Analysis of Terbuthylazine in Soil Samples by Two Test Strip Immunoassay Formats Using Reflectance and Luminescence Detection

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Two different immunoassay (IA) formats for terbuthylazine analysis were developed and optimized using the same monoclonal antibodies. The measuring range for the dipstick IA (using an alkaline phosphatase tracer, a 5-bromo-4-chloroindolylphosphate/nitro blue tetrazolium substrate and a portable reflectometer) was from 3 to 300 μg/kg. The teststrip IA (investigating a horseradish peroxidase tracer, a luminol substrate, and a portable luminometer prototype developed by Immunotek, Moscow, Russia) had a measuring range from 0.05 to 10 μg/kg. From 24 soil samples collected in the Veneto area, Italy, 18 samples contained different amounts of terbuthylazine (but <1 μg/kg atrazine) as was analyzed by gas chromatography with mass selective detection. Eight soil samples (six positive, two negative controls) were analyzed according to the two IA formats. Whereas the dipstick with reflectance detection yielded satisfying results, the test strip IA using luminescence detection has failed so far for soil samples.

Keywords: *Terbuthylazine residue; soil sample; immunoassay; reflectance detection; chemiluminescence detection*

INTRODUCTION

Terbuthylazine belongs to the class of *s*-triazine herbicides. The *s*-triazines act as inhibitors of photosynthesis and disturb enzymatic processes in plants. After the *s*-triazine atrazine was put on the market in 1958 by Ciba Geigy, it became the most commonly used *s*-triazine herbicide worldwide to control broad-leaf weeds in different plant cultures. Mainly it was applied to corn fields. Due to its high persistence, evoking a long-lasting load in the water body and in the ecosystem, it was banned in Germany in the spring of 1991. Although it was forbidden in application, atrazine is still detected in ground and drinking water because of its very slow degradation. In agricultural practice other *s*-triazine compounds became significant, mainly terbuthylazine in Germany, but the same in other European countries as in Italy, where terbuthylazine became one of the successors of atrazine.

The methods generally used for trace analysis of pesticides in water, soil, plant, and food samples are gas chromatography (GC) with a nitrogen−phosphorus selective detector (NPD), an electron capture detector (ECD), or a mass selective detector (MSD) or high-performance liquid chromatography (HPLC) connected with UV−vis, diode array, fluorescence, or mass spectrometric detection. Compared to these classic methods in residue analysis, immunochemical methods as immunoassays (IAs) offer many advantages over chromatographic procedures and even capillary electrophoresis (CE). These advantages include the speed of analysis, the high number of samples that can be processed in a day, and time reductions in sample preparation and cleanup procedures. In fact, IAs used in residue analysis are mostly based on microtiter plates as the solid support. They allow the quantification of analytes using photometers, often combined with computer-automated calculation. However, these methods are normally restricted to laboratories. Therefore, our aim was to investigate a suitable IA format for field testing that can be achieved by a dipstick IA format with reflectance detection.

The sensitivity and selectivity of the immunoassay strongly depends on the properties of specific antibodies (e.g., high affinity constants) and the sensitivity of the detection method. The enhanced chemiluminescence reaction (ECL) is described to be one of the most sensitive and rapid detection methods in medical and analytical biochemistry (Kricka and Thorpe, 1983). Therefore, in addition to transforming the dipstick IA format for atrazine analysis [cf. Giersch (1993), Wittmann et al. (1996), and Mosiello et al. (1998)] to a system appropriate for terbuthylazine measurement, a detection principle other than reflectance measurement, in our case chemiluminescence, was studied. In addition, we investigated a portable scanning luminometer (with changeable holders especially adopted for measuring luminescence in polystyrene strips) developed and provided by Immunotek (Moscow, Russia), which is based on a highly sensitive photomultiplier. This device was successfully applied to 2,4-dichlorophenoxyacetic acid (2,4-D) analysis in water by Rubtsova et al. (1997).

The aim of our work is to compare the two detection methods for immunoassay, chemiluminescence, and reflectance, in terms of accuracy, precision, sensitivity, and robustness by measuring real environmental soil samples. In contrast, the aspect of field testing is discussed by comparing the two IA formats with conventional IA.

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MATERIALS AND METHODS

Chemicals. The monoclonal terbuthylazine-specific antibodies P6A7 were kindly provided by Dr. Thomas Giersch (CSIRO, Canberra, Australia). Triazine standards were kindly provided by Riedel de Haen (Seelze, Germany). The triazine derivative for tracer synthesis, 4-chloro-6-(isopropylamino)-1,3,5-triazine-2-(6-aminohexanecarboxylic acid), was synthesized by Dr. U. Doht, Riedel de Haen. In addition, the following reagents were used: alkaline phosphatase from calf intestine (aP, 2500 U mg^{-1} = 41675 nkat; Boehringer, Mannheim, Germany); *N*,*N*′-dicyclohexylcarbodiimide (DCC; Sigma, Deisenhofen, Germany); goat anti-mouse immunoglobulin G (IgG; Sigma); horseradish peroxidase (HRP; 1350 U mg^{-1} = 22505 nkat; Serva, Heidelberg, Germany); hydrogen peroxide, 30% (Merck); *N*-hydroxysuccinimide (NHS; Aldrich, Gillingham, Dorset, U.K.); poly(oxyethylenesorbitan)monolaurate (Tween 20; Merck). All other reagents were of the highest purity grade available.

Chemicals for Dipstick IA: HRP substrate with 3,3′,5,5′-tetramethylbenzidine (TMB, Sigma) and dioctylsulfosuccinate, sodium salt (DSS, Sigma); BM purple aP substrate (Boehringer Mannheim) consisting of 5-bromo-4-chloroindolylphosphate/nitro blue tetrazolium; BM-TETON HRP substrate (Boehringer Mannheim) containing 4-(1,4,7,10-tetraoxadecyl)-1-naphthol; DAB HRP substrate Tablet Set (Sigma) consisting of 3,3′-diaminobenzidine and urea hydrogen peroxide.

Chemicals for Chemiluminescence IA: BM chemiluminescence ELISA reagent for HRP (Boehringer Mannheim) consisting of luminol, 4-iodophenol, and hydrogen peroxide.

Buffers and Solutions. The following were used for the optimized dipstick IA with the alkaline phosphatase tracer: (1) carbonate buffer, 50 mmol L^{-1}, pH 9.6, for coating; (2) Tris-buffered saline (TBS), 50 mmol L^{-1}, pH 7.2 (containing 8.5 g L^{-1} NaCl), for the dilution of the alkaline phosphatase tracer; (3) TBS washing buffer, 5 mmol L^{-1}, pH 7.2 (containing 0.85 g L^{-1} NaCl and 0.5 mL L^{-1} Tween 20), for washing the test strips; (4) BM purple precipitating substrate solution [insoluble substrates for aP, 5-bromo-4-chloroindolylphosphate/nitro blue tetrazolium (BCIP/NBT); stock solutions (a) 0.5 g NBT in 10 mL 70% dimethylformamide and (b) 1.0 g of BCIP in 10 mL of 100% DMF; just before use, add 66 μL each of solutions a and b to 10 mL of 0.1 mol L^{-1} Tris, 0.1 mol L^{-1} NaCl, and 5 mmol L^{-1} $MgCl_2$, pH 9.5].

The following were used for the performance of the optimized microtiter test strip IA with chemiluminescence detection: (1) carbonate buffer, 50 mmol L^{-1}, pH 9.6, for coating; (2) phosphate-buffered saline (PBS), 40 mmol L^{-1}, pH 7.2 (containing 8.5 g L^{-1} NaCl), for the dilution of the peroxidase tracer; (3) PBS washing buffer, 4 mmol L^{-1}, pH 7.2 (containing 0.85 g L^{-1} NaCl and 0.5 mL L^{-1} Tween 20) for washing the test strips; (4) chemiluminescence substrate mixture of 1.0 mmol L^{-1} sodium luminol, 0.5 mmol L^{-1} *p*-iodophenol, and 1.0 mmol L^{-1} hydrogen peroxide in 100 mmol L^{-1} borate/NaOH buffer, pH 8.5.

Preparation of Standards. Ten milligrams of terbuthylazine or related *s*-triazine compounds was dissolved in 10 mL of absolute ethanol with the aid of an ultrasonic bath (1 min). Starting with this solution, a stock solution was prepared consisting of 10 mg L^{-1} terbuthylazine (= excess). A standard series was prepared by making several dilutions of the stock solution to obtain the following terbuthylazine concentrations: 0.01, 0.1, 1, 10, 100, and 1000 μg L^{-1}. The stock solution and the standard series were made up in distilled water.

Equipment. The laboratory equipment used consisted of a photometer for 96-well microtiter plates (ICN, Eschwege, Germany), a microtiter plate washer with 96 channels and a stacker (ICN, Eschwege, Germany), an ultrasonic bath (Sonorex, Bandelin Electronic, Berlin, Germany) and for dipstick IA measurement an RQflex reflectometer (Merck), including the barcode "testroutine". This barcode permitted the output of absorption values in percent measured in the transmission mode at 570 nm. In addition, for chemiluminescence detection a portable scanning luminometer prototype suitable for 8-well test strips (Immunotek, Moscow, Russia, together with Taurus, Weimar, Germany) was used.

Support Materials. For the dipstick IA, the antibodies were immobilized on a membrane Biodyne B (a nylon membrane, pore size = 0.45 μm) from PALL (PALL Filtrationstechnik, Dreieich, Germany). The microtiter strip IA was performed in breakable strips with eight cavities provided by Labsystems (Finland). Further support materials used for comparison were 96-well microtiter plates, type F-form, high binding capacity (Nunc, Roskilde, Denmark).

Other Materials. These included the following: Sephadex G-25 PD10 columns (Pharmacia, Uppsala, Sweden), inert plastic sheets (transparencies, Tartan), and double-sided adhesive tape (3M Deutschland, Neuss, Germany).

Environmental Soil Samples. The soil samples were provided by Dr. Bertin, Castelfranco del Veneto, Italy. Soil samples were collected in the Veneto region, Italy. Twelve sampling sites were selected. Two soil samples (at different depths: top and 20−40 cm) were obtained from each sampling point. Subsamples were collected in each sampling site according to standardized soil sampling procedures. The analytical sample was obtained from the subsamples, ensuring their homogeneity. Twenty-four analytical samples were analyzed in the laboratory of Dr. Bertin (Castelfranco del Veneto, Italy) by GC.

Soil Sample Analysis. Each sample was subdivided into three aliquots. After pretreatment and extraction [following the method described by Steinwandter (1991)], the purified organic solvent extract was subdivided into two aliquots. The first one was directly analyzed by GC, and the second one was diluted with distilled water to obtain "acceptable" organic solvent concentrations for the dipstick and test strip IA analyses.

Methods. The production of the monoclonal antibody P6A7 directed against terbuthylazine has been reported previously by Giersch et al. (1993). The appropriate aP and HRP tracers were synthesized according to the method of Wittmann and Hock (1989) with DCC−NHS activation.

Different membranes (e.g., nylon membranes containing functional groups on their surface from Gelman), various enzyme tracers (using aP and HRP as the enzymes) in combination with various partly commercially available precipitating substrates and different procedures to immobilize the antibodies onto the membranes (e.g., via the system avidin/biotin) were studied [cf. also Giersch (1993) and Wittmann et al. (1996)]. The enzyme immunoassay for terbuthylazine analysis was performed according to the procedure described by Giersch et al. (1993).

Performance of the Optimized Dipstick IA with Reflectance Detection [Modified Method; cf. Wittmann et al. (1996)]. *(i) Preparation of Dipsticks.* To prepare the test strips, the nylon membrane Biodyne B was first coated with a primary antibody (goat anti-mouse IgG). For this purpose, a section of the membrane (10.6 cm × 10.6 cm = 110 cm^2) was cut (using a scalpel, tweezers,and gloves) and incubated in the goat anti-mouse IgG solution (1 μL cm^{-2}, i.e., 1 μL IgG diluted in 60 mL of coating buffer) for 2 h at room temperature on a horizontal shaker with gentle agitation. After being washed twice with TBS washing buffer, the membrane was incubated with the specific monoclonal antibody P6A7 at an antibody concentration of 0.08 μL mL^{-1} (i.e., 6 ng/mL lyophilized serum). Another washing step (see above) followed. Subsequently, to prevent unspecific binding to the membrane surface, a blocking step with 0.5% m/v casein solution for 1 h was performed, which could be omitted in most instances. After a final washing step, the membrane was dried in air at room temperature for ∼30 min. In the last step, the outer 0.5 cm of the membrane edges was removed and 0.8 cm squares were cut, which were mounted onto an inert plastic support using double-sided adhesive tape. At this stage the test strips (144 dipsticks were obtained) were ready for use in the assay and could be stored at 4 °C for at least 1 month.

(ii) Assay Protocol. To perform the dipstick assay all incubation steps were performed in 2 mL glass tubes. Prior to the assay the glass tubes were rinsed with 2 mL of TBS washing

buffer for 10 min, and the washing buffer was thoroughly removed. The test strips were then dipped into the solutions for as long as required. For the immunoreaction, the test strips were incubated with a mixture of 800 μL of the standard or sample and 200 μL of the aP enzyme tracer (dilution = 1:5000) for 10 min. After three washes with TBS washing buffer, 800 μL of the BCIP/NBT substrate solution was added and incubation was continued for 30 min. The reaction was stopped by shortly rinsing the test strips with distilled water. Total assay time was 45 min. The dipsticks were inserted in the RQflex reflectometer and measured at 570 nm (green LED) as the absorption maximum of the colored product lies at 600 nm (determined by a scanning densitometer, DESAGA, Heidelberg, Germany).

(iii) Estimation of Dipstick IA Results. The measured absorption data were normalized according to the equation

$$R_{NORM}\,(\%) = [(R - R_0)/(R_E - R_0)] \times 100 \qquad (1)$$

where R_{NORM} = normalized remission, R_0 = remission of the zero control, R = measured remission of the standard or sample, and R_E = remission of the analyte excess concentration (excess = concentration where the signal almost reaches its maximum).

Performance of the Optimized Microtiter Strip IA with Chemiluminescence Detection [cf. Rubtsova et al. (1997)]. The microtiter strip IA was performed in breakable strips (eight cavities). The IA was carried out as follows: the wells of the microtiter strips were coated with 200 μL of goat anti-mouse IgG (dilution = 1:1000 in carbonate buffer) by incubation at 4 °C overnight. All further steps were carried out at room temperature. The wells were washed three times with PBS washing buffer. Two hundred microliters of the monoclonal antibody P6A7 (in a dilution of 1:20000 in PBS buffer) was then added to each well. The microtiter strips were incubated for 2 h. After three washes with PBS washing buffer, 200 μL of standard solution or sample together with 50 μL of the HRP enzyme tracer (dilution 1:20000 in PBS buffer) was added to each cavity and incubated for 1 h. After a final washing step, three times with PBS washing buffer, the enzyme activity was determined. For chemiluminescence detection, 200 μL of the substrate mixture was added to each well. The substrate mixture consisted of the following compounds/reagents: 1.0 mmol L^{-1} sodium luminol, 0.5 mmol L^{-1} *p*-iodophenol, 1.0 mmol L^{-1} hydrogen peroxide in 100 mmol L^{-1} NaOH/borate buffer, pH 8.5. Each 8-well strip was inserted in the holder of the portable scanning luminometer prototype, and the light intensity was measured. The value of the maximum light intensity in each well was used for the calculations (cf. eq 1, where remission is replaced by the maximum light intensity or relative luminescence units, respectively).

GC Analyses. The soil sampling and subsequent GC analyses were performed by Dr. Placido Bertin, Ente di Sviluppo Agricolo del Veneto, Centro Agrochimico, Castelfranco Veneto, Italy. For GC analysis a Hewlett-Packard device was used with nitrogen−phosphorus detector (NPD) and for peak identification a mass selective detector (MSD) in selected ion mode using three ions (molecular ion, base peak, and confirming ions). The GC separation was performed with a 30 m quartz capillary column covered with methyl silicone with He carrier gas for the MSD. The detection limit for terbuthylazine with the GC/MSD was 1 μg/L.

RESULTS

The dipstick IA format with reflectance detection was optimized using an aP tracer and a commercially available enzyme substrate. With this format, the most promising results were obtained using the monoclonal antibodies P6A7 immobilized by a direct method to Biodyne B membranes. The measuring range was 3−300 μg/L terbuthylazine or kilograms, respectively

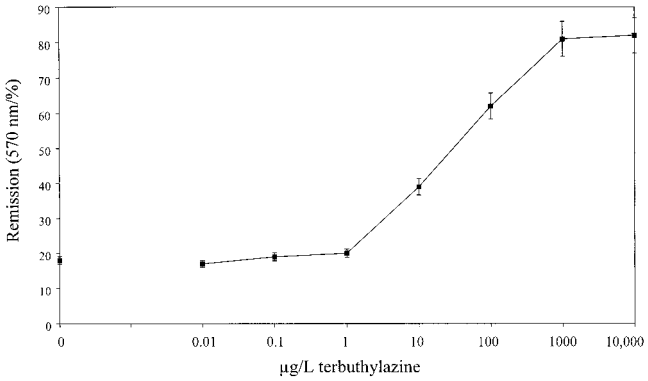

Figure 1. Representative terbuthlyazine calibration graph obtained with the dipstick IA based on the monoclonal antibodies P6A7 using reflectance detection. The tests were run six times. The 2-fold standard deviations (±2s, representing 95% confidence intervals) are indicated as error bars.

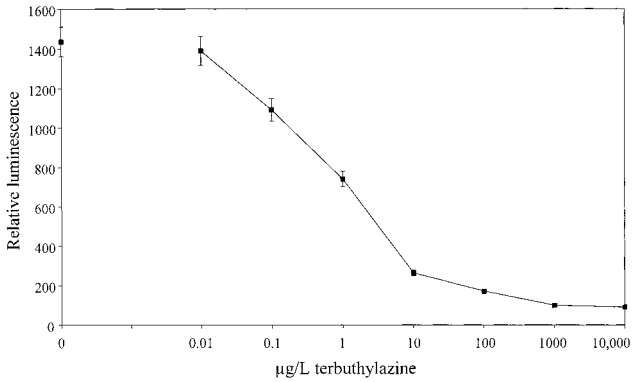

Figure 2. Representative terbuthylazine calibration graph obtained with the eight-well test strip IA based on the monoclonal antibodies P6A7 using luminescence detection. The tests were run six times. The 2-fold standard deviations (±2s, representing 95% confidence intervals) are indicated as error bars.

(cf. calibration curve in Figure 1). With the dipstick IA format used for atrazine measurements [cf. Wittmann et al. (1996)] transferred to terbuthylazine analysis using the monoclonal antibody P6A7 problems arose with the dye used for reflectance detection. When an HRP tracer was used in combination with tetramethylbenzidine as the enzyme substrate, the blue color developed in the enzymatic reaction did not remain on the dipstick (although dioctylsulfosuccinate, sodium salt, was taken for precipitation) but leached out into the solution. In addition, using this IA format the detection limit for terbuthylazine was 10 μg/L with a measuring range up to 500 μg/L. The measuring range for terbuthylazine by the original ELISA [as described by Giersch et al. (1993)] was only 0.3−3 μg/L and, thus, a factor of 10 lower than with the optimized dipstick IA format.

In contrast, the measuring range for the terbuthylazine test strip IA with chemiluminescence detection using the luminometer prototype was from 0.05 to 10 μg/L ELISA (cf. calibration curve in Figure 2), showing a lower detection limit and a greater dynamic range as compared with that originally developed.

Table 1 shows the results of soil sample measurement (the samples containing terbuthylazine) by the classical GC with NPD. The positive results (i.e., peaks with retention times identical to those of the terbuthylazine standard) were confirmed by GC using a mass selective detector. From 24 soil samples collected, 18 samples

Table 1. Environmental Soil Samples[a]

sample	terbuthylatrazine concentration (μg/L)	sample	terbuthylatrazine concentration (μg/L)
1151	**13.9**	1172	165
1162	**22.2**	1173	9.2
1163	11.4	1174	15.6
1164	10.1	1175	6.4
1165	19.2	1176	6.9
1166	**4.77**	1177	12.6
1168	**9.1**	1178	1.96
1170	**7.1**	1179	3
1171	139	**1180**	**2.4**

[a] Twenty-four samples were collected in the Veneto region, Italy, and analyzed by GC/NPD and GC/MSD for peak identification. The sampling and the GC analyses were performed by Dr. Placido Bertin, Castelfranco Veneto, Italy. From these samples 18 were positive for terbuthylazine. The atrazine concentration detected in all soil samples was <1 μg/L. The detection limit for atrazine and terbuthylazine was 1 μg/L by GC/MSD.

Table 2. Environmental Soil Samples Analyzed by GC/MSD and by the Two Immunoassay Formats[a]

sample	μg/kg terbuthylazine by GC/MSD	μg/kg terbuthylazine by dipstick IA with reflectance detection	μg/kg terbuthylazine by microtiter test strip IA with chemiluminescence detection
1151	13.9	15 ± 0.8	20.9 ± 0.02
1158	<1	<3	3.0 ± 0.03
1159	<1	<3	10.8 ± 0.002
1162	22.2	23 ± 0.9	53.5 ± 0.05
1166	4.8	5 ± 0.6	4.7 ± 0.01
1168	9.1	10 ± 0.4	18.7 ± 0.03
1170	7.1	7 ± 0.5	12.3 ± 0.08
1180	2.4	3 ± 0.6	1.8 ± 0.01

[a] The atrazine content in all samples was <1 μg/L.

were positive for terbuthylazine (2 samples exceeded 100 μg/kg and 9 samples exceeded 10 μg/kg). The atrazine concentration detected in all soil samples was <1 μg/kg.

Table 2 shows the results of soil sample analysis by the two test strip IA formats, with reflectance and luminescence detection. From the 18 soil samples containing terbuthylazine, 6 were chosen and analyzed by the two IA formats. In addition, two (surely) negative soil samples were used in the soil sample set for IA measurement to check for false positive results. Although it was expected that due to the lower detection limit and the greater dynamic range of the test strip IA with chemiluminescence detection this format should yield the more precise and accurate results, this was obviously not the case. As can be clearly seen from Table 2, disturbances leading to false positive results (in the case of the negative controls) occurred. These could derive from matrix effects, although the probability of the soil matrix affecting the analysis was quite low because the samples had to be diluted prior to the measurement at least by a factor of 10. The aspect that the antibody could have cross-reacted with other *s*-triazines or relevant metabolites could be neglected as the monoclonal antibodies used exhibited no major cross-reactivity with deethylterbuthylazine or deethylatrazine (the major degradation products of terbuthylazine) as shown in Table 3. Surprisingly, there was a satisfying correlation of dipstick IA results (using reflectance detction) with the GC/MSD data, although the relative standard deviation was relatively high (up to 20%).

Table 3. Cross-Reactivity Pattern of the Monoclonal Antibodies P6A7 with Different *s*-Triazines (Determined Using the Two IA Formats Described)[a]

compound	% cross-reactivity	compound	% cross-reactivity
atrazine	7	dichlorosimazine	0.3
dichloroatrazine	0.4	propazine	1.0
deethylatrazine	0.5	terbuthylazine	100.0
hydroxyatrazine	0.1	cyanazine	6.5
simazine	3		

[a] Deisopropylatrazine, deethylterbuthylazine, deethyldeisoproplyatrazine, <0.1%; ametryn, simetryn, prometryn, terbutryn, aziprotryn, atratone, not detectable.

DISCUSSION

From the two test strip IA formats developed, only the dipstick IA with reflectance detection could so far successfully be applied to the measurement of environmental soil samples. The test strip IA with luminescence detection showed a better measuring range but failed in the measurement of the soil samples. Partly, severe overestimations could be observed. This was not expected as due to the more sensitive measuring range the soil extracts could be diluted at least by a factor of 10. Therefore, potential matrix effects should be prevented by the dilution step, but this was not the case. The suitability of this IA format was proved by Rubtsova et al. (1997) for the analysis of 2,4-D in spiked water samples. However, in our case, even using a high dilution yielded a greater deviation, whereas the relative standard deviations of the calibration curve for terbuthylazine were quite low. It can be assumed, however, that either matrix effects or the detection principle using the luminometer prototype caused the overestimations. As a consequence, the method should be improved prior to the study of real samples. The existence of matrix effects was verified by the measurement of spiked soil samples in the study of two different kinds of soil (a sandy loam and a clay soil), in which the majority (80%) of the results were wrong (unpublished data). The data are not shown here as about half of the samples were false positive and several samples turned out to be false negative without any clear tendency. Therefore, no explanation was possible and further experiments have to be performed to elucidate the reason.

However, from the 24 soil samples analyzed 18 samples contained terbuthylazine, but atrazine contents were <1 μg/kg in all 24 soil samples. As Bowman et al. (1989) showed in their experiments with field lysimeters studying the mobility and persistence of commercial formulations of the herbicides atrazine, metolachlor, and terbuthylazine, both atrazine and terbuthylazine eluted within 24 h after application, resulting from a heavy water application. The authors suggested that agricultural chemicals (such as terbuthylazine) might be quite vulnerable to leaching for a period following application. It can be assumed that in our case, the terbuthylazine contents in the soil samples resulted from an application several months earlier. Sahid and Teoh (1994) investigated the persistence of *s*-triazines in soil and showed the dependency on the rate of application and environmental factors such as soil moisture, temperature, pH, and constituents. The result of their study was that under tropical conditions, terbuthylazine is not expected to accumulate in the agricultural soil ecosystem or to cause any deterimental effect on subsequent crops. The latter is quite remarkable, as the terbuthylazine concentrations in two soil samples in our case exceeded 100

μg/kg. According to the findings of Molinari et al. (1995), even with these terbuthylazine concentrations found in soil, the risk of a possible migration of terbuthylazine into plants cultivated (being the raw material for food productions) could be estimated to be low. Although Molinari et al. (1995) analyzed the possibility of migration of atrazine, simazine, terbuthylazine, molinate, and bentazon herbicides from contaminated water used in food manufacturing of gorgonzola and mozzarella cheeses (in an agricultural plant in an area in northern Italy), this might be transferred to the influence of terbuthylazine "contaminated" soil on food production. In all water samples they detected herbicide residues, but in the finished products they could not determine herbicide residues (although residues were found in some intermediate products).

Comparing the two test strip formats with the original enzyme immunoassay (EIA) as developed by Giersch et al. (1993), one must concede that with the EIA a higher sample throughput could be realized, guaranteeing an easier handling. The main advantage of the test strip IA format is in allowing, in principle, a field test. However, this field assay could be realized only for water samples for which no sample pretreatment or cleanup was required prior to the analysis.

It is often stated that IA, in general, is less precise and accurate than chromatographic methods such as HPLC and CE (Dinelli et al., 1995), especially in ground water samples. The main reason for this is described to be the cross-reactivities of the antibodies, which might result in overestimations. On the contrary, Dankwardt et al. (1997) showed that a terbuthylazine EIA could be used for a more rapid analysis of water samples, although the results were confirmed by additional GC/MSD analyses.

In conclusion, from the two test strip IA formats developed, only the dipstick IA with reflectance detection could be applied to the measurement of environmental soil samples, although the possible field testing character of the dipstick IA did not provide a big advantage in the case of soil analysis.

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