Evaluation of a Field Test Kit for Triazine Herbicides (SensioScreen® TR500) as a Fast Assay to Detect Pesticide Contamination in Water Samples

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Abstract

A field-test kit (*SensioScreen*[®] *TR500*) for the determination of triazine herbicides in water samples has been evaluated. The test is based on an ELISA method performed on a membrane that allows the visual estimation of the presence of triazine herbicides in less than ten minutes. The kit contains all necessary reagents and equipment, and operates on a very simple protocol. The detection level of this semiquantitative kit has been set at 0.5 µg L⁻¹ (for the sum of all triazines), although concentration levels of 0.1 µg L⁻¹ for atrazine can be detected. This is the first time that a commercial field-test kit to control water contamination by herbicides performs in compliance with the EU and USA legislations regarding the limit of detection reached. Evaluation and validation studies have been performed using spiked, certified (AquaCheck) and real environmental samples comparing the results with chromatographic methods. In spite of the semiquantitative-qualitative character of the kit, the results demonstrate that the test *SensioScreen TR500* provides a very good estimation of the atrazine concentration in the sample. This test format could be adapted to the detection of other contaminants of environmental relevance.

Keywords: Atrazine, triazines, on site, rapid field test, immunomembrane, ELISA

1. INTRODUCTION

Atrazine and other s-triazine compounds are widely used selective herbicides for weed growth control in agriculture. The chemical features of these herbicides provoke a good mobility of these compounds in the aquatic media and a high persistence in the environment[1-5]. Their continuous presence is a risk for the equilibrium of numerous ecosystems. Therefore an accurate and efficient control of the presence of s-triazine herbicides and its residues in the environment is necessary.

In a previous work we described the development of a microplate ELISA (enzyme-linked immunosorbent assay) and demonstrated its performance as a quantitative method for the determination of atrazine in environmental water samples [6]. The ELISA had an IC_{50} (concentration producing a 50% reduction of the maximum absorbance of the assay) of 0.28 nM (60 ng L⁻¹) and a detection limit of 0.043 nM (9 ng L⁻¹). Immunochemical screening methods raise many advantages to the analytical environmental field since many samples can be processed simultaneously, the tests do not require large volumes of sample and often, direct determination of low concentration levels is possible with a high specificity. However, isolated farming areas or small councils often lack of specific laboratories to perform, on a frequent-manner, control of the quality of their well waters, particularly when those are the source for edible water. Monitoring on almost daily basis, especially during pesticide spraying seasons, would improve health safety of the population. Thus, the possibility to perform *in situ* qualitative estimations of the quality of the waters, regarding pesticide contamination, would help to take safe decisions such as to use other sources of water or taking samples to perform the necessary analysis in well-equipped laboratories. With this purpose we have developed a fast and simple field-test kit that is in agreement with the European legislation regarding maximum overall pesticide level (0.5 µg L⁻¹) in drinking waters.

During the last years, several membrane-based assays, both in test strip and immunofiltration formats have been described for the rapid field determination of pesticides in environmental samples [7-13]. However, their limits of detection not always reach the requirements of the UE legislation. Due to their extensive use, triazine herbicides have been considered as indicators of pesticide contamination. Thus, detection of triazines may indicate potential contamination of the water by other pesticides. Based on polyclonal antibodies against atrazine prepared as previously

reported [6] a new field-test assay has been developed and made on a commercial format. The present paper describes performance of the test (SensioScreen® TR500, Sension GmbH, Germany) and the work made for its evaluation. The results demonstrate that visual estimation of contamination by triazine herbicides can be accomplished in less than 10 min reaching limits of detection near 0.1 $\mu\text{g L}^{-1}$ for atrazine and 0.5 $\mu\text{g L}^{-1}$ for mixtures of triazine herbicides.

2. MATERIALS AND METHODS

2.1. Chemicals.

Chemical reagents were purchased from Aldrich (Milwaukee, WI). Pesticide standards such as terbutylazine and ametryne were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Atrazine, simazine and irgarol 1051 were obtained as a gift from Ciba-Geigy (Barcelona, Spain). Deethylatrazine and propazine were purchased through Riedel-de-Haën (Seelze-Hannover, Germany). Prometryne was from PolyScience (Niles, IL). Stock solutions (1 mg mL⁻¹) of atrazine, terbutylazine, propazine, simazine, deethylatrazine, prometryne, ametryne, and irgarol were prepared in DMSO and stored at 4 °C. Diluted solutions were prepared from the stock in PBST for the selectivity studies. Mixture of sea salts used to prepare artificial seawater was obtained from Sigma (St. Louis, MO).

2.2. Biochemicals and Immunochemicals.

Proteins and enzymes were acquired from Sigma (St. Louis, MO). The antisera (As46 and As47) against atrazine were prepared as previously reported [6] by immunizing white New Zealand rabbits with 2d-KLH conjugate (*N*-(4-chloro-6-isopropylamino-[1,3,5]triazin-2-yl)-6-aminohexanoic acid coupled to keyhole limpet hemocyanin by the mixed anhydride method. The antisera (As) were used without further purification and were stored frozen in the presence of NaN₃. Working aliquots were stored at 4 °C. The enzyme tracer 2d-HRP (hapten 2d coupled to horseradish peroxidase) was synthesized by the active ester method as described [6].

2.3. Buffers used for the microplate ELISA.

Unless otherwise indicated PBS is 10 mM phosphate buffer, 0.8 % saline solution and unless otherwise indicated the pH is 7.0-7.2. Coating buffer is 50 mM carbonate-

bicarbonate buffer pH 9.6. PBST is PBS with 0.05 % Tween 20. 2X PBST is PBST double concentrated. Citrate buffer is a 40 mM solution of sodium citrate pH 5.5. The substrate solution is Enhanced K-Blue TMB (tetramethylbenzidine) Substrate (Neogen, Lexington, USA) and is used directly without prior dilution.

2.4. Instrumentation.

The pH and the conductivity of all buffers and solutions were measured with a pH-meter pH 540 GLP and a conductimeter LF 340, respectively (WTW, Weilheim, Germany). For the *Microplate ELISA*, polystyrene microtiter plates were purchased from Costar (Bodenheim, Germany). Washing steps were carried out on an 812 SW1 microplate washer (SLT Labinstruments GmbH, Salzburg, Austria). Absorbances were read using a Spectra microplate reader (SLT Labinstruments GmbH) at a single wavelength mode of 450 nm. The competitive curves were analyzed with a four-parameter logistic equation using the software Easyfit (SLT Labinstruments GmbH). Unless otherwise indicated, data presented correspond to the average of at least two well replicates.

2.5. Microplate ELISA protocol As46 / 2d-HRP.

Microtiter plates were coated with As46 in coating buffer (1/20000, 100 µL/well) overnight at 4 °C covered with adhesive plate sealers. The following day the plates were washed with PBST (3 times, 300 µL/well). Atrazine standards (0.03 nM to 2000 nM) or samples were added to the coated plates (50 µL/well) followed by the solution of the enzyme tracer 2d-HRP (1/40000 in PBST, 50 µL/well) and the mixture incubated 15 min at RT on a shaker. The plates were washed as described before and a solution of the substrate tetramethylbenzidine (TMB in citrate buffer) was added (100 µL/well). The enzyme reaction was stopped after 10-15 min at room temperature with 4M H₂SO₄ (50 µL/well) and the absorbances were measured at 450 nm.

2.6. Description of the kit (*SensioScreen*[®] TR500).

Each commercial package consist of: a) three cassettes and on each of them there is space for five samples or standards; b) four dropping bottles with the necessary solutions to perform the test (enzyme tracer (solution A) whashing solution (solution B), substrate (solution C), color stabilizer (solution D); c) three dropping bottles containing atrazine standards (0, 0.5 and 10 µg L⁻¹) and d) dropping bottles to place samples. *SensioScreen*[®] TR500 is stable for about six month when stored in the refrigerator.

After storage at ambient temperature the activity remains unchanged for about 48 h (see figure 1).

2.7. Kit Working Protocol.

The test is performed in less than ten minutes as shown in table 1 starting by adding samples, standards and immunoreagents in the following order: standards or samples (1 drop), solution A (1 drop), solution B (3 drops) and solution C (1 drop). Wait for 3 to 5 minutes to obtain acceptable color development. Finally add solution D (1 drop) and evaluate the results visually comparing the color of the samples to the one of the standards.

2.8. Evaluation Studies.

Several parameters such as selectivity, effect of the pH, matrix effect, accuracy, etc. were evaluated with the *SensioScreen*[®] TR500 using different kinds of samples.

2.8.1. Description of the samples.

Spiked Samples were prepared in MilliQ water, artificial seawater (**M1**, 35 ‰ sea salts in MilliQ water) and 15 real samples (**M2-M6**, groundwater; **M7-M11**, drinking water, **M12-M16**, surface water) from different geographical origins (see table 6)

Certified Samples from AquaCheck n° 8 were obtained from AquaCheck (WRC, Medmenham, UK) and contained mixtures of several pesticides (PCB(28), PCB(52), PCB(101), PCB(118), PCB(138), PCB(153), PCB(180), simazine, atrazine, propazine, MCPA, MCPB, mecoprop, 2,4-D, isoproturon, diuron, linuron, clortoluron, azinphos methyl, diclorvos, phenitrothion, malathion, mevinfos, clorpheninfos, diazinon, ezinfos ethyl, phenthion, parathion ethyl, and parathion methyl) at different concentrations.

Real Samples. Four environmental samples were collected from the Ebre Delta zone (Tarragona, Spain) at different sampling points (**1**, channel; **2**, cane field; **3**, harbor) and times (**1**, May 98; **2**, April and May 98; **3**, May 98)

2.9. Selectivity Studies.

2.9.1. Microplate ELISA.

Stock solutions (1 mg mL⁻¹) of different structurally related triazine pesticides (atrazine, ametryne, deethylatrazine, propazine, simazine, tertbutylazine) were prepared in DMSO. Standard curves for each of these compounds were build in PBST (0.03 nM to 2000 nM)

and for each curve the IC_{50} was determined in the optimized ELISA. The crossreactivity values were calculated according to the following equation: $[IC_{50}(\text{atrazine})/IC_{50}(\text{triazine derivative})] \times 100$.

2.9.2. Test kit

- (a) On a first set of experiments individual solutions of s-triazine herbicides (atrazine, terbutylazine, propazine, simazine, deethylatrazine, prometryne, and ametryne) were analyzed at two-concentration levels ($0.5 \mu\text{g L}^{-1}$ and $10 \mu\text{g L}^{-1}$) and the color obtained compared with the one developed by atrazine at five different concentrations (0, 0.1, 0.5, 5 and $10 \mu\text{g L}^{-1}$). The so-called *pseudo cross reactivity* coefficients were calculated according to the following formula: $\%CR = 100 \cdot (C_a / 10 \text{ or } 0.5)$, where C_a is the concentration of atrazine affording a similar color.
- (b) On a second set of experiments, solutions containing mixtures of atrazine (constant concentration, $0.5 \mu\text{g L}^{-1}$) and terbutylazine (three different concentrations, 0.5, 1, and $10 \mu\text{g L}^{-1}$) were prepared and analyzed with the test kit.

2.10. Effect of the pH.

Atrazine solutions ($0.5 \mu\text{g L}^{-1}$) were prepared in PBST with the pH adjusted to 3, 4, 5, 6, and 11 and measured by the test kit to visually compare with color developed by the standards (pH 7.5).

2.11. Matrix Effects.

Samples from different origins were spiked with atrazine and terbutylazine solutions (0; 0.3; 0.5; 1; 3 and $10 \mu\text{g L}^{-1}$). All samples were measured before and after spiking and the color developed compared with the one produced by the atrazine standards prepared in PBS.

2.12. Semi-Quantitative Accuracy Studies performed with the Test Kit.

2.12.1. Analysis of Spiked Blind Samples.

Eight blind samples were prepared by a third person in MilliQ water by spiking them with atrazine at different concentrations. The samples were analyzed with the test kit to visually estimate the concentration comparing the color with that developed by the standards provided by the supplier (0, 0.5 and $10 \mu\text{g L}^{-1}$).

2.12.2. Analysis of Certified Samples.

Fifteen AquaCheck certified samples of the group n° 8 containing different pesticides were analyzed and the total concentration of triazines estimated with the test kit. The visual estimation was compared later with the real value.

2.12.3. Correlation Studies.

Real natural water samples were analyzed using the field-test kit and the estimated concentration results compared to those obtained using an optical evanescent wave (EW) immunosensor [14, 15] with fluorescent detector (RIANA) and by liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry (LC-APCI-MS)[15].

2.13. Study of the Objectivity of the Test Kit.

Seven persons were selected to participate on a joint study evaluating individually, by the visual estimation, the triazine concentration present in the AquaCheck samples mentioned above. Only four of the persons had previous experience using immunochemical methods.

3. RESULTS AND DISCUSSION

SensioScreen® TR500 is a field test designed for the rapid and highly sensitive detection of the contamination of water samples by triazine herbicides. The kit is mainly addressed to have a direct and fast semi-quantitative estimation of the potential contamination of potable waters by pesticides. The test can be performed in less than 10 minutes and does not need additional instrumentation or pipetting equipment (see figure 1). According to the EC-drinking water guidelines, the total amount of pesticides in drinking water is not allowed to exceed $0.5 \mu\text{g L}^{-1}$. As we will show in this paper other frequently occurring triazine herbicides are also detected, and therefore the test can be considered as a total triazine qualitative-semiquantitative test method. A triazine-positive result will indicate necessity of confirmation by more robust analytical methods. Most of the widely used triazine herbicides are detected at concentrations of $0.5 \mu\text{g L}^{-1}$, but for atrazine a detection limit near $0.1 \mu\text{g L}^{-1}$ can be achieved.

The test is based on the selective recognition of triazine herbicides by antibodies and works according to the protocol described in table 1. The triazine herbicides in the sample and a triazine-enzyme conjugate compete for the binding sites of the antibodies immobilized on a membrane (see figure 1). After rinsing the membrane, a color signal

is created by the enzymatic turnover of the substrate. The intensity of the blue color is inversely proportional to the concentration of triazines in the sample. This means, negative samples show a dark blue color (as the blank standard), positive samples a light blue or no color (as the standards). The samples are evaluated by direct visual comparison to the standards.

The field-test kit was developed after investigation of the immunoassay performance on a microplate format using antibodies and immunoreagents prepared as described [6]. The microplate ELISA allowed establishing the combination As46 and enzyme-tracer 2d-HRP as the most appropriate to accomplish detection of atrazine at the trace level. The optimized microplate assay showed an IC_{50} of 47 ng L⁻¹ and a LOD (80% B/B₀) of 10 ng L⁻¹ (see figure 2).

3.1. Selectivity of the Test Kit.

The *selectivity* of the test was estimated by calculating the so-called *pseudo-cross reactivity* coefficient of several s-triazine herbicides at two concentration levels (0.5 and 10 µg L⁻¹). The coefficient correlates the concentration of the cross-reactant by comparing visually the color developed, with the color produced by different concentrations of atrazine. The results shown in table 2 demonstrate that at both concentration levels the selectivity estimated with the *SensioScreen*® TR500 test is very similar with the selectivity calculated using the microplate ELISA. As observed in other reported atrazine ELISAs [16-18], the herbicide propazine was better recognized than atrazine, with a crossreactivity value of 313% in the microplate format. The herbicide terbutylazine, frequently used in Germany, alone or in combination with other triazine herbicides, is also recognized. Therefore mixtures of atrazine and terbutylazine were investigated. The later herbicide only interfered with the estimation of atrazine concentration at levels of 10 µg L⁻¹ or higher (data not shown), demonstrating the possibility to use the test as a semiquantitative-method for atrazine under these conditions.

3.2. Effect of the pH.

Regarding the *pH*, it was observed that the response of *SensioScreen*® TR500 was stable at pH values ranging from 4 to 11 (see figure 3). No significant differences in the blue color intensity were observed when comparing the color to the atrazine standard prepared at the same concentration (0.5 µg L⁻¹) but at pH 7.8. The assay tolerates better basic pH solutions. Thus, at pH 11 the color was slightly more intense, but at pH

3 an important decrease of the signal was observed. These results are in agreement with the behavior observed on the microplate ELISA.

3.3. Evaluation of the Accuracy of the Test Kit.

To assess *semi-quantitative test accuracy* we performed different types of experiments. Initially, we measured 11 blind samples prepared in milliQ water and analyzed the correlation of the estimated results with the real concentration values by comparing the blue color intensity of the samples and standards. As it is shown in table 3, the results of the visual estimation of the atrazine concentration match very well with the real spiked concentration value. Only sample 5 was underestimated.

A second set of experiments consisted on estimating the concentration of AquaCheck certified samples. These samples contained mixtures of several environmental contaminants including three triazine herbicides (see experimental section for description of these samples). Table 4 shows the results of the visual estimation compared to “total amount” of s-triazines in the samples. The “total amount” was calculated using the corresponding cross-reactivity coefficients determined in the microplate ELISA. From fifteen samples, the concentration of 6 were erroneously estimated (see table 5) when compared to the atrazine standards (0, 0.5 and 10 $\mu\text{g L}^{-1}$). These samples had triazine concentration values very close to the standard of concentration 0.5 $\mu\text{g L}^{-1}$, On this situation the visual test allows prediction of a concentration near 0.5 $\mu\text{g L}^{-1}$, but fails to discriminate when it is only slightly higher or lower than the standard.

3.4. Evaluation of the Objectivity of the Test Kit.

Due to the fact that the concentration is estimated visually, we were concerned about the possibility that a certain degree of subjectivity could determine the results obtained, due to our familiarity with the test. To assess the degree objectivity of the *SensioScreen® TR500* we designed an experiment consisting on a panel of seven persons (only four had experience using microtiter plate ELISA methods) evaluating individually the concentration of the Aquacheck Certified samples above mentioned. Since the estimation of the concentration was performed by comparison of the samples with the atrazine standards provided by the supplier (0, 5, 10 $\mu\text{g L}^{-1}$) we defined three ranges of concentration possible: A) 0 to 0.5 $\mu\text{g L}^{-1}$; B) 0.5-10 $\mu\text{g L}^{-1}$ and C) more than 10 $\mu\text{g L}^{-1}$ and ask the persons involved in the experiment to classify the samples into the A, B or C groups. As it can be seen in table 5, the results showed a suitable objectivity for the test. In some cases the differences observed could be attributed to

the fact that the concentration is placed near of the middle point of the intervals above defined (ex. around $0.5 \mu\text{g L}^{-1}$), which made difficult to classify the sample in one or another group (*A* or *B*). The option for the middle point should had been included in the initial design of the experiment. Nevertheless, in almost all cases more than 50% of the analyzers assigned the expected value to the concentration and the rest gave a value close to it.

3.5. Analysis of Environmental Samples.

To evaluate potential *effects of the matrix*, artificial seawater and 15 environmental samples (surface water, groundwater and drinking water) from different origins (see table 3) were initially assayed unspiked with the *SensioScreen*[®] *TR 500*. Most of the unspiked samples showed a color development similar to the blank atrazine standard except for the seawater (**M1**) and for one (**M6**) from the five groundwater samples that showed a slight color reduction (see table 6). For the case of the artificial seawater, this behavior was attributed to its high salinity (108.5 mS cm^{-1} , pH= 8.06) compared if compared to PBS (15.6 mS cm^{-1} , pH 7.5) or any other water sample (i.e. **M11** 12.04 mS cm^{-1} , pH 7.42). For the case of **M6** the matrix effect observed could not be explained as a consequence of its conductivity value (48.6 mS cm^{-1} , pH 7.13) since atrazine standard samples prepared in PBS with a conductivity value near 50 mS cm^{-1} did not produce an inhibition in the color development. Therefore, in this case, it must be contemplated the existence of a matrix effect or a slight contamination by triazines. When some of these samples not showing matrix effect, were spiked with atrazine, a reduction of the color development was observed in accordance to the concentration value (see table 6). The triazine contamination level observed by the *SensioScreen*[®] *TR 500* was matched very well the spiked value. Similar experiments were performed spiking with terbutylazine. In this case the decrease in color that was less compared to atrazine (data not shown), in agreement to the lower cross-reactivity value observed for this analyte (see above).

Finally, contamination by triazines of real water samples was tested using the *SensioScreen*[®] *TR500* and the estimated concentration values compared to results obtained when analyzing these same samples by LC-APCI-MS and an EW (evanescent wave) immunosensor under evaluation in our laboratory at that moment [15]. The estimation of the triazine concentrations of those samples using the semiquantitative test-field matched very well the results obtained by the other two quantitative methods (see table 7).

4. CONCLUSIONS

SensioScreen[®] *TR500*, a field-test for the rapid and sensitive detection of s-triazine herbicides has been evaluated. This test is intended to the qualitative or semi-quantitative screening of water samples (tap water, surface water, etc.) and does not require previous sample preparation. With the test *SensioScreen*[®] *TR500* the samples are evaluated by direct visual comparison to the standards. It is not supposed to replace determinations by conventional methods but allows estimating if the samples are negative or having concentration lower, near or higher than 0.5 µg L⁻¹, the limit tolerated by the EC for total pesticide concentration in edible waters.

Due to the variety of water samples, matrix effects causing test interferences cannot be excluded. The experiments carried out and described in this paper demonstrate that *SensioScreen*[®] *TR500* field test is a fast tool to easily check the quality of potable waters, regarding pesticide contamination, in rural farm and agricultural areas. These controls would be particularly interesting during pesticide spraying seasons since regular monitoring of the quality of the waters in well-equipped laboratories is usually not possible.

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Figure Legends

Figure 1. *Sensio Screen*[®] TR500 commercial kit. Each commercial package consist of: **a)** three cassettes with space on each of them for five samples or standards, **b)** four dropping bottles with the necessary solutions to perform the test (enzyme tracer (solution A), washing solution (solution B), substrate (solution C, color stabilizer (solution D); **c)** three dropping bottles containing atrazine standards (0, 0.5 and 10 $\mu\text{g L}^{-1}$) and **d)** dropping bottles to place samples. On each well, the assay is based on an immunofiltration ELISA format

Figure 2. Calibration curve obtained for atrazine using the microtiter plate ELISA (As 46/2d-HRP). A four-parameter logistic equation was used to fit the standard curve according to the following formula: $y=(A-D)/[1+(x/C)^B]+D$, where A is the maximal absorbance of the assay, D is the minimal absorbance, B corresponds to the slope of the sigmoid curve and C is the concentration in $\mu\text{g L}^{-1}$ inhibiting the binding of the enzyme-tracer to the antibody by 50%. Represented points correspond to the average of standard curve with three well replicates.

Figure 3. Digitalized picture showing the effect of the pH on the test result when analyzing atrazine at 0.5 $\mu\text{g L}^{-1}$ at different pH values (atrazine standard 1 is 0.5 $\mu\text{g L}^{-1}$ and standard 2 is 10 $\mu\text{g L}^{-1}$, both at pH 7.5).

Table 1. Working protocol of the SensioScreen® TR500 commercial kit.

step	reagent	drops	aperture
1	blank ($0 \mu\text{g L}^{-1}$)	1
2	sample 1	1
3	standard 1 ($0.5 \mu\text{g L}^{-1}$)	1
4	sample 2	1
5	standard 2 ($10 \mu\text{g L}^{-1}$)	1
6	solution A (enzyme tracer)	1
7	solution B (washing buffer)	3 x 1
8	solution C (TMB substrate)	1
9	wait 3-5 minutes		
10	solution D (stop solution)	1
11	evaluate sample concentration by comparing with blank and standards		

Table 2. Selectivity of the *SensioScreen*[®] TR500 at two concentration levels compared with that of the microplate ELISA.

sample	SensioScreen TR500 ⁽¹⁾		microplate ELISA
	(0.5 µg L ⁻¹)	(10 µg L ⁻¹)	As46 / 2dHRP
atrazine	100	100	100
propazine	100	>100	313
terbutylazine	20	30	37
simazine	10	10	10
ametryne	30	20	4
prometryne	30	30	9
deethylatrazine	0	5	1
irgarol	0	0	-

⁽¹⁾Values corresponding to a *pseudo cross reactivity* coefficient: %CR = 100·(C_a / X) where C_a is the extrapolation of the corresponding color intensity to the atrazine concentration, and X is the spiked concentration (0.5 or 10 µg L⁻¹).

Table 3. Analysis of blind samples using the field test *SensioScreen TR500*.

sample	visual estimation ($\mu\text{g L}^{-1}$)	Spiked value ($\mu\text{g L}^{-1}$)
1	>1	5
2	<0.1	0.07
3	0	0.001
4	0.1	0.1
5	0.1	1
6	0.1-0.5	0.5
7	<0.1	0
8	>>1	10
9	<0.5	0.3
10	>0.5	1.5
11	0.5	0.8
12	>>1	6.5

Each sample was prepared in milliQ water and analyzed two times. The color was compared with the color produced by four different concentrations of atrazine standards (0, 0.1, 0.5 and 1 $\mu\text{g L}^{-1}$)

Table 4. Results of the analysis of the certified samples.

Sample	AquaCheck distribution number	Real value ($\mu\text{g L}^{-1}$)			Total ⁽¹⁾ ($\mu\text{g L}^{-1}$)	Visual estimation ($\mu\text{g L}^{-1}$)
		simazine	atrazine	propazine		
1	55	0.121	0.075	0.175	0.612	>0.5
2	59	0.748	0.211	0.145	0.735	>0.5
3	64	0.134	0.180	0.109	0.531	<0.5
4	68	0.173	0.643	0.176	1.206	>0.5
5	72	0.606	0.138	0.698	2.362	>0.5
6	76	0.210	0.191	0.126	0.603	≤ 0.5
7	104	0.151	0.171	0.094	0.477	≥ 0.5
8	108	0.220	0.140	0.110	0.503	<0.5
9	112	0.170	0.210	0.139	0.658	>0.5
10	116	0.076	0.110	0.219	0.796	≈ 0.5
11	120	0.107	0.173	0.074	0.413	≥ 0.5
12	124	0.195	0.086	0.141	0.543	≥ 0.5
13	128	0.230	0.190	0.116	0.573	≥ 0.5
14	132	0.085	0.230	0.190	0.827	≥ 0.5
15	140	0.186	0.095	0.110	0.455	≤ 0.5

Samples from the group n° 8 of AquaCheck series were analyzed with the test *SensioScreen®TR500*. The real concentration values of simazine, atrazine and propazine are shown in the table. ⁽¹⁾ Total amount of s-triazine herbicides. The simazine and propazine concentration values have been corrected by a factor related to its cross reactivity in the microplate ELISA (0.1 for simazine and 3.1 for propazine).

Table 5. Distribution in percentage of a panel of persons regarding the triazine concentration given by when estimating visually the Aquacheck samples using field test kit *SensioScreen*[®] *TR500*

Sample	Total ⁽¹⁾ , ng L ⁻¹	A, % 0-0.5 µg L ⁻¹	B, % 0.5-10 µg L ⁻¹	C, % >10 µg L ⁻¹
1	612	28	44	28
2	735	28	72	
3	531	100		
4	1,206	14	72	14
5	2,362	44	56	
6	603	100		
7	477	57	43	
8	503	100		
9	658		72	28
10	796	43	57	
11	413	72	28	
12	543	57	43	
13	573	57	43	
14	827	28	72	
15	455	100		

Seven persons participated individually estimating the concentration of atrazine by comparing the color of the samples to the color of the standards. ⁽¹⁾Total amount of s-triazine herbicides. The simazine and the propazine concentration values shown in table 4 have been corrected by a factor related to their cross-reactivity in the microplate ELISA (0.1 for simazine and 3.1 for propazine).

Table 6. Results from the evaluation of the effect of the matrix in the test-kit

Type of Matrix	Sample #	Origin	Spiking level	Observed level
Seawater	M1	artificial	0	≈ 0.5
Groundwater	M2	Wolfsberg, well 1	0	0
			0.3	<0.5
			0.5	≈ 0.5
			1	>0.5
			3	>0.5
			10	≥10
	M3	Wolfsberg, well 2	0	0
	M4	Deimhausen, well 1	0	0
	M5	Deimhausen, well 2	0	0
			0.3	<0.5
			0.5	≈ 0.5
			1	>0.5
			3	>0.5
			10	≥10
	M6	Barcelona	0	≈ 0.5
Drinking water	M7	Lechhausen/Augsburg	0	0
			0.3	<0.5
			0.5	≈ 0.5
			1	>0.5
			3	>0.5
			10	≥10
	M8	Innenstadt/Augsburg	0	0
	M9	Göggingen/Augsburg	0	0
	M10	Diedorf/Augsburg	0	0
			0.3	<0.5
			0.5	≈ 0.5
			1	>0.5
			3	>0.5
			10	≥10
	M11	Barcelona	0	0
Surface water	M12	Lech/Hochablass	0	0
			0.3	<0.5
			0.5	≈ 0.5
			1	>0.5
			3	>0.5
			10	≥10
	M13	Schmutter/Diedorf	0	0
			0.3	<0.5
			0.5	≈ 0.5
			1	>0.5
			3	>0.5
			10	≥10
	M14	Autobahnsee/Augsburg	0	0
	M15	Kuhsee/Augsburg	0	0
	M16	Mountain creek/Garmisch	0	0

All concentrations are expressed in $\mu\text{g L}^{-1}$. The atrazine concentration of the samples was estimated by comparing visually the color development with the one produced by the atrazine standards at 0, 0.5 and 10 $\mu\text{g L}^{-1}$. All samples were taken in Germany except M2 and M11 that were taken in Barcelona, Spain.

Table 7. Comparison of the results obtained from the analysis of environmental real samples with the *SensioScreen*[®] TR500 and with the EW immunosensor and LC-APCI-MS [15].

Sample	SensioScreen [®] TR500, ng L ⁻¹	EW immunosensor ^(a) (ng L ⁻¹)	LC-APCI-MS (ng L ⁻¹)			
			total	atrazine	simazine	DEA
1, May	< 500	410 (9)	470	70	370	30
2, April	<< 500	180 (5)	90	30	40	30
2, May	<< 500	370 (4)	180	80	50	50
3, May	0			0		

^(a) A detailed description of the immunosensor has been published by Brecht *et al.*[14]. The methodology has been already reported [15]. Description of the origin of the samples is given in the experimental section.