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*original scientific paper*

# Automated Immunochemical Analysis of Specific s-Triazine and Phenylurea Herbicides in Drinking Water Supplies

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***Summary***

*Automated immunochemical analysis holds out the prospect of offering an inexpensive on-line monitoring or off-line screening of different drinking water sources without extensive sample preparation and without the usage of organic solvents. For this purpose, two prototype systems, one as on-line and another as off-line instrument are currently investigated within an European project (program Life). The results presented here were performed with the off-line device, where the standards and samples were supplied in separate vials. Automated analysis is dependent upon an extended time of operation without the attendance of personnel, therefore all reagents, especially the immunoreagents (antibody and enzyme-tracer), should have a stability of at least 14 days. Different stabilization methods were investigated. The best stability (at least 14 days) was achieved with the addition of 0.5% BSA (bovine serum albumin) and 0.5% micro-O-protect, together with storage at 4 °C.*

*For reduction of buffer salts, which will be introduced into the environment during continuous FIIAA (flow injection immunoaffinity analysis), a comparison of 40 and 4 mM PBS (phosphate buffered saline) as carrier buffer was investigated. The results showed, that there was no difference in performance between the two carrier buffers.*

*As an example, the system was applied to the analysis of diuron in real water samples that contained high amount of humic substances (9.5 mg/L DOC (dissolved organic carbon)). The samples were collected from groundwater and different water treatment stages of the water supply station in Fuhrberg, Germany and afterwards spiked with diuron. As a reference method, the samples were analyzed by conventional microtiterplate ELISA (enzyme-linked immunosorbent assay), using the same immunoreagents. The determined amounts were generally in good agreement with the amounts spiked, with FIIAA producing the better results.*

*Keywords:* automation, biosensors, flow injection analysis, herbicides, immunochemical analysis, water monitoring, stabilization

## Introduction

Pestidice residue analysis is routinely carried out by conventional methods, such as high performance liquid chromatography (HPLC) and gas chromatography with mass spectrometry (GC/MS), or with combinations of these chromatographic techniques. These methods usually require sample preconcentration, derivatization, and/or clean up procedures, which are time consuming, expensive and cause an environmental hazard of their own, for example, when large amounts of organic solvents are used. Complementary to the conventional strategy is the usage of immunochemical analysis, because it is less time consuming, needs no or only minor sample pretreatment, it is especially applicable to hydrophilic compounds, and normally does not require expensive and sophisticated equipment.

Immunochemical analysis in the environmental field is usually carried out by enzyme immunoassay in microtiterplate or test tube formats. In these formats, im-

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munoassays are especially applicable, when many samples have to be analyzed in a short period of time and for a small amount of compounds. These formats can be used as field screening methods, but they still require pipetting steps and automation is lacking.

Conventional analysis is very useful, when a smaller number of samples has to be analyzed for a variety of compounds. A drawback of conventional analysis is that it is generally difficult to use for on-line monitoring, because it needs preconcentration. One example, where *in situ* preconcentration and automated conventional analysis (e.g., SPE-LC-DAD or GC) is applied, is the SAMOS (System for Automated Monitoring of Organic micropollutants in Surface water) for pesticide monitoring used in the international Rhine Basin program *(1–3)*. This equipment is quite expensive, however, and is not likely to become installed at many locations. Immunochemical analysis would be very advantageous in this respect, because it is sensitive enough to avoid sample preconcentration.

Until today, automation in immunochemical analysis has been achieved through the usage of laboratory robotics. This is applicable to high sample throughput normally existing in clinical diagnostics, but it is less suitable for on-line monitoring needed *e.g.*, in water monitoring. One possibility with the objectives of automation and on-line monitoring is the so-called flow injection immunoanalysis, which was initiated in our laboratory in 1988, gradually developed further *(e.g., 4–6)*, and lately transferred to a prototype instrument by the company Meta GmbH (now Jüke GmbH, Altenberge, Germany) *(7,8)*. Automated immunochemical analysis offers various advantages, *e.g.*, inexpensive off-line screening of samples in the laboratory and on-line monitoring, *e.g.* at water control stations or even at individual effluents of pesticide production plants. For environmental monitoring it would be extremely beneficial to have a screening tool that could monitor specific compounds of interest in an environmentally safe way. Additionally, in the online prototype, the sampling protocol could be integrated together with the protocol for the analysis, by which mistakes in this important part of analysis could be avoided *(9)*. Lately, special attention was paid to the presence of pesticides in surface waters and their effect on aquatic organisms. Therefore, besides the automation in drinking water control, automation of pesticide monitoring in other water bodies is of importance as well.

This paper presents parts of the currently ongoing optimization and demonstration of the applicability of this technique in the scope of an European project (program *Life**). The latest results obtained for the analysis of diuron in spiked real water samples will be presented. The results with these water samples spiked with atrazine have been published elsewhere *(7)*. Diuron and atrazine are widely used herbicides, that occur in surface water or in groundwater, respectively. Both water systems serve as supplies of drinking water in Europe. Occasionally, pesticide residues of these herbicides exceed the maximum allowable concentration of 0.1 μg/L (MAC), which is set by the European drinking water directive *(10)*.

## Experimental

### *ELISA (Enzyme-Linked Immunosorbent Assay) for Atrazine*

The ELISA for atrazine (s-triazine herbicide) was performed with some modifications according to Giersch *(11)*. Protein A (2 μg/mL, in 50 mM carbonate buffer, pH = 9.6; 250 μL/well) was incubated on microtiterplates (MaxiSorp, Nunc, Denmark) overnight at 4 °C. After the plates were washed (4 mM PBST, phosphate buffered saline with 0.5% Tween 20, pH = 7.6), monoclonal antibody (mab) K4E7 was added (200 μL/well; 1:100,000 in 40 mM PBS, pH = 7.6) and incubated for 2 h at room temperature. Plates were washed again with 4 mM PBST, 150 μL of analyte (water samples or standards in distilled water) together with 50 μL of enzyme-tracer (1:100,000 in 40 mM PBS, pH=7.6) were added into each well and incubated for 1 h at room temperature. After another washing step, 200 μL/well of substrate (0.4 mM TMB (3,3', 5,5'-tetramethylbenzidine), 1.3 mM $H_2O_2$ in 100 mM sodium acetate buffer, pH = 5.5) were added and the enzyme reaction was stopped after 15 min with 50 μL/well 1 M $H_2SO_4$. Plates were read at 450 nm (reference 650 nm) with a microtiterplate reader (Molecular Devices Corp., Sunnyvale, CA, USA).

### *ELISA for Diuron*

Immunoanalysis for diuron (phenylurea herbicide) was done as described by Schneider *et al. (12)* with modifications. Microtiterplates were precoated with 150 μL/well protein A (2 μg/mL, 50 mM carbonate buffer, pH = 9.6) overnight at 4 °C. After a washing step with 4 mM PBST, polyclonal antibody 2238 (100 μL/well; 1:10,000 in 40 mM PBS, pH = 7.6) was added and incubated for 2 h at room temperature. After another washing step, 50 μL of analyte (water samples or standards at different concentrations in distilled water) and 50 μL of enzyme-tracer (1:15,000 in 40 mM PBS, pH = 7.6) were added into each well and incubated for 1 h at room temperature. After another washing step, the substrate for the enzyme reaction was added (100 μL/well, 0.4 mM TMB, 1.3 mM $H_2O_2$ in 100 mM sodium acetate buffer, pH = 5.5). The color reaction was stopped after approximately 15 min with 50 μL/well 1M $H_2SO_4$ and read in a microtiterplate reader at 450 nm (reference 650 nm).

### *Stabilization of Immunoreagents*

Different stabilization procedures were tested. These tests were the continuation of earlier investigations *(6–8)*. These experiments had to be carried out both for the atrazine and the diuron assay. All tests were performed by ELISA as described above, because this format suits best for side-by-side comparisons.

As stabilizers, different concentrations of BSA (0.5% and 1% mass fraction), bovine serum albumin (fraction V, Boehringer Mannheim, FRG; Sigma, Deisenhofen, FRG) were studied. The addition of MP (micro-O-protect, Boehringer Mannheim, FRG) was investigated, because it is usually used to prevent bacterial growth. Dif-

* A financial instrument to support the development and implementation of the Community environmental policy.

ferent storage temperatures (room temperature and 4 °C) were compared.

### *Flow Injection Immunoaffinity Analyzer*

The instrument is one of two prototypes, namely the laboratory type (off-line) version (Jüke GmbH, formerly Meta GmbH, Altenberge, FRG), where the standards and samples are introduced in separate vials and analyzed sequentially. The instrument is computer-controlled by a newly written software package (Jüke GmbH, Altenberge, FRG), which can be used under Windows 3.11. The software could only control the system. A conventional printer was used for the recording of signal peaks. Data analysis was done by Excel 6.0. The features for automated analysis of the peaks and a possible feedback operation, which would change the sequence of steps after a defined event, are still under development.

The principle set-up and function of the FIIAA instrument was described already in detail *(7,8)*. Briefly, the system consists of the following parts: two pumps (one constant pump for filling of all injection loops, one variable for carrier buffer and regeneration buffer, and for time and flow controlled transport of all reagents into the column reactor), one three-way selection valve, two selection valves (one six port, one ten port), one temperature controlled column. This column contains immunoaffinity material with protein A on a polymetacrylate support (Toyopearl affinity chromatography resin AF-Protein A-650M, ligand density 4 mg/mL, adsorption capacity ≥20 mg/mL, TosoHaas, PA, USA**). The fluorescence signal [mV] is measured in a flow-through fluorimeter (RF-551, Shimadzu, Japan) after the column.

### *FIIAA (Flow injection immunoaffinity analysis) for Atrazine and Diuron*

The principle of the FIIAA for atrazine and diuron has been published earlier *(7,8)*. This assay format is basically an automated ELISA (enzyme-linked immunosorbent assay) with fluorescence detection of the enzyme product. However, the most important difference is, that in contrast to conventional ELISA, the sample and enzyme-tracer are incubated sequentially. The sequential immunoanalysis by FIIAA consists of the following steps: (1) injection and incubation of anti-pesticide specific antibodies at the affinity column coated with protein A; (2) injection and incubation of analyte (standard or water sample); (3) injection and incubation of enzyme-tracer, (4) injection and incubation of substrate for the enzyme reaction; (5) downstream measurement of the product of the enzyme reaction in a flowthrough fluorimeter; (6) regeneration of the affinity column with 100 mM sodium citrate (pH = 2.5) and start with step (1). Between all steps, the carrier stream (4 or 40 mM PBS, pH = 7.6) was used for transport and rinsing. This is due to the heterogeneous format, where bound and unbound material have to be separated.

The fluorescence signal of the product of the enzyme reaction was registered as peak height in mV. Data analysis of standard curves and calculations of unknown analyte concentrations in the water samples were performed by the software Excel 6.0 and Origin 3.5 using the following 4-parameter curve fitting equation *(13)*:

$$y = ((A-D)/(1+(x/C)^{\wedge}B))+D$$

where D is the Y-value corresponding to the asymptote (i.e., the flat part of the curve) at high (analyte) values on the X-axis and A is the Y-value corresponding to the asymptote at low (analyte) values on X-axis. The coefficient C is the X-value corresponding to the midpoint between A and D (midpoint of the standard curve). The coefficient B describes how rapidly the curve makes its transition from the asymptotes in the center of the curve *(13)*.

This equation is also used for ELISA standard curve fittings, but in FIIAA control values/% = (fluorescence signal in mV of standard or sample / fluorescence signal in mV of preceding zero standard) × 100), are used instead of absorbance values in ELISA.

### *Reduction in Buffer Salts Concentrations*

In order to reduce the amount of buffer salts in the carrier stream (PBS; 40 mM sodium phosphate and 1 M sodium chloride), we ran standard curves with both 40 mM PBS and 4 mM PBS (4 mM sodium phosphate, 100 mM sodium chloride). Immunoreagents (antibody and enzyme-tracer) were kept in 40 mM PBS, because only small amounts were used (100 and 200 μL, respectively).

## Results and Discussion

### *Stabilization of Immunoreagents for Atrazine and Diuron*

Fig. 1 shows the different stabilization methods for the atrazine assay, which were tested for the antibody and for the enzyme tracer. These results are a continuation of earlier experiments *(6–8)*. Here, new combinations were tested and compared with the ones studied earlier. Stability was guaranteed for at least 14 days with storage at 4 °C and the addition of BSA regardless of whether 1% or 0.5% BSA were used. The addition of MP is recommended: although it did not show a noticeable effect, it prevents growth of microorganisms. It has to be mentioned though, that already the storage at 4 °C was very effective against bacterial growth. Storage in 40 mM PBS alone showed a fast decline in signal and cannot be recommended for more than 1 day.

Stabilization experiments of diuron immunoreagents (data not shown) resulted in even more stable immunoreagents than those for the trazine assay. Here, after 14 days of storage at 4 °C (without addition of BSA and/or MP) the remaining activity was still about 50%. The addition of BSA, MP and storage at 4 °C showed

** This resin from TosoHaas, PA, USA, was excellent in its performance (more than 1000 regenerations), worked in four different laboratories, and it is still working. Unfortunately, the company must have changed their production procedure for this material. We tried with a new batch of the same material (different lot number) and did not get the same performance. For this reason, we are currently testing new affinity materials.

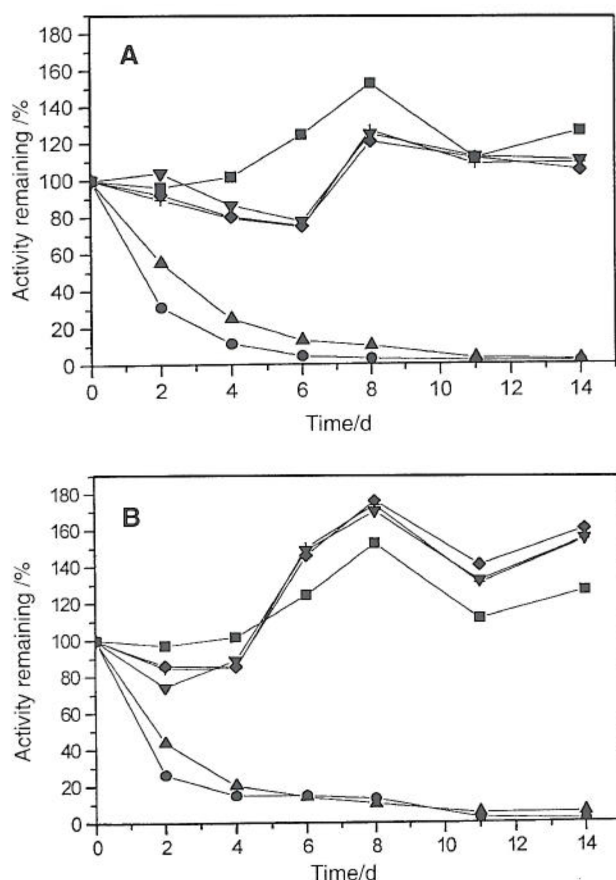


Fig. 1. Comparison of different stabilization methods of the atrazine immunoreagents. Day 0 is set as 100%. A: Stabilization of mab K4E7; B: stabilization of enzyme tracer. ■ mab K4E7 and enzyme tracer both stabilized with 0.5% BSA and 0.5% MP, stored at 4 °C (this curve is identical in both graphs); ● storage of the corresponding reagent in 40 mM PBS at room temperature; ▲ storage of the corresponding reagent in 40 mM PBS at 4 °C; ▼ storage of the corresponding reagent in 40 mM PBS with 1% BSA at 4 °C; ◆ storage of the corresponding reagent in 40 mM PBS with 0.5% BSA and 0.5% MP at 4 °C; ✚ storage of the corresponding reagent in 40 mM PBS with 1% BSA and 0.5% MP at 4 °C.

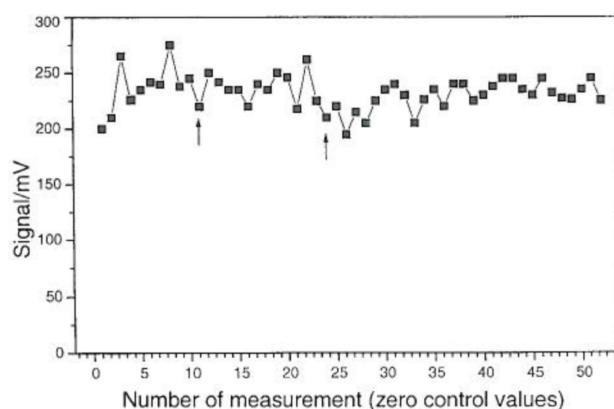


Fig. 2. Zero control values (without pesticide) for atrazine-FIIAA. Assay conditions: mab K4E7 1:100 000 and enzyme-tracer 1:100 000, both stored at 4 °C in 40 mM PBS with 0.5% BSA and 0.5% MP. This figure shows a time period of about 3 $^1/_2$ days of continuous operation. The irregular values of some peaks (arrows) have their origin in malfunctions of the hardware, they do not correspond to instability of immunoreagents.

prolonged stability. There are two possible explanations for this improved stability of the diuron immunoreagents. One is, that the dilution of immunoreagents is not as high as in the atrazine assay, and the second is, that the anti-diuron antibody is a polyclonal serum which contains additional proteins.

All assays showed very consistent control values/% over this time interval (data not shown). These values were tested with 0.1 µg/L atrazine or diuron, respectively, which is for both assays in the linear range of the standard curve (45–60% control).

Fig. 2 shows a continuous operation of FIIAA during nearly 4 days (104 assays), showing only the zero control values (52 assays with distilled water). Here, the best stabilization method with 0.5% BSA, 0.5% MP and storage at 4 °C was used. A few irregular peaks can be seen; they do not reflect the instability of immunoreagents, but rather correspond to two aspects during this operation. One was, that some problems with the hardware occurred (arrows), and the second is, that we still see an influence of preceding high concentrations of analyte (standard/sample) on the following zero control value, which is probably due to residues in the tubing. Nevertheless, the signal was really stable (232.3 ± 15.6 mV; n = 52).

With these stabilization methods, immunoreagents for both assay would certainly remain stable for an even longer period of time, but then the limitation could be the substrate solution for the enzyme reaction. HPPA (3-(*p*-hydroxyphenyl) propionic acid) and $H_2O_2$ were premixed and a slight increase in fluorescence over time was observed. For about 5 days, this mixture was usable, after that time the usage is not recommended. The usage of a commercially available, premixed and stabilized substrate would be a solution to this problem. Until now, this was not studied.

### *Reduction in Buffer Salt Concentrations*

No difference in the performance of the standard curves for diuron was observed whether 40 or 4 mM PBS buffer were used as carrier buffers (Fig. 3). This was also the case for atrazine-FIIAA (data not shown), suggesting that for future experiments, 4 mM PBS might be used. This will avoid the input of high amounts of salts into the environment. In addition, the reduction is also beneficial for the operation of the instrument itself, because salts can crystallize in the injection valves.

### *Analysis of Spiked Real Water Samples (Fuhrberg)*

Standard curves for diuron in distilled water had a linear range from 0.02 to 0.5 µg/L *(8)*, which is the needed range for the European drinking water directive. With this standard curve, it was possible to measure natural water samples. These samples were collected from groundwater wells and from different stages of the water treatment process in the water supply station in

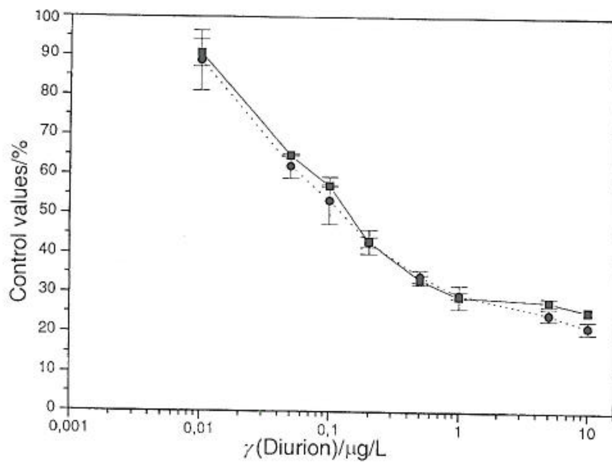


Fig. 3. Comparison of diuron standard curves with different carrier buffers. ■ 40 mM PBS (40 mM sodium phosphate, 1 M sodium chloride), ● mM PBS (4 mM sodium phosphate, 100 mM sodium chloride). Antibody and enzyme-tracer solutions were set up in 40 mM PBS with the addition of 1% BSA and 0.5% MP.

Fuhrberg, which is located in the north of Hannover, Germany. The samples were analyzed by conventional methods (high pressure liquid chromatography) as »free of diuron and atrazine« (≤0.05 μg/L, respectively). They were spiked with diuron at different concentrations (0.02; 0.05; 0.1 and 0.5 μg/L). Table 1 shows, that the amounts spiked and the amounts determined were generally in good agreement. With ELISA, there was a tendency of too low recovery data. This was very prominent especially with the water samples, that still had high amounts of the DOC (dissolved organic carbon, 9.5 mg/L). With drinking water samples, which were sampled at the end of the water treatment process (5 mg/L DOC), results with ELISA showed better recoveries. It should be noted, that the ELISA was done without the addition of BSA in antibody and enzyme-tracer solutions, whereas in FIIAA BSA/MP as stabilizers in these solutions where used. More assay comparisons with and without BSA have to be performed to find out, if this has an effect on the diuron ELISA determination.

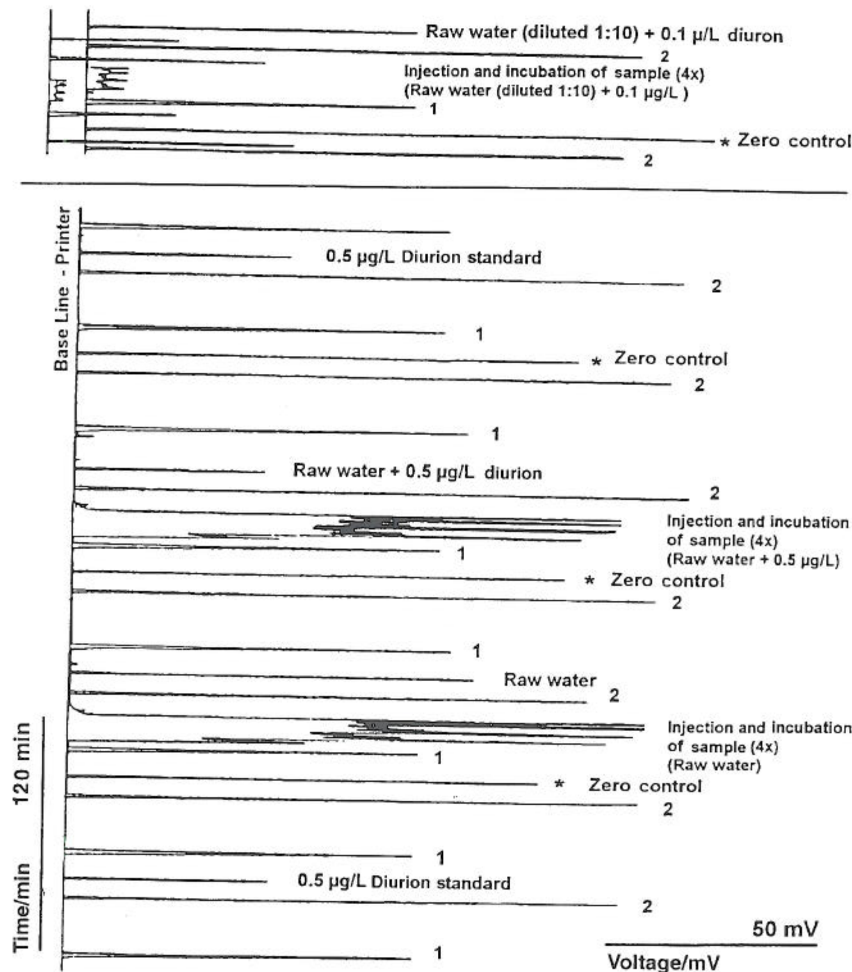


Fig. 4. Scans of original traces of FIIAA of real water samples, either with or without diuron spiked. The injections and incubations of the raw water sample were visible in the fluorescence detector, because this water source contained high amounts of humic substances (DOC 9.5 mg/L). Injections of raw water, raw water with 0.5 μg/L diuron (bottom), and raw water (diluted 1:10) spiked with 0.1 μg/L diuron (top) are presented. Top: 2 printer outputs; 500 mV (left), 200 mV (right). When the matrix is diluted 1:10, a reduced signal of the sample incubations and injections is visible.
Step 1: injection of antibody solution (signal is caused by BSA in this solution); step 2: injection of enzyme-tracer solution (signal is caused by BSA in this solution). *Zero control corresponds to distilled water and is set as 100% control.

An original trace of the water analysis by FIIAA is shown in Fig. 4. This trace represents about 3 hours of actual time operation. It clearly shows the high amount of humic substances, that gave a very high signal in the fluorescence detector during injection and incubation of these samples. As a proof of this, the injection and incubation of the raw water (diluted 1:10 with distilled water) is shown in a separate trace (upper part of Fig. 4; two printer output tracers are shown here: 500 mV and 200 mV). Here, the fluorescence intensity is clearly reduced. Incubations of standards (or zero control = distilled water) show no fluorescence in the trace. Fortunately the fluorescence of the samples does not affect the actual signal of pesticide determination, because it is occurring at a different time. Normally, these signals are not needed for the pesticide residue analysis, but they are good control signals for the performance of the system itself. Nevertheless the actual signal is influenced by this matrix, as can be seen in decrease of signal compared to the zero control value (set as 100%). The control value (in %) of raw water is only 86%, which can be interpreted as a matrix effect.

Fortunately, the influence of the matrix on the determination of diuron was only insignificant in FIIAA. As an example, this can also be seen in Fig. 4 (lower part), where the signals are shown both for the 0.5 μg/L diuron standard and for the 0.5 μg/L diuron spiked into the raw water matrix ($40.3 \pm 2.03$ ($n = 3$) and $37.2 \pm 2.77$ control value/% ($n = 4$), respectively). It seems, that the matrix effect was higher, when no target analyte was present.

In monitoring situations, one possibility to avoid this problem could be to use 'non contaminated' water itself as zero control, if it is available. This would be set as 100%, and if the pesticide of interest would come to pass, this would automatically show a decrease in the control value (in %). Here, a certain cut-off value could be set as acceptable, and an alarm point could be set as well. Care should be taken though, that before starting the monitoring, the water source is extensively analyzed and characterized by conventional analysis to make sure, that it is 'pesticide free'.

### *Conclusions and Future Prospects*

FIIAA is a technique, which has the potential of automated screening of water samples. It has to be mentioned though, that conventional ELISA on a microtiterplate is faster than FIIAA, because ELISA measures several samples (up to 20 in quadruplicate) in parallel on one microtiterplate. The FIIAA system on the other hand – operated in the mode as it is at the moment – needs about 2 hours per sample (including the preceding zero control value). For on-line monitoring of waterways this frequency is enough. For off-line determination of different water samples, in combination with the use of an autosampler, the measurement frequency may be higher. Here, one solution would be to use several columns as well as a software for controlling in parallel the pumping and incubation steps. This should be possible, because most of the assay time is needed for incubations.

One of our European project partners put already some efforts into the shortening of the timing of the program and succeeded in performing the analysis in 25 min per cycle (S. Gort, personal communication).

Tests for the stabilization of immunoreagents have been completed. Compared to standard curves in ELISA without BSA/MP, no noticeable difference was observed when these stabilizers were added. This was true for both the atrazine and the diuron ELISA. However, they had to be calculated as control values/%, because there were differences in absorbance values. In contrast, it was observed that BSA had a minor effect on the analyte determination in real water samples, at least in the ELISA format. First results indicate that with higher DOC-values, the atrazine-ELISA shows a tendency toward minor overestimation of the analyte concentration in the water sample when no BSA is present *(7)*. This was also described by Dankwardt *et al.* *(14)*, who used the same assay. The authors assumed, that BSA adsorbs humic substances, so that it is no longer available for the antibodies.

With the diuron-ELISA, this effect was not observed. Generally, an underestimation of the diuron concentration was obtained when humic substances were present (see Table 1). Differences in matrix effects might be possible and this has to be examined with the different immunoreagents used. Additional eperiments will have to be carried out, also with FIIAA, to get more data about this effect.

So far, we demonstrated that FIIAA of real water samples is possible without major sample pretreatment and preconcentration, and without the usage of organic solvents. More work has to be done, though, to demonstrate the real potential of this system by analyzing more sources of water samples with different matrices, e.g., surface water, rain water, ground-water and drinking water from various locations. This will be the task of the near future.

With the incorporation of an A/D converter in the FIIAA systems, the newest version of the software can now register the output of the detector (mV). In the future this will allow storing the data and transferring them directly to the Excel software, where all calculations can be done.

On the one hand immunochemical analysis is very specific, but on the other hand it is also affected by the occurrence of structurally related compounds. This could be demonstrated both for the atrazine-FIIAA and the diuron-FIIAA. An influence on the analysis of atrazine was seen by the presence of simazine *(7)*. Although this has not been examined yet, it is most likely, that the FIIAA-signal will be decreased by the presence of propazine in the sample, because this monoclonal antibody has the highest cross reactivity with propazine *(11)*. The analysis of diuron will be affected by the presence of monuron *(8)*, and presumably also by the presence of linuron and neburon, because this antiserum has cross reactivities to these compounds *(12)*. Although there are always cross reactivities within these specific assays, one still has to focus on a few compounds of interest. In the future, the development of recombinant antibodies might be able to change this, because antibodies could be designed with specificities for a group of compounds, *e.g.* for all phenylurea herbicides with equal cross reactivities. This would

Table 1. Analysis of diuron in spiked water samples from the water supply station in Fuhrberg, Germany. Comparison of ELISA and FIIAA results

| Sample name | Concentration Spiked | Concentration determined ± σ | |
|---|---|---|---|
| | | ELISA[a,b] | FIIAA[c] |
| | μg/L | μg/L | |
| Raw water (mixture of 5 groundwater wells) | 0.02 | <0.001 | 0.036 ± 0.004 |
| | 0.05 | <0.001 | –[d] |
| | 0.1 | 0.014 ± 0.021 | –[d] |
| | 0.5 | 0.160 ± 0.023 | 0.381 ± 0.12 |
| Raw water, dilluted 1:10 | 0.1 | 0.051 ± 0.028 | 0.108 ± 0.022 |
| Raw water after the first water treatment process (first part of flocculation) | 0.05 | 0.014 ± 0.018 | 0.047 ± 0.004 |
| | 0.1 | 0.035 ± 0.011 | 0.103 ± 0.008[a] |
| Water of the accelerator (coagulation/ flocculation process) | 0.05 | 0.026 ± 0.008 | 0.064 ± 0.017 |
| | 0.1 | 0.055 ± 0.009 | 0.116 ± 0.018[a] |
| Filtrate from sand filter No. 2 | 0.05 | 0.034 ± 0.022 | 0.045 ± 0.002 |
| | 0.1 | 0.057 ± 0.015 | 0.095 ± 0.019[a] |
| Mixture of filtrate from all sand filters | 0.05 | 0.06 ± 0.027 | 0.044 ± 0.001 |
| | 0.1 | 0.092 ± 0.031 | 0.115 ± 0.028 |
| Drinking water | 0.02 | 0.039 ± 0.023 | 0.019 ± 0.001 |
| | 0.05 | 0.059 ± 0.024 | 0.045 ± 0.01 |
| | 0.1 | 0.109 ± 0.026 | 0.106 ± 0.008 |
| | 0.5 | 0.431 ± 0.085 | –[d] |

[a]n = 3; [b]No BSA/MP added to antibody and tracer solutions; [c]n = 4; [d] Sample not tested

shift immunochemistry into a completely new direction. In the meantime, though, we have to focus more or less on a handful of major target analytes, that might be indicators of the occurrence of additional pesticides. But getting this information on a routinely and environmentally friendly basis could be at least in the short run of great benefit.

This FIIAA system would be also suitable for additional monitoring tasks, *e.g.* the screening for endogenous disruptors, that are presently of great interest. Antibodies for different hormones and 'hormone-like' compounds exist already, so the adaptation of this system should be quite easy.

## *Acknowledgements*

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# Automatizirana imunokemijska analiza specifičnih s-triazinskih i fenilurea herbicida u postrojenjima za pitku vodu


### *Sažetak*

*Automatizirana imunokemijska analiza omogućava jeftin izravan nadzor ili neizravnu zaštitu izvora pitke vode, bez velikih priprema uzoraka i korištenja organskih otapala. U tu svrhu ispitana su dva prototipna sustava, unutar europskog programa »Life«, jedan kao izravni, a drugi kao neizravni uređaj. Prikazani rezultati dobiveni su s neizravnim uređajem, gdje su se standardi i uzorci nalazili u posebnim bočicama. Automatizirana analiza provodi se određeno dulje vrijeme bez nadzora, pa stoga svi reagensi, osobito imunoreagensi (antitijelo i enzimski indikator), moraju biti stabilni barem 14 dana. Ispitani su razni postupci stabilizacije. Najbolja stabilnost (barem 14 dana) postignuta je dodatkom 0,5% BSA (goveđi serum albumin) zajedno s 0,5% mikro-O-protektom uz pohranu pri 4 °C.*

*Ispitano je i smanjenje količine puferskih soli koje se unose tijekom kontinuiranog postupka FIIAA (flow injection immunoaffinity analysis), i to usporedbom 40 i 4 mM PBS (fosfatom puferirana otopina soli) kao pufera nosača. Rezultati su pokazali da ne postoji razlika između ova dva pufera. U radu je naveden sustav za analizu diurona u stvarnim uzorcima vode koja je sadržavala veliku količinu huminskih tvari (9,5 mg/L DOC (otopljeni organski ugljik)). Uzorci su potjecali od podzemne vode i od pojedinih stupnjeva obradbe vode u stanici za opskrbu vodom u Fuhrbergu, kojima je poslije dodavan diuron. Za usporedbu uzorci su analizirani postupkom ELISA koristeći iste imunoreagense. Utvrđena količina diurona općenito je odgovarala količini dodanog herbicida, ali su postupkom FIIAA dobiveni bolji rezultati.*