Dipstick Immunoassay Format for Atrazine and Terbuthylazine Analysis in Water Samples

Lucia Mosiello, Carlo Cremisini, Lia Segre, Salvatore Chiavarini, and Marcello Spanò

ENEA, Department of the Environment, C.R. Casaccia, via Anguillarese 301, 00060 Rome, Italy

Thorsten Kimmel, Antje J. Bäumner, and Rolf D. Schmid*

Institute for Technical Biochemistry, University of Stuttgart, Allmandring 31, D-70569 Stuttgart, Germany

A new dipstick immunoassay format for terbuthylazine determination, based on the use of polystyrol strips as antibody coating support, is described. The terbuthylazine calibration curve shows a linear measuring range between 1.2 and 10 μg/L. Real water samples were analyzed, and results correlate well with those from gas chromatography (GC/MS) and ELISA. No false negative results were evidenced. The dipstick immunoassay format for atrazine determination (Wittmann et al. *Analyst* **1996**, *121*, 863−869) was also used for the analyses of the same samples. Some false positive results were obtained, probably due to cross-reactivity of the atrazine monoclonal antibody for terbuthylazine. At this developmental stage, these dipsticks can be very useful as a qualitative/semiquantitative "field test" for identifying "positive" samples, reducing the number of samples to be reanalyzed in the laboratory, according to analytical standard methods (GC). Further improvements are possible to optimize the whole system on the strictly analytical aspects.

Keywords: *Atrazine; terbuthylazine; dipstick immunoassay; enzyme immunoassay; environmental monitoring; water samples*

INTRODUCTION

The availability of rapid and reliable methods for "in field" determination of pesticides and other organic micropollutants in water samples is an increasing need for environmental monitoring purposes. Gas chromatographic (GC) methods are generally used, due to their low detection limits and high selectivity. Laboratories generally have to analyze a large number of samples requiring adequate storage conditions and time-consuming sample pretreatment and preconcentration procedures. Recently, immunoassays have seen a great development in the field of organic micropollutant analytical determination (Vanderlaan et al., 1991) and specifically in the analysis of *s*-triazines (Bushway et al., 1988; Dankwardt et al., 1995; Thurman et al., 1990; Wittmann and Hock, 1991, 1993), but these methods are generally restricted to laboratories.

Dipstick assays, widely used as a diagnostic tool to detect toxins (Schneider et al., 1991), hormones, or drugs (Aeppli et al., 1989), allow a rapid determination of the analytes. These dipstick assays, following the ELISA procedural schemes, are based on a membrane, affixed to a plastic strip, as the antibody coating support (Stott 1989).

Surface and ground water pollution by triazine herbicides has been a severe problem in the past two decades in Europe. The extensive use of atrazine in Italy started in th 1960s corresponding to the widespread application of corn monocultures, mainly present in the northern part of the country. A monitoring program at the end of the 1980s showed widespread contamination of ground water in the northern part of Italy, and atrazine was found at considerable levels (>1 μg/L) in a significant number of wells (Funari et al., 1989). Sale and use of atrazine was banned in Italy in 1990. A similar situation can be described for Germany, where the application of atrazine has been prohibited since 1991. Nevertheless, recent monitoring programs showed that atrazine is, in some cases, still present in the effluent of rural waste water treatment plants. The ratio between atrazine and its metabolite desethylatrazine is an interesting parameter to be considered for evaluating the real entity of the contamination cases (Nitschke and Schüssler, 1998). The presence of terbuthylazine in surface and ground water samples collected from the Veneto region (Italy) indicates the use of other triazines in agricultural practices.

A qualitative rapid immunoassay for the determination of atrazine in water samples was previously introduced by Giersch (1993). Further modifications were introduced to enhance its performance as a quantitative test (Kettling, 1994; Wittmann et al., 1996).

In this paper a new dipstick assay for terbuthylazine, using a monoclonal antibody (P6A7) showing a high specificity for the analyte, was developed and the analytical procedure for atrazine determination (using K4E7 dipstick) was improved. The competitive reaction between the pesticide and the enzyme tracer for binding the membrane-coated specific monoclonal antibody (mAb) involves a change in color intensity, which can be measured as remission values at 657 nm by a portable reflectometer. The terbuthylazine dipstick assay is suitable as a qualitative or semiquantitative method to select and identify "in field" all "positive" water samples and to obtain preliminary significant information on the

* Author to whom correspondence should be addressed (fax +49 711 685 3196).

S0021-8561(97)00966-7 CCC: $15.00
Published on Web 08/25/1998

concentration levels. Both dipstick assays are rapid, easy to perform, and inexpensive and could be advantageous in comparison with ELISA or GC/MS for in field environmental analysis. Further improvements of analytical parameters such as precision, accuracy, and detection limits (especially for terbuthylazine) are required.

The evaluation of the analytical performance of both dipstick assays for terbuthylazine and atrazine, in comparison with other analytical methods such as GC/MS and ELISA, is reported.

MATERIALS AND METHODS

Antibodies and Chemicals. The murine hybridoma clones secreting mAbs K4E7 (atrazine specific) and P6A7 (terbuthylazine specific) as well as the *s*-triazine derivative [4-chloro-6-(isopropylamino)-1,3,5-triazine−2-(6-amino)caproic (atrazine−caproic acid)] were kindly provided by Prof. B. Hock and Dr. T. Giersch [K4E7 and P6A7 mAbs, protein A affinity purified, producer T. Giersch (Department of Botany, Technical University of Munich, Freising, Germany, 1993].

Protein A, goat anti-mouse IgG, casein (sodium salt from bovine milk), dioctylsulfosuccinate (sodium salt) (DSS), dimethyl sulfoxide (DMSO), and 3,3′,5,5′-tetramethylbenzidine (TMB) were purchased from Sigma (St. Louis, MO).

Tween 20 and 30% hydrogen peroxide were from Merck (Darmstadt, Germany).

Atrazine, terbuthylazine, propazine, and desethylterbuthylazine standards were from Supelco (Bellefonte, PA). Methylene chloride and absolute ethanol (for pesticide residue analysis) were from Carlo Erba (Milan, Italy).

Anhydrous sodium sulfate (reagent grade) was from Rudi-Pont (Milan, Italy).

All other reagents were of the highest purity grade.

Monoclonal Antibodies Production and Purification. Mass production of mAbs (K4E7 and P6A7) was obtained from Teflon bags and from a roller bottle device (Miniperm). Using Teflon bags, during a 6 week culture period, a total yield of 90 mg of IgG in 1.6 L of culture medium was obtained, whereas by using the Miniperm device 35 mg of mAbs in 35 mL of final supernatant volume was collected, after a 15 day production cycle. Although the total amount of mAbs produced by Miniperm is lower, the smaller supernatant volume makes the successive purification procedures easier.

K4E7 and P6A7 mAbs were purified from the supernatant by a protein A affinity chromatography column, after sulfate ammonium precipitation.

Buffers and Solutions. Carbonate buffer (50 mM, pH 9.6), 40 mM phosphate-buffered saline (sodium chloride, 0.150 mM) (PBS), pH 7.2, and PBS washing buffer containing 0.05% Tween 20 were used. The enzyme tracer was prepared according to the literature method (Hock et al., 1992) and diluted 1:10000 in PBS to be used for the dipstick assay. TMB substrate (2 mM) for dipstick assay consisted of 400 μL of TMB stock solution (6 mg/mL in DMSO) plus 1 mL of DSS solution (8 mg/mL in ethanol) plus 3.6 mL of 0.1 M sodium acetate solution, pH 5.5, plus 10 μL of 1% hydrogen peroxide. Casein blocking solutions of 0.5% in PBS for the atrazine dipstick immunoassay and 2% in PBS for the terbuthylazine dipstick immunoassy were used.

Standard Solutions. Atrazine (10 mg) was dissolved in a volumetric flask (10 mL) in absolute ethanol. The same procedure was repeated for terbuthylazine and propazine. Stock solutions at 10 mg/L concentration were prepared by dilution in absolute ethanol. Standard solutions were prepared by serial dilutions of the stock solutions to obtain the following herbicide concentrations: 0.01, 0.1, 0.5, 1, 10, and 100 μg/L.

Other Materials. Ninety-six-well microtiter plates were provided by NUNC (Roskilde, Denmark). Double-sided adhesive tape was from 3M Deutschland (Neuss, Germany). The test kit for atrazine (EnviroGard high-sensitivity triazine plate kit, ENVR P00 48) was purchased from Millipore (France). Nylon membranes (Immunodyne), used for the immobilization of the antibodies on the dipstick, were from Pall GmbH (Dreieich, Germany). Polystyrol 500 μm (transparent) was bought from SKK (Denzlingen, Germany).

Equipment. An RQflex reflectometer (Merck, Darmstadt, Germany) including the barcode "test routine" was used for dipstick immunoassay measurements. This barcode permits the output of absorption values in percentage measured in the transmission mode at 657 nm.

A Multiskan Plus II (Flow Laboratories, Milan, Italy) ELISA reader for 96-well microtiter plates was used for immunoassay measurements at 450 nm.

GC/MS analyses were performed on an HP 5890 GC/HP 5970/B MSD system with electron impact ionization (70 eV).

A Rotavapor Buchi 461 (Flawil, Switzerland) and a Miniperm Heraeus (Heraeus Instruments GmbH, Hanau, Germany) were also used.

Dipstick Assay. *Preparation of Dipsticks.* The strips for dipstick assay were prepared as follows.

Atrazine. Nylon membranes (10 cm square pieces) were precoated with goat anti-mouse IgG (5 μg/mL in carbonate buffer) overnight at 4 °C. After washing with a PBS/Tween solution, the membranes were incubated with the mAb K4E7 (0.2 μg/mL in PBS, purified by protein A affinity chromatography column) for 3 h. The membrane was then blocked with 0.5% casein solution for 30 min and washed three times, as above. After drying for 1 h at room temperature, the membrane was cut into 0.8 × 0.8 cm square pieces, which were mounted onto an inert plastic support using double-sided adhesive tape. Finally, the test strips were ready for use in the assay and can be stored at 4 °C for at least 6 months without loss of efficiency.

Terbuthylazine. As for analysis of environmental water samples, no suitable calibration curve was obtained by applying the same protocol as for K4E7 (polystyrol sheets instead of nylon membrane were used). Polystyrol sheets (cut into strips of 0.7 × 7 cm) were soaked in acetone, washed (with PBS solution), and incubated with the mAb P6A7 (2 μg/mL in carbonate buffer, purified by protein A affinity chromatography column) at 4 °C for 24 h. After being washed twice as above, the polystyrol strips were blocked with 2% casein solution for 30 min. The test strips were washed three times and dried for 1 h at room temperature.

All of the incubation steps were carried out at room temperature under constant shaking. Finally, the test strips were ready for use in the assay and can be stored at 4 °C for at least 6 months without loss of efficiency.

Assay Protocol. The test strips are incubated in 2 mL glass test tubes with a mixture of 200 μL of enzyme tracer and 800 μL of standard solution (or sample). The reaction is allowed to proceed [15 min (atrazine) and 45 min (terbuthylazine)], and the strips are washed three times with PBS/Tween (atrazine assay) or PBS (terbuthylazine assay). The strips are incubated for 10 min (atrazine assay) or 20 min (terbuthylazine assay) in the TMB substrate buffer (800 μL). All incubations are carried out at room temperature under constant shaking and, as a general principle, 30 strips can be analyzed in the same experiment, including a sufficient number of replicates for both standard solutions and samples.

The absorption of the colored product was measured with a portable reflectometer at 657 nm. The remission values, relative to the standard solutions (concentration in a range from 0.01 to 100 μg/L), were fitted to a four-parameter logistic function using a commercial software package (ORIGIN).

The remission values were normalized as $\%B/B_0$, according to the following equation, and elaborated with the same software package:

$$\%B/B_0 = (R - R_{xs}/R_0 - R_{xs}) \times 100$$

R is the remission at 657 nm, R_0 is the remission at zero concentration of the hapten, and R_{xs} is the remission at an excess of the hapten.

The IC_{50} (the amount of hapten required to occupy 50% of mAb binding sites) was 0.7 μg/L for atrazine and 4.5 μg/L for terbuthylazine.

ELISA. The commercial kit EnviroGard and ELISA tests using the same mAbs K4E7 (atrazine) and P6A7 (terbuthylazine) used on the dipstick membrane surface were used for the comparison with the data from the dipstick assays.

Data were obtained on four replicates of each standard solution in three different experiments and substantially confirmed literature data (Giersch, 1993; Giersch et al., 1993). A good agreement with the data from the commercial kit was also seen.

GC Analysis. Water samples (200 mL) were fortified with 500 ng of propazine as an internal standard and extracted in a separatory funnel with 2 × 30 mL of CH_2Cl_2. The extract was filtered through anhydrous sodium sulfate and concentrated in a rotary evaporator to a small volume (~5 mL) and reduced to 250 μL under moderate nitrogen flow. The concentrated extract was analyzed by GC/MS. The gas chromatography was performed under the following conditions: electron impact ionization mode, 70 eV; carrier gas, helium, 100 kPa head pressure; column, PTE5-Supelco, 0.25 mm i.d., 0.25 μm film tickness, 30 m length; temperature program, 60 °C (hold for 1 min), 25 °C/min to 140 °C, 5 °C/min to 240 °C, and then hold for 3 min; injector, splitless, 260 °C; transfer line temperature, 280 °C; selected ion monitoring (SIM) with *m*/*z* values of 214−216 for propazine, 200−202 for atrazine, 214−216−229−231 for terbuthylazine, and 186−188−201−203 for desethylterbuthylazine. For both atrazine and terbuthylazine the detection limit is 0.01 μg/L.

Analysis of Water Samples. Surface and ground water samples were collected in an agricultural area in northern Italy (Veneto). All samples were analyzed by GC/MS, ELISA, and dipstick assays.

One sample, previously tested by GC/MS for the absence of atrazine, terbuthylazine, other *s*-triazines, their relevant degradation products, and other related compounds, was spiked with atrazine at three different concentrations or with atrazine and terbuthylazine for the evaluation of possible matrix effects.

RESULTS AND DISCUSSION

Different immobilization methods were investigated for dipstick preparation. The antibodies were immobilized via absorption and via specific binding such as protein A and primary antibodies, or they were covalently coupled to the surface via glutaraldehyde. However, immobilization via absorption proved to be the best method.

For atrazine dipstick immunoassay, nylon membranes, positively charged, were used according to the method of Wittmann et al. (1996), which gave a better performance of the K4E7 antibody among different materials investigated [nitrocellulose, poly(vinylidene fluoride), nylon].

No acceptable data were obtained with terbuthylazine dipstick prepared using the same protocol as for K4E7 on nylon membrane.

Several factors may combine to determine the difference in performance between K4E7 and P6A7 mAbs coated on nylon membrane, and the following hypotheses can be made. The different binding behaviors of the antibodies toward their haptens were investigated in the microtiter plate; the anti-atrazine antibody bound better than the anti-terbuthylazine one. The different isotype classes to which K4E7 and P6A7 antibodies belong may involve differences in the surface charge and/or protein glycosylation with a subsequently different binding affinity for the nylon membrane. Moreover, the different spatial orientations that the mAbs coated on the membrane could present consequently

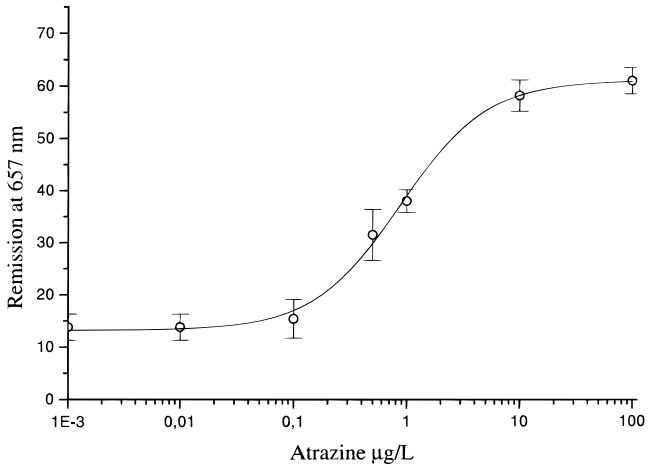

Figure 1. Representative atrazine calibration curve obtained with K4E7 dipstick assay. The 2-fold standard deviation ($\pm 2\sigma$ representing 95% confidence interval) is indicated as error bars.

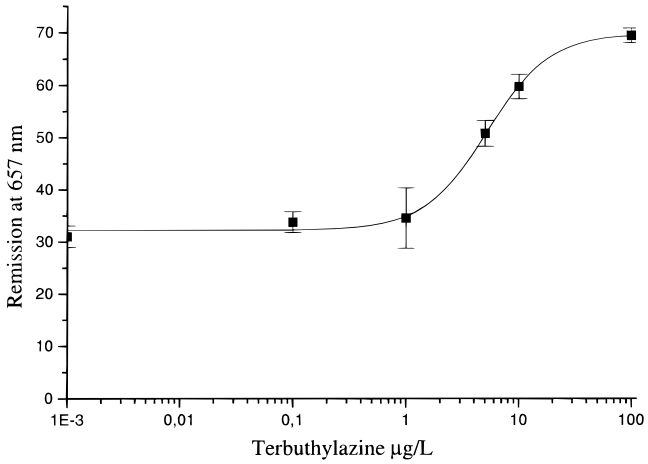

Figure 2. Representative terbuthylazine calibration curve obtained with P6A7 dipstick assay. The 2-fold standard deviation ($\pm 2\sigma$ representing 95% confidence interval) is indicated as error bars.

showed a binding affinity for the analyte different from that showed in the microtiter plate. All of these causes could lead to different sensitivities of the competitive atrazine and terbuthylazine dipstick assays performed on nylon membrane. Therefore, anti-terbuthylazine immobilization via absorption was investigated on various supports [such as polystyrol, poly(vinyl chloride), polycarbonate, and nitrocellulose], and, even though most of the plastic materials gave positive results, polystyrol was chosen for terbuthylazine dipstick assay development, due to the best color precipitation obtained.

Figures 1 and 2 show typical calibration graphs for atrazine and terbuthylazine.

Several calibration curves were analyzed to evaluate their reproducibility. A relatively high coefficient of variation (CV) suggested the necessity to include an internal calibration curve for each set of analyses in the analytical protocol. Because the color developed on the membrane is relatively unstable (especially for the atrazine dipstick), the timing of instrumental readings should be considered critical and it is advisable to analyze no more than 30 test strips in a single set of analyses to avoid loss of analytical consistency of the data. Therefore, the best compromise is based on the

Table 1. Terbuthylazine Determination on Real Water Samples with ELISA (P6A7), Dipstick (P6A7), and GC/MS[a]

sample	ELISA P6A7 (μg/L)	CV%	dipstick P6A7 (μg/L)	CV%	GC/MS[b] (μg/L)	GC/MS[c] (μg/L)
855/96	<0.3		<1.2		0.29	0.05
856/96	<0.3		<1.2		0.24	0.05
857/96	<0.3		<1.2		0.32	0.10
858/96	<0.3		<1.2		0.29	0.10
859/96	<0.3		<1.2		0.27	0.05
860/96	<0.3		<1.2		0.02	<0.01
861/96	<0.3		<1.2		0.14	0.38
862/96	<0.3		<1.2		0.23	0.42
863/96	0.7	2.8	<1.2		0.34	0.39
864/96	0.7	2.0	<1.2		0.50	0.45
865/96	2.0	3.0	2.1	6.2	2.13	1.55
866/96	2.3	1.2	2.0	12.7	2.12	4.88
867/96	3.0	3.7	3.8	4.5	2.19	4.48
868/96	3.0	5.8	1.2	5.8	1.91	1.45
869/96	<0.3		<1.2		<0.01	0.02
870/96	0.6	3.9	<1.2		0.25	0.45
871/96	0.7	1.9	<1.2		0.06	0.52
872/96	0.7	5.0	<1.2		<0.01	0.56
873/96	<0.3		<1.2		0.08	0.48
874/96	<0.3		<1.2		0.10	<0.01
896/96	<0.3		<1.2		0.03	<0.01
897/96	<0.3		<1.2		0.04	<0.01
898/96	<0.3		<1.2		0.04	0.05
899/96	<0.3		<1.2		0.03	0.02
900/96	<0.3		<1.2		0.03	<0.01

[a] All analyses were performed on four replicates. [b] GC/MS: data on terbuthylazine. [c] GC/MS: data on desethylterbuthylazine.

simultaneous analysis of five samples (three replicates each) and five different standard solution concentrations (three replicates each) to obtain significant quantitative results.

On the basis of this analytical protocol terbuthylazine was determined in 25 real water samples with terbuthylazine dipstick assays (Table 1).

Four samples gave positive results for terbuthylazine at 1.2−3.8 μg/L concentration level (with a practical detection limit of 1.2 μg/L). ELISA, using mAb P6A7 anti-terbuthylazine specific (with a practical detection limit of 0.3 μg/L), confirmed the four positive samples and showed five more terbuthylazine positive samples at 0.6−0.7 μg/L concentration level. GC/MS analyses showed the presence (even at trace level) of terbuthylazine in 23 of 25 samples (with a detection limit of 0.01 μg/L), which were negative in ELISA because the terbuthylazine concentrations were under the ELISA detection limit.

The differences between results from GC/MS and immunoassays for terbuthylazine determination could be justified with the P6A7 mAb cross-reactivity to desethylterbuthylazine, the main terbuthylazine degradation product [calculated as ∼2% (A. Marx, Technical University of Munchen, personal communication, 1997)], or to other compounds (to be confirmed), such as metanitron and tribenuron, which were used for cultural treatment in the selected sampling area.

The same 25 water samples were reanalyzed with the atrazine dipstick as well. Four samples yielded positive detections at 0.5−0.6 μg/L concentration level, with a detection limit for atrazine of 0.1 μg/L (Table 2). Almost the same result was obtained with ELISA (K4E7). GC/MS analyses showed the absence of atrazine in all samples (under a detection limit of 0.01 μg/L).

This presumably can be explained by the mAb K4E7 cross-reactivity to terbuthylazine (26%) (Giersch, 1993).

Table 2. Atrazine Determination on Real Water Samples with ELISA (K4E7), Dipstick (K4E7), and GC/MS[a]

sample	ELISA K4E7 (μg/L)	CV%	dipstick K4E7 (μg/L)	CV%	GC/MS (μg/L)
865/96	0.7	2.7	0.6	9.0	<0.01
866/96	0.7	5.6	0.6	14.7	<0.01
867/96	0.8	5.2	0.5	19.7	<0.01
868/96	0.8	2.8	0.5	7.8	<0.01

[a] All analyses were performed on four replicates. The other 21 samples resulted in atrazine negative with all analytical methods.

Experiments on samples spiked with both atrazine and terbuthylazine confirmed, in fact, the known cross-reactivity of K4E7 (26%) to terbuthylazine, overestimating the atrazine concentration.

No matrix effects were seen under specific tests.

CONCLUSION

The proposed dipstick assay seems to be a fast and reliable field test for a preliminary qualitative/semiquantitative screening of environmental water samples. The real samples can be analyzed without any preliminary enrichment or cleanup treatment, and the method is relatively rapid, easy to perform, and quite inexpensive. All positive samples can be successively analyzed in the laboratory according to an analytical standard method, to confirm the presence of the analytes and obtain officially acceptable quantitative data.

From a strictly analytical point of view, at this stage of development, the main existing problems seem to be a relatively poor precision, probably due to problems in the homogeneity of the monoclonal antibody coating on the nylon membranes or polystyrol transparents, and the instability of the colored TMB charge-transfer complex. The practical analytical protocol proposed in this paper permits minimization of these problems as shown by the reasonably good agreement among results from GC/MS, ELISA, and dipstick assays.

Dipstick assay detection limits are suitable for environmental monitoring purposes (runoff water, surface water, etc.), but they are still too high for drinking water analysis according to European Union Guidelines for drinking water (0.1 μg/L).

Further improvements are needed to optimize the system in terms of both the screening field test point of view (reducing the number of sample replicates and standard solutions to be processed) and the strictly analytical aspects (increasing precision and accuracy of the method and lowering the detection limits).

ACKNOWLEDGMENT

We thank T. Giersch and B. Hock for providing us with clones of antibodies.

LITERATURE CITED

Aeppli, R. E.; Bargetzi, M. J.; Binz, H. Diagnostic screening of multiple antigen−antibody reactions in a new single assay on nitro-cellulose: the line immunobinding assay (LIBA). *J. Immunol. Methods* **1989**, *120*, 93−98.

Bushway, R. J.; Perkins, B.; Savage, S. A.; Lekousi, S. J.; Ferguson, B. S. Determination of atrazine residues in water and soil by enzyme immunoassay. *Bull. Environ. Toxicol.* **1988**, *40*, 647−654.

Dankwardt, A.; Pullen, S.; Rauchalles, S.; Kramer, K.; Just, F.; Hock, B. Atrazine residues in soil two years after the

atrazine ban- A comparison of enzyme immunoassay with HPLC. *Anal. Lett.* **1995**, *28* (4), 621−634.

Funari, E.; Acquafresca, G.; Arca, F.; Baldi, M.; Bastianetti, J.; Cappelli, A.; Carli, G.; Carniel, A.; Chierici, S.; Fanuzzi, A.; Ferraro, P.; Lopez, A.; Mattioni, R.; Narese, M.; Peretti, A.; Salamana, M.; Zapponi, G. Preliminary report on the atrazine and molinate water supply contamination in Italy. *Chemosphere* **1989**, *18*, 2339−2343.

Giersch, T. A new monoclonal antibody for the sensitive detection of atrazine with immunoassay in microtiter plate and dipstick format. *J. Agric. Food Chem.* **1993**, *41*, 1006−1011.

Giersch, T.; Kramer, K.; Hock, B. Optimization of a monoclonal antibody-based enzyme immunoassay for the detection of terbuthylazine. *Sci. Total Environ.* **1993**, 435−448.

Hock, B.; Giersch, T.; Kramer, K. Monoclonal antibody-based enzyme immunoassays for the sensitive detection of s-triazines in water. *Analysis* **1992**, *20*, M29−M33.

Kettling, U. Untersuchungen zur Entwicklung von Immunteststreifen zum Nachweis von s-Triazinherbiziden. Studienarbeit Technische Universitat Carolo-Wilhelmina zu Braunschweig, Fachbereich fuer Chemie, Pharmazie und Biowissenschaften, 1994.

Nitschke, L.; Schüssler, W. Surface water pollution by herbicides from effluents of waste water treatment plants. *Chemosphere* **1998**, *36*, 35−41.

Schneider, E.; Dietrich, R.; Martlbauer, E.; Usleber, E.; Terplan, G. Detection of aflatoxins, trichothecenes, ochatoxin A and zearalenone by test strip enzyme immunoassay: a rapid method for screening cereals for mycotoxins. *Food Agric. Immunol.* **1991**, *3*, 185−193.

Stott, D. I. Review article: immunoblotting and dot blotting. *J. Immunol. Methods* **1989**, *119*, 153−187.

Thurman, E. M.; Meyer, M.; Pomes, M.; Perry, C. A.; Schwab, A. P. Enzyme-linked immunosorbent assay compared with gas chromatography/mass spectrometry for the determination of triazine herbicides in water. *Anal. Chem.* **1990**, 2042−2048.

Vanderlaan, M.; Stanker, L. H.; Watkins, B. In *Immunassay for Trace Chemical Analysis*; Vanderlaan, M., Stanker, L. H., Watkins, B., Roberts, D. W., Eds.; American Chemical Society: Washington, DC, 1991.

Wittmann, C.; Hock, B. Development of an ELISA for the analysis of atrazine metabolites deethylatrazine and deisopropylatrazine. *J. Agric. Food Chem.* **1991**, *39*, 1194−1200.

Wittmann, C.; Hock, B. Analysis of atrazine in food by an enzyme immunoassay. *J. Agric. Food Chem.* **1993**, *41*, 1421−1425.

Wittmann, C.; Bilitewski, U.; Giersch, T.; Kettling, U.; Schmid, R. D. Development and evaluation of a dipstick immunoassay format for the determination of atrazine residues on-site. *Analyst* **1996**, *121*, 863−869.

Received for review November 12, 1997. Revised manuscript received July 6, 1998. Accepted July 9, 1998. We thank the European Union for financial support under the E.U. Environment and Climate Programme, EV5V-CT94-0358.

JF9709663