassay has the advantages of being more sensitive, quicker and less expensive. Thus far, there has been one immunoassay procedure reported for atrazine and that was for water. The other reference (Dunbar et al. 1985) was a patent obtained by Colorado State for an atrazine antibody developed there to be used for the specific detection and quantification of atrazine.

This paper describes an immunoassay for determining atrazine in water and soil which can be adapted to field testing. The method is excellent for screening with fairly good accuracy when compared to HPLC. Because of the cross-reactivity of the antibody, this immunoassay test is nonspecific and several other triazine herbicides will also react.

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MATERIALS AND METHODS

Atrazine and all other triazine pesticides were obtained from the EPA and Ciba-Geigy. Stock solutions of each pesticide were prepared by weighing 20 mg of each into a 100-mL volumetric flask and bringing to volume with methanol. Intermediate standard solutions were obtained by pipetting 0.1 mL of stock solution into a 10-mL volumetric flask and using water to bring to volume. Actual working standards were prepared by removing 2.5, 5, 10, 25, 50, 125, 250 and 2500-uL aliquots from the intermediate standard solutions and putting each aliquot into a separate 5-mL volumetric flask and bringing to volume with water. This gave working standards of 1, 2, 4, 10, 20, 50, 100 and 1000 ppb which were used to test linearity and cross-reactivity. Buffer used was comprised of 12 mL of methanol, 88 mL of 0.02 M ammonium acetate in pH 7.2 phosphate buffer and 100 mL of water. The phosphate buffer was prepared by adding 28 mL of 0.2 M monobasic sodium phosphate (anhydrous) and 72 mL of 0.2 M dibasic sodium phosphate (anhydrous) to a 200-mL volumetric flask and bringing to volume with water.

Atrazine antiserum was prepared by derivatizing atrazine at the 2-chloro position and covalently conjugating it to bovine gamma globulin through a modified carbodiimide crosslinking procedure. The final molar ratio of hapten to globulin was 30:1. Antiserum was prepared in rabbits by multiple sub-cutaneous injections over several months. Blood was collected from the rabbits on a monthly schedule and the serum separated and stored frozen at -10°C.

Antibodies to atrazine were coated to the walls of polystyrene test tubes by a proprietary method developed by ImmunoSystems. Shelf-life of the dried and stabilized antibody-coated tubes was greater than one year. Horseradish peroxidase was covalently bound to atrazine (the "enzyme conjugate") also by a modified carbodiimide conjugation technique and is stable in liquid for over one year at 4°C. The substrate and chromogen were stabilized, buffer preparations of hydrogen peroxide and tetramethylbenzidine (TMB), respectively.

Analysis of water by immunoassay was performed by adding 100 uL of the water sample to one of the test tubes followed by 160 uL of atrazine "enzyme conjugate". The mixture was allowed to incubate 5 min at room temperature before rinsing (4 times) the unreacted mixture away with water. Substrate (160 uL hydrogen peroxide) was added, followed by 160 uL of chromogen (TMB). After 5 min of incubation the reaction was stopped with 1 drop of 2.5N sulfuric acid. The amount of yellow color was measured by reading the difference in optical density (ΔOD) between the control and each sample at 450 nm with a hand-held battery-powered differential photometer from Artel, Inc. As many as 7 samples plus a control can be run at once without losing accuracy. A control sample (no atrazine present) must be run with each set of tubes since it is used to determine the background value.

Soil analysis by immunoassay uses the same procedure with the exception of an additional extraction step. Water or a mixture of water/organic solvent (90 % acetonitrile/10% water; Vickery et al. 1980) was used to extract atrazine from soil that had been passed through a 30-mesh sieve. The soil was not dried before extraction. Five grams of soil were extracted with 10 mL of water or solvent either by shaking or sonicating for 5 min. If water is used then the sample can be filtered through a syringe type 25 mm 0.45 u aqueous filter (Gelman, Inc.) and a 100-uL aliquot removed for testing. If an organic solvent is used then a 500-uL aliquot was removed, evaporated to dryness and redissolved in 500 uL of buffer. A 100-uL aliquot was taken from the buffer solution and run like the water samples.

HPLC was performed by using a Rheodyne injector with a 50-uL loop, a Waters 510 pump and a Hewlett-Packard 1040A photodiode array detector/integrator system. The column was an Ultremex C18, 150 mm x 4.6 mm (Phenomenex, Inc.). Mobile phase was methanol/acetonitrile/water (40 + 20 + 40 v/v) with a flowrate of 1 mL/min. Detection was at 223 nm and 0.01 AUFS. Whenever possible, ultraviolet spectral scans were run on the atrazine peaks to check the purity.

RESULTS AND DISCUSSION

The immunoassay showed a linear relationship from 0.5 to 10 ng/mL (50 to 1000 pg/tube) which was observed between the logarithm of the atrazine concentration and the ΔOD at 450 nm. For samples containing greater than 10 ng/mL a dilution must be made. A ΔOD of 1.0 or greater indicates a sample concentration of more than 10 ng/mL and should be diluted and analyzed again.

Table 1. Reproducibility of the atrazine immunoassay for atrazine-fortified water samples.

	Amount added in ppb					
	1	2	4	10	20	50
Number of Samples Analyzed	14	14	14	14	14	14
% Coefficient of Variation	23.8	15.7	10.0	8.2	6.5	4.1

Table 2. Reproducibility of the atrazine immunoassay for atrazine-fortified soil samples.

	Amount added in ppb					
	2	4	10	20	40	80
Number of Samples Analyzed	11	11	10	9	11	11
% Coefficient of Variation	20.3	16.3	4.0	4.1	8.4	6.8

The reproducibility of this immunoassay in the detection of atrazine residues in water and soil is shown in Tables 1 and 2. For both types of samples, the reproducibility was excellent with percent coefficients of variation (% CV) ranging from 23.8 to 4.1.

Table 3. Comparison of the HPLC and immunoassay methods for the determination of atrazine in 6 different water sources fortified with atrazine.

Water Source	Amount Added ppb	Amount Found, ppb	
		Immunoassay	HPLC
River	0	0	0
	1	1	1
	4	8	5
	10	11	12
	20	21	20
	100	93	100
Well	0	0	0
	1	2	0
	4	3	4
	10	6	1
	20	8	23
	100	93	100
Ocean	0	0	N
	1	1	N
	4	2	N
	10	7	N
	20	19	N
	100	106	N
Blueberry Waste Water	0	0	0
	1	2	2
	4	2	3
	10	9	8
	20	12	17
	100	105	100
Pond	0	0	0
	1	3	1
	4	5	3
	10	10	10
	20	18	16
	100	106	100
Rain	0	0	0
	1	1	2
	4	4	3
	10	7	7
	20	10	17
	100	93	77

N = was not detectable by direct injection HPLC because of interference caused by the salt water.

The % CV of 23 and 20 may seem high, but they were obtained by doing a wide variety of samples containing 1 and 2 ppb atrazine analyzed over a period of 4 weeks. For example, Table 1 is comprised of data from 14 different water sources that varied a great deal in water type and quality (well water to farm pond water) while Table 2 consists of 11 different soil samples ranging from clay to sandy soils. This type of data indicates the ruggedness of the method plus different sample matrices do not have a significant effect on the atrazine immunoassay. However, it is recommended for quantification purposes that a 3 point standard curve be run each day at the 0.5, 4 and 10 ppb concentrations.

Comparisons were made between the HPLC and immunoassay methods for atrazine determination in water and soil (Tables 3, 4, and 5). For water (Table 3), 6 different sources were fortified at 5 atrazine levels in a blind study. For the majority of samples, the techniques compared well at both low and high atrazine concentrations. Also from Table 3, it can be seen that diluting the samples for immunoassay analyses did not have an effect since those samples also compared well with the HPLC results.

Also, well water samples from ten Central Maine areas that use triazine herbicides were analyzed. Of these ten, three showed positive with the test kit (concentrations of 2-5 ppb triazine). HPLC analyses of those three samples showed that it was not atrazine since the retention time was too long. We are presently looking at other triazines.

Table 4. Comparison of the immunoassay and HPLC methods for determination of atrazine residues in soil after organic solvent extraction.

	ppb Atrazine Found	
Sample	Immunoassay	HPLC
Soil-1	1900	1370
Soil-2	1700	1630
Soil-3	1100	810
Soil-4	880	660
Soil-5	86	95
Soil-6	4	6
Soil-7	42	10
Soil-8	37	42
Soil-9	ND	ND
Soil-10	2	ND
Soil-11	12	7
Soil-12	20	21
Soil-13	52	83
Soil-14	42	20
Soil-15	26	13
Soil-16	7	5
Soil-17	5	15
Soil-18	22	11

ND = None Detected

Table 5. Comparison of the immunoassay and HPLC methods for determination of atrazine residues in soil after water extraction.

Sample	ppb Atrazine Found	
	Immunoassay	HPLC
Soil-1	16	21
Soil-2	6	14
Soil-3	130	150
Soil-4	120	150

The soil comparison study (Tables 4 and 5) was done with water and organic solvent extracted soil since both methods may be used to determine atrazine levels in soil.

Table 6. Summary of atrazine cross-reactivity data.

Compound	50% B_0 Dose[1]	LDD[2]
Atrazine	0.4	0.1
Simazine	2.5	1.0
Propazine	0.5	0.1
Prometryn	0.4	0.1
Ametryn	0.7	0.1
Simetryn	2.5	0.3
Cyanazine	40.0	1.0
Cyprazine	0.7	0.2
Terbuthylazine	4.0	0.1
Dipropetryn	0.4	0.1
Atraton	1.0	0.5
Trietazine	10.0	1.0
Prometon	0.7	0.1
6-Hydroxy Atrazine	28.0	1.0
De-ethylated Atrazine	10.0	0.4
Di dealkylated Atrazine	Does not react	
Hexazinone	80.0	20.0
Procyazine	90.0	15.0
Terbutryn	15.0	1.0
Diazinon	Undetectable below 100 ppb	

The Following Compounds Were Undetectable at 1000 ppb:

Metribuzin	Glyphosate
Maleic Hydrazide	Azinphos Methyl
Ethirimol	Carbaryl
Anilazine	Triclopyr
Alachlor	Methamidophos
Metolachlor	Maneb
Carbofuran	Mancozeb
Aldicarb	Chlorpyrifos
2,4-D	

[1] 50% B_0 Dose = dose that causes 50% inhibition (50% of zero signal). Values given in ppb.

[2] LDD = Least detectable dose (lowest standard or dose at 90% B_0).

Recent research indicates that the water extraction value is the more important value for crop carry over (Ferris and Haigh 1987). Soil samples for this study were obtained from fields in Maine and Michigan treated with atrazine (and in some cases other triazines). Results of the methods comparison with soil (Tables 4 and 5) were not as good as with water but when the cross-reactivity of the immunoassay is taken into account, the comparisons are favorable. Soil samples giving the poorest correlation (soils 1, 3, 4 and 7) were treated with both simazine and atrazine; this fact accounts for the discrepancies in the results. Other higher immunoassay values might be attributed to the presence of atrazine degradation products that react with the antibody.

As mentioned above, the immunoassay has cross-reactivity with several triazines. The reactivity seems to be with the 2 and 4 position diamine side chains containing the ethyl and isopropyl groups since metribuzin, anilazine and ethirimol do not react at even 1000 ppb. As for non-triazines, diazinon is somewhat reactive (at greater than 100 ppb) while the others showed no reactivity at 1000 ppb (Table 6). It is not surprising that diazinon has some reactivity since it has an isopropyl group at the 2 position with ring nitrogens at the 1 and 3 positions. This broad cross-reactivity is an advantage for screening samples.

Although tube type immunoassays are limited by the number of samples that can be run simultaneously (8 samples with this test) compared to the plastic well type systems, it does have the advantages of less incubation time and is field adaptable. With this tube system, actual samples can be analyzed on site by using a hand-held battery-powered photometer. Even soil samples can be run in the field after they are water or solvent extracted. On-site analyses can save much time and money in that not as many samples will have to be brought back to the lab to run by the instrumental techniques.

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