Determination of Trace Atrazine Levels in Water by a Sensitive Magnetic Particle-Based Enzyme Immunoassay

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Atrazine is a pre- and post-emergence selective herbicide specific for broad-leaved weeds and grasses. Although extensively applied in the United States, its use in Europe is limited and restricted. European drinking water regulations are strict: allowable atrazine contamination levels are 100 parts per trillion (ppt, ng/L). As a result of European regulations, increased method sensitivity is needed to detect atrazine-contaminated water supplies. A sensitive and rapid enzyme immunoassay using specific atrazine antiserum covalently coupled to a magnetic particle solid phase has been developed for the quantitation of atrazine in water at parts per trillion levels. The immunoassay provides accurate detection to as low as 15 ppt of atrazine directly from a 250 μL water sample. Recovery from water samples without sample pretreatment averaged 100% and compared favorably to samples with GC/MS results ($r = 0.993$). The immunoassay represents a valuable method to detect trace amounts of atrazine in ground, surface, and drinking waters.

Keywords: *Atrazine; immunoassay; ELISA; triazine; herbicide*

INTRODUCTION

Atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine], one of the most commonly used agricultural pesticides in the world, provides the pre- and post-emergence control of broad-leaved weeds and grasses in corn, asparagus, fruit orchards, and citrus groves. Atrazine residues are often detected in environmental water samples, mostly as a result of spills, spraying, or agricultural runoff (Hall et al., 1993; Thurman et al., 1992; Goolsby et al., 1991; Frank et al., 1987). The widespread application, stability, and relatively high solubility of atrazine in water allow it to leach from soil and be a relatively persistent environmental contaminant (Cai et al., 1993). Despite a ban on the agricultural application of atrazine in Germany in 1990, residues are repeatedly detected in drinking water at concentrations above mandated European Community (EC) tolerance levels (Giersch, 1993). The EC regulations state that the maximum admissible concentration (MAC) of atrazine or any other single pesticide in drinking water is 100 parts per trillion (ppt), and the total combined concentration of all pesticides, regardless of toxicity, is 500 ppt (EC Council, 1980). The U.S. Environmental Protection Agency (U.S. EPA) has determined atrazine as a Class CQ possible human carcinogen, suggesting evidence of carcinogenicity based on animal toxicity research and inconclusive results from available clinical data (U.S. EPA, 1991). The U.S. EPA (1991) has therefore regulated atrazine by setting a practical quantitation limit (PQL) of 1 ppb and a maximum contaminant level (MCL) and maximum contaminant level goal (MCLG) of 3 ppb in drinking water. The use of atrazine in the United States is currently under special review by the U.S. EPA and may result in further restrictions of the pesticide.

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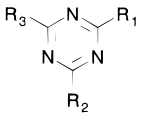
Programs for continued atrazine residue testing in Europe and elsewhere are valuable for compliance monitoring, especially when one considers the extent of application, the environmental persistence, and the fact that atrazine is still allowed and frequently used in most of the world (Giersch and Hock, 1990). The need for continued testing in combination with the expense and extended turnaround time associated with currently available GC methods have made rapid, inexpensive methods to detect small quantities of atrazine desirable. Immunological methods suitable for both laboratory and field analysis provide a unique opportunity to screen large numbers of samples quickly and cost effectively while accurately detecting pesticide residues at parts per trillion levels (Van Emon and Lopez-Avila, 1992).

The advantages and principles of enzyme immunoassays for the detection of pesticide residues have been previously described (Hammock and Mumma, 1980) and applied to the detection of atrazine in water (Giersch, 1993; Muldoon et al., 1993; Weller et al., 1992; Bushway et al., 1991; Thurman et al., 1990; Huber, 1985), food (Wittman and Hock, 1993), plants and soil (Wittman and Hock, 1990), and urine (Lucas et al., 1993). These methods describe immunoassay systems that utilize polystyrene microtiter wells or tubes as the solid support for passively absorbed antibody. Antibody immobilized in this manner has been shown to increase variability and compromise assay sensitivity (Howell et al., 1981) by desorption or leaching off from the polystyrene surface (Engvall et al., 1980; Lehtonen and Viljanen, 1980).

Magnetic particle-based immunoassays have been developed and previously applied to the detection of pesticide residues (Lawruk et al., 1992, 1993a, 1994; Itak et al., 1992) including the *s*-triazine herbicides cyanazine (Lawruk et al., 1993b) and atrazine (Gruessner et al., 1995; Aga and Thurman, 1993; Hall et al., 1993; Rubio et al., 1991). These immunoassays utilize specific antibody covalently coupled to a magnetic particle solid phase which has been shown to be more precise than methods using polystyrene-coated wells

S0021-8561(95)00737-0 CCC: $12.00

Table 1. Triazine Enzyme Conjugate Derivatives[a]



triazine derivative	R_1	R_2	R_3	B/B_0[b]
compd **I**	$NHCH(CH_3)_2$	Cl	$NH(CH_2)_5CO_2H$	0.635
compd **II**	NH_2	Cl	$NH(CH_2)_5CO_2H$	nd[c]
compd **III**	$NH(CH_2)_5CO_2H$	Cl	$NHCH_2CH_3$	0.780
compd **IV**	$NHCH(CH_3)_2$	$S(CH_2)_5CO_2H$	NH_2	0.796
compd **V**	NH_2	$S(CH_2)_5CO_2H$	$NHCH_2CH_3$	nd
compd **VI**	NH_2	$S(CH_2)_5CO_2H$	NH_2	nd
compd **VII**	$NHCH_2CH_3$	$S(CH_2)_5CO_2H$	$NHCH_2CH_3$	0.675

[a] Each triazine compound was conjugated to HRP by the active ester method and the performance examined in the immunoassay. [b] Displacement at 100 ppt atrazine in the immunoassay (B/B_0). B/B_0 is defined as the absorbance at 450 nm of a sample or standard divided by the absorbance of the zero standard. [c] Unable to determine B/B_0 value due to low immunoassay absorbance signal produced by the enzyme conjugate.

and tubes (Aga and Thurman, 1993). The magnetic particles permit the precise addition of antibody which is uniformly dispersed throughout the reaction mixture providing the means for non-diffusion-limited reaction kinetics and improved precision. Described is the development and performance of a sensitive, competitive magnetic particle-based immunoassay that takes <1 h to perform and is accurate for the detection of atrazine in ground, surface, and drinking water at parts per trillion levels.

MATERIALS AND METHODS

Antibody Solid-Phase Synthesis. Atrazine antiserum was produced by immunizing sheep with a keyhole limpet hemocyanin (KLH) hapten prepared from a 2-aminohexanecarboxylic acid−4-(isopropylamino)-6-chloro-1,3,5-triazine derivative according to the procedure of Wüst and Hock (1992). The atrazine antiserum was covalently attached to amine-terminated superparamagnetic particles (Perseptive Diagnostics, Cambridge, MA) by glutaraldehyde (Sigma Chemical Co., St. Louis, MO) activation of the solid phase as described by Rubio et al. (1991). The magnetic particles, approximately 1 μm in diameter, quickly separate in magnetic fields and retain no magnetic memory, allowing for repeated separation and resuspension. The efficiency of the antiserum coupling to magnetic particles, which exceeded 90%, was determined by measuring the absorbance of the IgG concentration of the postcoupled supernatant at 280 nm and comparing to the total concentration of IgG added to the reaction. The anti-atrazine magnetic particles were diluted 1:1000 in Tris-buffered saline (TBS, pH 7.4) for use in the immunoassay.

Enzyme Conjugate Synthesis. Seven triazine-derived compounds (Table 1) were coupled to horseradish peroxidase (HRP) by an active ester method (Langone and VanVunakis, 1975) in which 0.25 μmol of each triazine derivative together with 0.25 μmol (28.9 mg) of *N*-hydroxysuccinimide (Sigma) and 0.25 μmol (52.5 mg) of dicyclohexylcarbodiimide (Sigma) was dissolved in 2.0 mL of anhydrous *N*,*N*-dimethylformamide (Aldrich Chemical Co., Milwaukee, WI). The mixture was stirred overnight at room temperature and centrifuged, and the supernatant was collected for use as the active ester. The active ester (100 μmol of triazine derivative/μmol of HRP) was slowly added to 10 mg of HRP (1 μmol/42 mg) dissolved in 2 mL of 0.5 M sodium bicarbonate buffer (pH 9.5) and stirred overnight. The unbound derivative was removed by gel filtration on a Sephadex G-25 (Sigma) column (1 × 20 cm) equilibrated with TBS (pH 7.4), and fractions were collected every 0.5 mL. Fractions containing visible HRP were combined and diluted 1:1 with glycerol (Sigma) to prevent crystallization during −20 °C storage. Each triazine enzyme conjugate was diluted in TBS (pH 7.4) for use in the immunoassay.

Additional Chemicals and Reagents. Atrazine, related triazines, and other nonrelated agrochemicals were purchased from Riedel-de-Haen (Hanover, Germany) or Chem Service (West Chester, PA) to evaluate assay cross-reactivity. Hydrogen peroxide and 3,3′,5,5′-tetramethylbendizine (TMB) were obtained from Kirkegaard and Perry (Gaithersburg, MD). All other materials were of reagent grade or suitable chemical purity.

Immunoassay Apparatus. The Gilson P-1000 adjustable pipet (Rainin, Woburn, MA) and Eppendorf repeating pipet (Eppendorf, Hamburg, Germany) were used to dispense all reagents. A 60-position test tube rack with a removable magnetic base containing permanently positioned rare earth magnets (Itak et al., 1992) was used to separate antibody magnetic particles from unreacted reagents (Ohmicron Environmental Diagnostics, Newtown, PA). The RPA-I Analyzer (Rubio et al., 1991) was used to determine spectrophotometric measurements (Ohmicron).

Immunoassay Procedure. All standards, controls, and water samples were tested by adding 250 μL of sample, 250 μL of enzyme conjugate, and 500 μL of anti-atrazine coupled magnetic particles to a 12 × 75 mm disposable polystyrene test tube and incubating at room temperature for 30 min. The reaction mixture was magnetically separated using the 60-position test tube rack and separation base before decanting. The separated magnetic particles were washed twice with 1.0 mL of preserved deionized water solution. The amount of enzyme-labeled atrazine conjugate bound to the antibody was determined by dispensing 500 μL of hydrogen peroxide/TMB substrate (1:1) into each tube and allowing color to develop for 20 min at room temperature. After stopping and stabilizing the color formation by the addition of 500 μL of 2 N sulfuric acid, the absorbance of each tube was measured at 450 nm using the semiautomated RPA-I Analyzer. The RPA-I calculated sample concentrations from a linear regression analysis using a log−logit standard curve constructed from 0, 35, 250, and 1000 ppt atrazine calibrators prepared in TBS (pH 7.4). Samples >1000 ppt were diluted with the zero calibrator before analysis and the sample concentrations were calculated by multiplying the results by the appropriate dilution factor.

Water Analysis. Over 300 water samples from ground, surface, and municipal sources were collected from various locations around the world and analyzed by the immunoassay. Thirty-seven of these samples were surface waters previously evaluated by a conventional GC/MS method (Thurman et al., 1992) and contained known levels of atrazine. The GC/MS results were not corrected for procedural recoveries, and a correlation between methods was determined by a linear regression analysis of the data. Specific immunoassay performance characteristics were defined by examining sensitivity, precision, sample accuracy, dilution linearity, triazine specificity, tolerance to compounds ordinarily found in ground water, pH, and frequently used analytical solvents.

RESULTS AND DISCUSSION

Enzyme Conjugate Selection. Improved immunoassay sensitivity was achieved by evaluating multiple triazine enzyme conjugates with various molecular structures to the atrazine specific antiserum (Table 1). To develop an immunoassay sensitive to parts per trillion levels of atrazine, a triazine enzyme conjugate was selected with a molecular structure which produced a sufficient zero atrazine absorbance signal and possessed a substantially lower affinity for the anti-atrazine magnetic particles than atrazine (Harrison et al., 1991). To evaluate each triazine enzyme conjugate performance, the displacement of a 100 ppt atrazine calibrator (measured in B/B_0) was examined in the immunoassay after each triazine enzyme conjugate concentration was adjusted to give an absorbance at 450 nm of 1.4 ± 0.6 OD units (Table 1). B/B_0 is defined as the absorbance at 450 nm of a sample or standard divided by the absorbance of the zero standard. Of the seven triazine

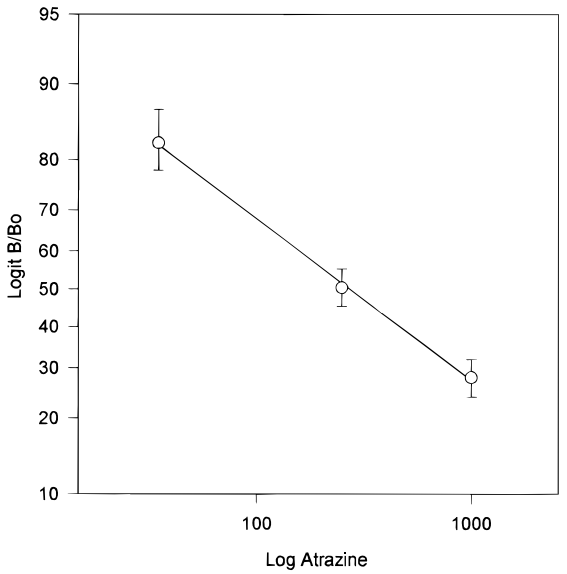


Figure 1. Atrazine calibration curve. Each point represents the mean of 68 runs with error bars indicating ±2 SD from the mean. B/B_0 is the absorbance at 450 nm of a standard or sample divided by the absorbance of the zero standard.

enzyme conjugates, only four bound to the anti-atrazine magnetic particles sufficiently to yield an adequate absorbance signal to determine B/B_0 displacement at 100 ppt atrazine (Table 1). The enzyme conjugate prepared from compound **I** produced the greatest sensitivity in the immunoassay, giving the most displacement at the 100 ppt calibrator, and was used for method development. The position of the carboxylic chain on this compound is distal to the isopropyl and chlorine moieties of the atrazine molecule. Triazine enzyme derivatives containing available isopropyl moieties have been previously shown to exhibit greater immunoassay sensitivity than those with available ethyl moieties (Schneider and Hammock, 1992). The antiserum demonstrated the strongest affinity for the enzyme conjugate prepared from compound **IV** but exhibited the least displacement at the 100 ppt calibrator. This compound has modifications to both the chloro and ethyl groups of atrazine. This together with the lack of binding from the conjugates prepared with compounds **II** and **V** suggests that antibody binding may be dependent on the recognition of the isopropyl group and, to a much lesser extent, the chloro and ethyl groups.

Dose Response Curve and Sensitivity. A standard curve for the atrazine calibrators was constructed from the mean values obtained in 68 runs and transformed by a linear regression line using a log−logit curve fit (Figure 1). The error bars at 2 standard deviations from the mean of each calibrator indicate the run to run variability (n = 68). Accurate sample concentrations were quantitatively reported within the linear range of the assay (15−1000 ppt) by including a calibration curve with each run to compensate for the variability resulting from small differences in timing, temperature, or reagent age. Immunoassay sensitivity (the lowest concentration that can be distinguished from zero) was estimated from the mean standard curve to be 15 ppt as the concentration corresponding to 90% B/B_0 (Midgley et al., 1969). The sensitivity can also be defined as the mass equivalent of 2 or 3 times the standard deviation of the B_0 from its mean absorbance. The minimum detectable concentrations according to this method are, respectively, 2 and 3 ppt of atrazine. The sensitivity of this method exceeds all government regulatory levels for drinking water including the U.S. EPA PQL of 1 ppb (U.S. EPA, 1991) as well as the EC limit of 100 ppt (EC Council, 1980).

Table 2. Precision of the Atrazine Immunoassay

	fortified sample concn[a]				
	50 ppt	100 ppt	350 ppt	500 ppt	750 ppt
replicates	5	5	5	5	5
days	5	5	5	5	5
N	25	25	25	25	25
mean[b] (ppt)	53	159	354	574	732
%CV (within assay)	8.4	6.2	4.0	5.3	3.7
%CV (between assay)	7.1	4.6	0.3	3.5	3.7
%CV (total assay)	10.6	7.5	4.0	6.2	5.0

[a] Atrazine-fortified drinking water samples were each assayed in five singlicates over 5 days. [b] The mean concentrations of some samples are greater than the fortified atrazine value probably due to atrazine in the unspiked sample.

Table 3. Accuracy of the Atrazine Immunoassay

amt of atrazine added (ppt)	atrazine recovered[a]			
	mean[b] (ppt)	*n*	SD (ppt)	% recovery
50	54	12	5.5	108
100	107	12	7.8	107
350	331	12	32	95
500	460	12	34	92
750	739	12	30	99
av				100

[a] Four water samples individually fortified at the above concentrations and assayed in duplicate in the immunoassay. [b] Each concentration represents the mean of three determinations in the immunoassay for each sample. All unfortified samples assayed as less than the detection limit of 15 ppt atrazine.

Precision. To determine the reproducibility of the immunoassay, within- and between-day variation was examined by testing drinking water samples fortified at 50, 100, 350, 500, and 750 ppt of atrazine as five singlicates per assay over 5 consecutive days (total n = 25 at each concentration). The within- and between-day variation was determined by analysis of variance (ANOVA) (Bookbinder and Panosian, 1986) and is summarized in Table 2. Coefficients of variation (%CV) for within- and between-day values were <9% and <8%, respectively, demonstrating the reproducibility of the method.

Accuracy. Immunoassay accuracy was evaluated by analyzing four water samples obtained from various municipal sources and a local well before and after the addition of atrazine. The samples were fortified with atrazine across the range of the method and evaluated three times in duplicate to verify reproducibility. Percent sample recovery was determined by subtracting the neat concentration from the spiked concentration and comparing to the original spike to calculate recovery. The average recovery for the water samples was 100% with a range of 92−108% (Table 3).

Sample Dilution. Four atrazine-fortified water samples were diluted at 1:2, 1:4, and 1:8 in the zero calibrator and analyzed to determine concentration linearity. The expected values were derived from the atrazine concentrations of the undiluted fortified samples. The results from the diluted samples agreed well with the expected values (Table 4). If the results were affected by either specific or nonspecific interferences, the sample dilution curves would not be parallel with the immunoassay standard curve and the diluted samples would not assay as expected (Jung et al., 1989).

Specificity. A variety of *s*-triazine analogs, including atrazine metabolites, and many structurally nonrelated

Table 4. Linearity upon Sample Dilution[a]

water sample	undiluted	1:2	1:4	1:8
1				
obtained (ppt)	745	374	173	93
expected[b] (ppt)	745	372	186	93
recovery (%)		100	94	100
2				
obtained (ppt)	806	408	194	85
expected (ppt)	806	403	201	100
recovery (%)		101	97	85
3				
obtained (ppt)	393	195	85	49
expected (ppt)	393	196	98	49
recovery (%)		100	88	100
4				
obtained (ppt)	458	233	103	53
expected (ppt)	458	229	114	57
recovery (%)		102	91	93

[a] Samples were diluted in the zero calibrator. [b] Expected concentrations were determined from a single assay in duplicate and derived from the atrazine concentration of each undiluted sample.

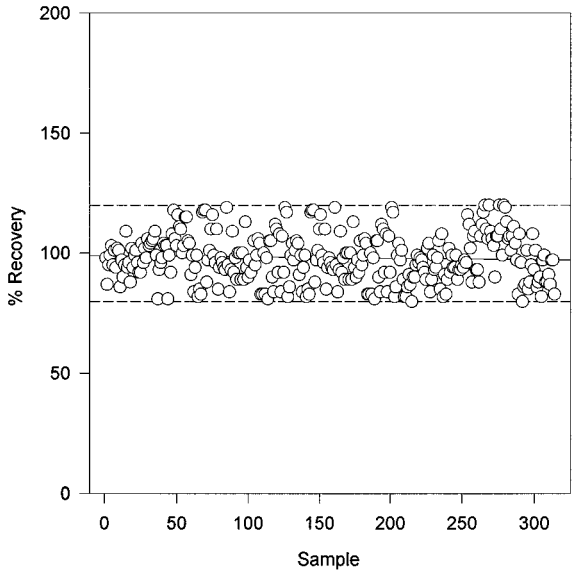
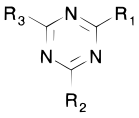


Figure 2. Percent recovery of 321 drinking, ground, and surface water samples from around the world after fortification with 100 ppt of atrazine. An acceptance range is plotted at 100 ± 20%. The mean recovery was 95% (SD = 9).

agricultural compounds were tested to determine the specificity of the anti-atrazine serum. Percent cross-reactivity was defined as the amount of analog necessary to displace 50% of the enzyme conjugate compared to the displacement of 50% atrazine (I_{50}). The least detectable dose (LDD) was determined as the amount of analog required to achieve 90% B/B_0 in the immunoassay. The immunoassay results indicate low reactivity to all atrazine metabolites; the greatest affinity was found for propazine, an *s*-triazine compound with a chloro and two isopropyl groups (Table 5). Considering that propazine is restricted in the United States and banned in most of Europe with the exception of The Netherlands and Belgium, the detection of this compound should be infrequent and allow for the accurate detection of atrazine in drinking and other environmental water samples (Wüst and Hock, 1992). The immunoassay is, therefore, capable of atrazine quantitation in the presence of expected concentrations of metabolites and other commonly applied pesticides.

Interferences. Immunoassay tolerance to typical water matrices, a broad range of common ground water components, pH, and frequently used analytical solvents were examined by evaluating water samples before and after fortification with atrazine. The potential of ground, surface, and drinking waters to interfere with the assay was examined by testing over 300 samples collected from around the world. These samples were tested both unfortified and fortified with 100 ppt of atrazine. The acceptable percent sample recoveries (80−120%) indicate that ground, surface, and drinking water matrices are an unlikely source for assay interference (Figure 2). To further evaluate potential inorganic and organic interferences, compounds ordinarily found in environmental water samples were added to deionized water and analyzed in the immunoassay (Table 6). The immunoassay demonstrated tolerance to these compounds in excess of the concentrations typically found in common water samples (American Public Health Association, 1989). The effects of sample pH on water blanks and atrazine recovery were also examined. It was determined that samples from pH 2 through 11 have no adverse effect on immunoassay performance. These results demonstrate that a broad range of envi-

Table 5. Specificity of Various *s*-Triazine Compounds and Unrelated Agrochemicals in the Atrazine Immunoassay



compd	R_1	R_2	R_3	LDD[a] (ppt)	I_{50}[b] (ppt)	cross-reactivity (%)
atrazine	$NHCH(CH_3)_2$	Cl	$NHCH_2CH_3$	15	220	100
deethylatrazine	$NHCH(CH_3)_2$	Cl	NH_2	27	870	25
didealkylatrazine	NH_2	Cl	NH_2	156	98100	0.22
deisopropylatrazine	NH_2	Cl	$NHCH_2CH_3$	156	128000	0.17
hydroxyatrazine	$NHCH(CH_3)_2$	OH	$NHCH_2CH_3$	158	6260000	<0.01
propazine	$NHCH(CH_3)_2$	Cl	$NHCH(CH_3)_2$	5	91	242
simazine	$NHCH_2CH_3$	Cl	$NHCH_2CH_3$	19	2030	10.8
terbuthylazine	$NHC(CH_3)_3$	Cl	$NHCH_2CH_3$	19	5090	4.3
cyanazine	$NHCCN(CH_3)_2$	Cl	$NHCH_2CH_3$	36	50700	0.43
prometryn	$NHCH(CH_3)_2$	SCH_3	$NHCH(CH_3)_2$	13	2380	9.2
prometon	$NHCH(CH_3)_2$	OCH_3	$NHCH(CH_3)_2$	15	2380	9.2
ametryn	$NHCH(CH_3)_2$	SCH_3	$NHCH_2CH_3$	19	6080	3.6
terbutryn	$NHC(CH_3)_3$	SCH_3	$NHCH_2CH_3$	27	63500	0.35

[a] Least detectable dose (90% B/B_0). [b] 50% inhibition concentration (50% B/B_0). The following pesticides were assayed at 10 000 ppb and found to have no reactivity in the immunoassay: alachlor, aldicarb, aldicarb sulfoxide, aldicarb sulfone, butachlor, butylate, captan, carbaryl, carbendazim, carbofuran, chlorothalonil, 2,4-D, dichloropropene, dicamba, dinoseb, folpet, metolachlor, metribuzin, pentachlorophenol, picloram, propachlor, terbufos, thiobendazole, thiophanat-methyl, and triclopyr.

Table 6. Effect of Possible Interfering Substances

interference	concn of interference tested[a] (ppm)	measured concn of atrazine[b] (ppt)
nitrate	250	101
copper	100	96
nickel	250	85
thiosulfate	250	99
sulfite	250	100
magnesium	250	106
zinc	100	102
mercury (+2)	100	101
manganese	250	109
phosphate	250	82
iron	100	83
calcium	500	85
silicates	500	103
sulfate	10 000	104
sodium chloride	0.65[c]	112
peroxide	250	111

[a] All unfortified samples assayed less than the detection limit of 15 ppt atrazine. [b] Samples were fortified with 100 ppt atrazine. The measured concentrations were determined from duplicate samples in a single assay. [c] Molar.

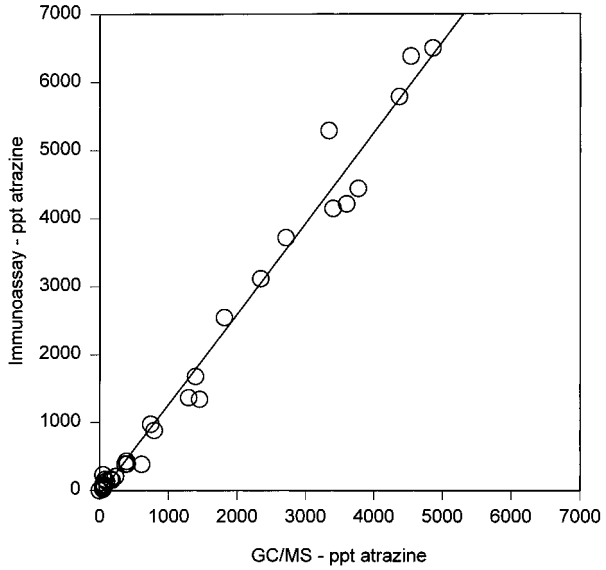


Figure 3. Correlation between atrazine water sample concentrations as determined by the magnetic particle immunoassay and the GC/MS method. $n = 37$, $r = 0.993$, $y = 1.33x - 62$ ppt.

ronmental water samples with various pH values can be accurately evaluated by this immunoassay. Also evaluated for compatibility in the immunoassay were frequently used extraction and elution solvents (methanol, acetonitrile, and acetone). The immunoassay tolerances to methanol, acetonitrile, and acetone were determined as 2%, 5%, and 1% (v/v), respectively.

Method Comparison. A correlation of 37 water samples analyzed by a conventional GC/MS method (x) and the atrazine enzyme immunoassay (y) is illustrated in Figure 3. Samples with atrazine concentrations >1000 ppt were diluted with the zero calibrator to be within the standard range of the assay. The actual sample concentrations were calculated by multiplying the results by the appropriate dilution factor. Linear regression analysis yielded a correlation coefficient (r) of 0.993, with an intercept at −62 ppt ($p = 0.328$) and a slope of 1.33 ($p < 0.001$) between methods. Statistical results from the linear regression analysis indicate that the intercept is not significantly different from 0 ($p = 0.328$) but that the slope is significantly different from 1 ($p < 0.001$). Taking into account the accuracy and specificity of the immunoassay, the positive slope bias observed could be the result of analyte loss during the sample extraction and concentration steps of the GC/MS method or the presence of cross-reacting compounds in the samples.

Conclusion. The advantages of immunoassay technology for the detection of trace atrazine residues using a magnetic particle solid phase are demonstrated. The immunoassay provides results in <60 min and is ideally suited for on-site or laboratory monitoring of trace atrazine levels in drinking, surface, and ground water samples taken from around the world. The method compares favorably to GC/MS determinations ($r = 0.993$, $y = 1.33x - 62$ ppt), exhibits within- and between-assay precision of <9%, and has an average method recovery of 100%. The assay sensitivity allows for the detection of atrazine from 15 ppt, which exceeds the EC drinking water limit of 100 ppt and the U.S. EPA PQL of 1 ppb, as well as the method detection limits of traditional technologies. The immunoassay yields accurate atrazine concentrations for samples containing organic and inorganic compounds ordinarily found in ground, surface, and drinking waters, while the specific antibody employed allows for the detection of atrazine in the presence of metabolites and other commonly applied *s*-triazine herbicides without the need for sample preparation.

ACKNOWLEDGMENT

We thank Dr. Bertold Hock of the Technical University of Munich at Weinenstaphen (Freising, Germany) for his generous contribution of the sheep anti-atrazine serum and Dr. Diana S. Aga of the U.S. Geological Survey (Lawrence, KS) for the GC/MS analysis of the surface water samples.

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Received for review November 7, 1995. Accepted August 20, 1996.[⊗]

JF950737L

[⊗] Abstract published in *Advance ACS Abstracts*, October 1, 1996.