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# Achievements of the RIANA and AWACSS EU Projects: Immunosensors for the Determination of Pesticides, Endocrine Disrupting Chemicals and Pharmaceuticals

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**Abstract** In the last 15 years the research projects supported by the European Commission River Analyser (RIANA) and Automated Water Analyser Computer Supported System (AWACSS) have developed quick, intelligent and cost-effective biosensors that allow the monitoring of different organic pollutants, ranging from regulated ones such as pesticides to emerging contaminants such as pharmaceuticals and endocrine disrupting compounds. Herein we give an overview of the aims, scope and main achievements of the RIANA and AWACSS projects as well as details about basic technology, immunoassays, software and networking developed within the research project. We also report on the systems performance, real water sample measurements and validation of the biosensors with conventional analytical methods. The biosensors developed were able to measure several organic pollutants at low nanogram per litre level in an analysis taking only a few minutes without any prior sample pre-concentration or pre-treatment steps. This work is a proof-of-concept that biosensors are a practical alternative or complementary methodology to traditional chromatographic techniques.

**Keywords** Automated Water Analyser Computer Supported System, Environmental monitoring, Emerging pollutants, Immunoassay, Network system, Optical immunosensor, River Analyser, Validation

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## 1 Introduction

Environmental and water pollution is a global problem which has led to increasing water quality demands and to more stringent legislation measures in the European Union (EU). Standardised methods for water monitoring need to be developed and implemented throughout Europe to ensure effective enforcement of EU water quality directives. Until now, environmental monitoring has been accomplished by the use of classical analytical tools such as liquid and gas chromatography, which are often lengthy and limit the number of analytes that can be determined in a single run to specific chemical families [1]. Therefore, new tools are demanded for the simultaneous monitoring of organic micro-pollutants in water in real-time and at reasonable cost. In the last 15 years two research projects supported by the European Commission – River Analyser (RIANA) and Automated Water Analyser Computer Supported System (AWACSS) – have developed quick, intelligent and cost-effective instruments that allow the simultaneous detection of different organic pollutants, ranging from regulated ones such as pesticides to emerging contaminants such as pharmaceuticals and endocrine disrupting compounds (EDCs). Herein we intend to provide an overview of the main practical achievements of these research projects. Two different biosensor prototypes were developed for a different set of trace organic contaminants and applied in different water matrices during the projects course. The biosensor RIANA (1995–1999; ENV4-CT95-0066) was developed within the project with the same name, funded by the European Commission under the Environment and Climate Program (Fourth Framework Program). Three RIANA biosensor prototypes were constructed by Perkin Elmer (Ueberlingen, Germany). The system was optimised toward ultra-sensitive detection of pesticides and EDCs in water samples. A new more advanced prototype was constructed by

Central Research Laboratories (CRL, Middlesex, UK) within the AWACSS project (2001–2004) (EVK1-CT-2000-00045) under the Fifth Framework Programme. The AWACSS consortium included nine European partners from four different European countries. Four partners out of the nine were also part of the former RIANA consortium. This new and versatile, multi-sensor system was designed based on reliable and low-cost and instrumentation for a quick multi-analyte analysis. Another objective of the AWACSS project was the design of an intelligent warning system with a number of remote measurement stations linked by a communications network.

## 2 Achievements of the RIANA and AWACSS Projects

The main achievements attained during the RIANA and AWACSS projects are summarised in the following sections. Key features of the two biosensors developed in the corresponding projects are also reviewed.

### *2.1 RIANA System: Key Features*

The RIANA immunosensor was described in detail by Klotz et al. [2]. Basically, the system consists of a flow injection system, a transducer mounted in a flow cell and an optical excitation and detection system. The flow injection system, FIAS 3000, from Perkin Elmer (Ueberlingen, Germany), consists of a six-way distribution valve equipped with a 1-mL syringe pump and connected through a Teflon tubing to the flow cell (dimensions of 40 × 1.7 × 0.06 mm). The transducer is mounted in this flow cell, which includes a flow channel of 1.7 mm width and 0.1 mm depth, and is sealed by an O-ring. Light from a collimated and modulated He–Ne laser source (633 nm, 7 mW) is directly coupled into a glass-slide via a bevelled end-face and guided through the length of the transducer by total internal reflection. An evanescent field is produced at each reflection spot and penetrates a few hundreds of nanometres into the external medium. This configuration avoids interferences from bulk fluorescence and permits the excitation of fluorescently labelled antibodies that are locally and specifically bound to the analyte derivatives. Fluorescence light is subsequently collected through optic fibres, filtered, and detected by photodiodes using lock-in detection. An autosampler AS90/91 from Perkin Elmer is used to deliver the samples to the FIA system. Fluid handling and data acquisition is fully automated and computer controlled.

Immunochemical techniques can be particularly suited for the measurement of organic pollutants because of their specificity, high sensitivity, adaptability for field use and ability to recognise a wide range of substances [1]. Both in the RIANA and AWACSS systems, a solid phase fluoroimmunoassay was selected as an appropriate option to meet the requirements of a useful monitoring immunoprobe. In this

immunoassay the immobilisation of an analyte derivate was preferred to the immobilisation of labile antibodies in order to increase the stability and shelf life of the transducer. The configuration also enables its regeneration for re-use without loss of activity, thus allowing semi-continuous water monitoring.

Polyclonal antibodies were prepared against target analytes by immunising sheep with the corresponding hapten–protein conjugates. After about 8 months pure antibodies were isolated from the polyclonal antisera of the sheep by affinity chromatography. After purification, the antibodies were labelled with a fluorescent marker. The immunochemistry utilised in the project takes advantage of a binding inhibition test that requires that either the antibodies directed against specific analytes or analyte derivatives can be covalently bound to a transducer surface. The immobilisation of the immunoassay component in the surface of the transducer was achieved by binding aminodextran derivatives of the target analytes on separated areas on the transducer glass slide following the procedure described by Barzen et al. [3]. The objective of achieving spatially resolved excitation and collection of fluorescence from fluorescently labelled antibodies locally bound at a planar interface can be met by the evanescent field excitation of the fluorophore. Excitation light is guided by total internal reflection within the transducer structure resulting in an evanescent wave which allows the excitation of the fluorophores bound to the transducer surface (Fig. 1). The total internal reflection fluorescence (TIRF) principle has important advantages compared to direct illumination of the active area of the transducer: it allows selective detection of surface bound fluorophores and, therefore, on-line monitoring of binding events [1].

## *2.2 AWACSS System: Advanced Features*

On the basis of the same immunoassay and optical detection principle of the biosensor RIANA, the new device AWACSS was designed to overcome RIANA drawbacks, with major improvements in three critical areas: (1) novel design approaches to the optical detection and fluidics including miniaturised integrated optics and micro-fluidics; (2) expanded multi-analyte analysis capability allowing for simultaneous measurements of up to 32 analytes; and (3) intelligent remote surveillance and control for unattended continuous monitoring [5].

With the RIANA design, up to six different analytes can be determined simultaneously, whereas the AWACSS instrument allows the performance of up to 32 different immunoassays simultaneously. The AWACSS biosensor is based on the same fluorescence detection principle but the fibre-pigtailed chip, driven by a semiconductor laser, consists of a waveguide circuit that distributes excitation light to 32 separate sensing patches on the chip surface. A fibre-coupled detection array is used to monitor the 32 separate fluorescence signals. The optics with 32 channels and 32 photodiodes was a major part of the AWACSS instrument design [5]. In addition, both the optical bench and the photodiode block were fitted with a semiconductor temperature detector. Algorithms to correct for temperature variation

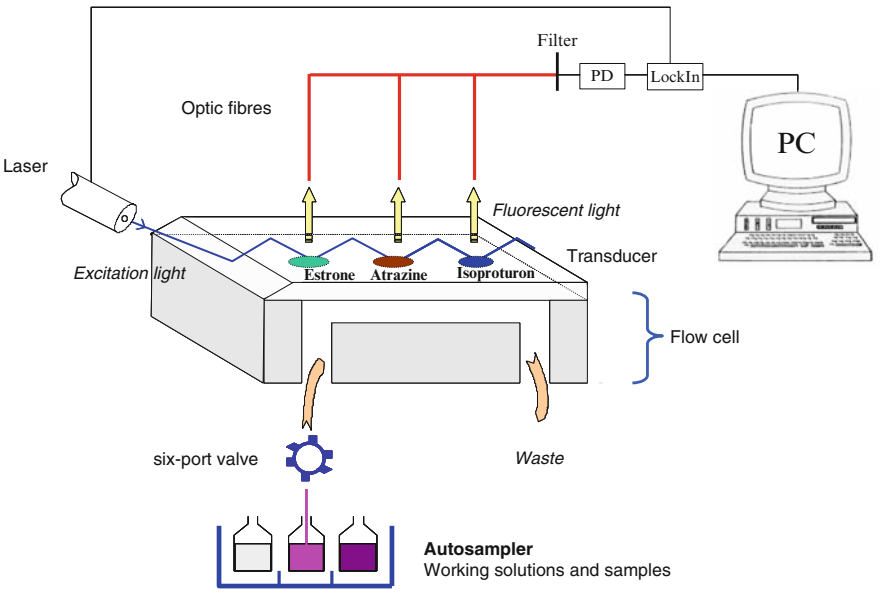


**Fig. 1** Scheme of the immunosensor RIANA for a particular application, the simultaneous analysis of atrazine, isoproturon and estrone. The FIA system, equipped with a six-port valve and a 1-mL syringe pump, delivers the buffer solution, the sample or the regeneration solutions to the flow-cell. The transducer – a 1.5-mm thick surface polished sheet glass –is mounted on the flow cell and is sealed with an O-ring. The excitation light (He–Ne laser) is totally internally reflected at the transducer surface. The collected fluorescent light is filtered and detected by photodiodes. Reprinted from [4] with permission of Elsevier

(positive or negative trends) can therefore be programmed into the measurement software. The electronics with low noise, linear amplifier and filter behaviour, external data conversion, and RS232 communications with an external data acquisition unit was the second major part. The electronics design required the assembly of 32 independent linear amplifier circuits. The fluidics box with fully automated sample handling, micro-fluidic flow channel, and interfacing to autosampler was the third major part of the instrument design. The enclosure part with high-quality RF shielding and three separate sections for optics/electronics/fluidics brought the three major parts together. The casing for the instrument was designed as three compartments, separating the main electrical components from the optical bench and amplifier circuits. In this way it was possible to screen the outputs from the 32 channels from digital electronic interference. The optical bench and electronic amplifier compartments were also made light tight to prevent stray light from impinging on the sensor chip or photodiodes. The inside surfaces of the light tight compartments were also blackened to eliminate the possibility of reflected light interfering with the signal [5].

## 2.3 Limit of Detection

Immunoanalytical methods with very low limits of detection (LOD) and low limits of quantification (LOQ) are becoming increasingly important for environmental analysis and, especially, for monitoring drinking-water quality. Tschmelak et al. devoted some efforts to improve the limit of detection of the biosensor assay. The basis of a sensitive assay is an antibody with a high affinity constant toward the analyte [6, 7]. Reducing the amount of antibody per sample results in better validation parameters and lower limits of detection [7]. During the optimisation process, Tschmelak et al. compared different surface modifications (four different immobilised derivatives) and reduced the amount of antibody per sample. The optimisation of the immunoassay resulted in very low limits of detection in water: 0.6, 0.37 and 2 ng $L^{-1}$ for propanil, [8] progesterone [6] and estrone [7], respectively, without any sample pre-treatment or pre-concentration. These low LOD achieved, in the nanogram per litre range, show that biosensors can compete with common analytical methods in the field of water analysis [6].

## 2.4 Multi-Analyte Determination

Sensors capable of determining several analytes simultaneously allow shortening of the time of analysis and the volume of sample and other reagents, and thus constitute a valuable tool for environmental monitoring. The development of multi-analyte immunoassays was one of the aims of the RIANA project. The spatially resolved immobilisation of the biomolecular recognition elements in transducer surfaces enabled the real-time parallel monitoring of multiple organic contaminants by means of separate immunoreactions. Aminodextran derivatives of the target analytes were covalently bound on separated areas on the transducer glass slide following the procedure described by Barzen et al. [3]. Briefly, a glass interference layer of 58 × 10 × 1.5 mm with a 45° bevel on the short side was cleaned by immersion in a freshly prepared hot mixture of concentrated $H_2SO_4/H_2O_2$ 2:1 for 30 min, rinsed with water afterwards and dried at room temperature. Silanisation of the surface was achieved by treatment with 3-glycidoxypropyltrimethoxysilanxysilane (GOPTs) for not more than 1 h. Thereafter, it was rinsed with acetone and dried in a nitrogen stream. Conjugates of the analyte derivatives with aminodextran were then placed in separated areas of the activated transducer surface allowing the reaction to take place. Such a multi-analyte transducer with predefined detection spots permits the performance of simultaneous multi-analyte measurements.

The first attempts at multi-analyte determination were carried out during the RIANA project [3]. Feasibility studies on multi-analyte measurements were performed with a mixture of atrazine, isoproturon and estrone [9]. The method developed was used to investigate the occurrence and removal of these three compounds throughout the treatment process (sand filtration, ozonation, activated carbon filtration and chlorination) in a waterworks [4]. In the frame of the AWACSS project further efforts were devoted to this multi-analyte aspect; different studies and assays were developed

for the determination of pharmaceuticals, antibiotics, hormones, endocrine disrupting chemicals and pesticides. In the final stage of the project, the multi-analyte determination capability of the AWACSS was successfully tested with a mixture of six analytes. The AWACSS chip was modified with derivatives of atrazine, bisphenol A, estrone, isoproturon, sulphamethizole and propanil according to the previously described immobilisation protocol. Subsequently, a simultaneous calibration in Milli-Q water with all six analytes mixed with an antibody stock solution containing the six corresponding polyclonal antibodies (anti-atrazine, anti-bisphenol A, anti-estrone, anti-isoproturon, anti-mixed sulphonamides and anti-propanil) was performed. For all compounds, the calculated LOD was below 0.020 $\mu g\ L^{-1}$ [10]. This multi-analyte calibration demonstrated the possibility to quantify pesticides from three different classes (triazines, phenylureas and anilides), EDCs (bisphenol A), steroid hormones (estrone), and pharmaceuticals (sulphamethizole) within one single measurement cycle, which only takes approximately 18 min. No cross-reactivity effect was observed for any of the tested analytes.

## *2.5 Automatisation*

Automation and minimal or no sample manipulation are among the most important features in any analytical technique to enhance the precision and accuracy of the measurements and to help diminish potential contamination problems in the analysis process. An autosampler AS90/91 from Perkin Elmer was used in the RIANA project, and initially during the AWACSS project, to deliver the samples to the FIA system of the biosensor. Fluid handling and data acquisition was fully automated and computer controlled. Later, this autosampler was replaced with an autosampler from CTC Analytics, the HTC PAS, which was coupled both to the RIANA and to the new AWACSS device. With this new system there was no need to prepare and incubate samples and standards before analysis since the new autosampler was able to select, mix and inject the samples following a programmed sequence. The new autosampler also permitted improvement in reproducibility, by lowering the standard deviations (RSD), and as a consequence, the LODs.

## *2.6 Network System*

Contaminant concentrations in water courses are dynamic, changing both as a result of receiving inputs and changes in water flow. With monthly sampling and analysis it is extremely unlikely that mapping of contamination profiles or obtaining rapid results will occur when it is important, such as after accidental spills or pollution events [11]. The establishment of network analytical systems generates huge interest. These network systems consist of multiple autonomous analytical stations that can control extensively the sites of interest in rivers, lakes, wells or even water treatment plants. In this respect, biosensors can be used as automated continuous

monitoring systems that can provide easy, rapid and on-site measurement. One of the main achievements of the AWACSS project was the establishment of an early warning system by means of a network of measurement and control stations [5].

Figure 2 illustrates the capabilities of the AWACSS monitoring network. The network concept is based on remote measurement stations and an internet-accessible database on the server station. The measurement station uploads the measurement data to the server and downloads new parameters for the next measurement. For each analyte, the incoming data on the server side is checked regarding the limit value concentration. Different limit value concentrations are held in the database depending on analyte type, sampling site location and additional factors. A comparison of these values with the incoming analyte concentration values originating from the different sampling stations is performed by a watch dog module. Automatic alert messages and e-mails are generated following the early-warning concept defined in the database and watch dog module [5].

## 2.7 *AWACSS–Marketing Aspects: Water Quality Survey*

A market survey covering 12 European countries and the USA was performed to check the feasibility of the AWACSS system in the water quality market [5]. A standardised questionnaire was completed by institutions dealing with water quality matters in the selected countries to help identify end-user requirements. The data from this survey represented the situation in analytical practice in the different

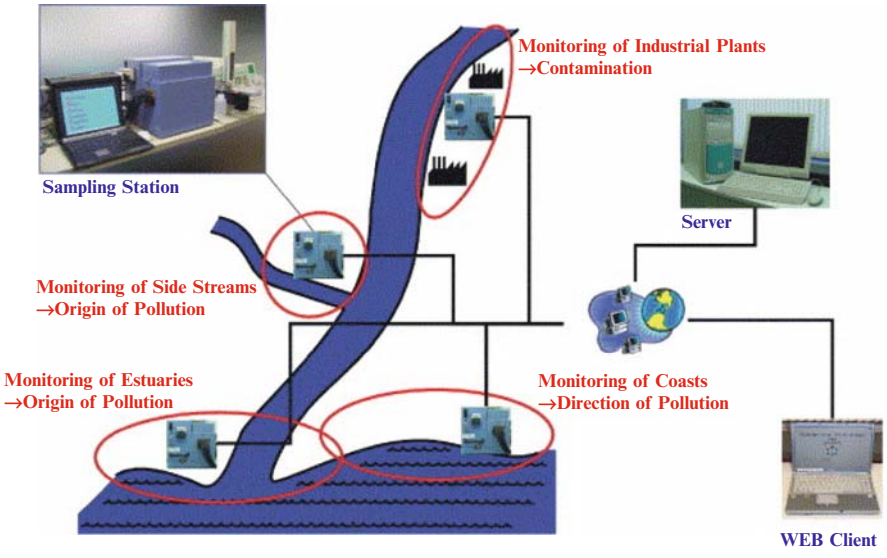


**Fig. 2** AWACSS monitoring network. Reprinted from [5] with permission of Elsevier

countries based on the present legislative needs and available laboratory equipment and assays. The collected data covered lists of substances, number of sampling sites, sampling frequencies, number of samples, detection limits, methods applied, number of positive findings, determinations below 0.1 μg $L^{-1}$, and analytical costs. Real-time sensing, improved regulatory and public acceptance and less expensive monitoring were found to be prime end-user requirements. The AWACSS system not only meets the market demand but exceeds the current competitive detection methods in terms of meeting end-user requirements for current and future expenditures and expectations: A system like AWACSS, equipped to measure up to 32 top compounds, could be well placed to the actual market with increasing acceptance in the future due to the flexibility of adaptation of new compounds as well as the capability of online, unattended and centrally controlled monitoring and surveillance.

## 3 Environmental Applications

In the previous section, biosensor features were described in terms of analytical achievements and contribution to biosensor field development. Practical aspects related to biosensor performance in the analysis of environmental samples as well as different aspects and achievements of the RIANA and AWACSS projects are reviewed in this section.

### *3.1 Target Pollutants*

A set of immunochemical reagents was developed and generated for selected organic micro-pollutants of interest to be analysed in environmental water samples. Major criteria for selection of target analytes were their presence in the environment, the existing environmental legislation, the increasing interest in certain emerging contaminants and the technical possibilities to prepare sufficiently selective polyclonal antibodies and their corresponding analyte. Analytical methods for the analysis of pesticides, EDCs, WFD priority substances, industrial pollutants and pharmaceuticals have been developed using the biosensor devices from the RIANA and AWACSS projects. Table 1 gathers together the organic pollutants and the water matrices studied and the limits of detection achieved.

### *3.2 Real-World Water Analysis*

Different assays using the RIANA and AWACSS biosensors have been developed in Milli-Q water in the process of developing and optimising the appropriate analytical method for the target compound analysis. This step is necessary before application

**Table 1** List of target organic pollutants analysed by RIANA or AWACSS using either single or multi-analyte configurations

| Analytes | LOD [ng $L^{-1}$] | Environmental matrix | References |
|---|---|---|---|
| Paraquat | 10 | Milli-Q and river water | [12] |
| Isoproturon | 10 | Milli-Q and river water | [13] |
| Triazines | 60–200 | River water | [14] |
| Propanil | 0.6 | Milli-Q water | [6] |
| Bisphenol A | 14 | Waterworks | [15] |
| Progesterone | 0.37 | Spiked Milli-Q, drinking and river water | [8] |
| Testosterone | 0.2 | Surface water | [16] |
| Estrone | 0.2 | Milli-Q water | [7] |
| | 1 | | [17] |
| Sulphonamides | 2.7–6.6 | Drinking, ground, and surface water | [18] |
| **Multi-analyte determination** | | | |
| Atrazine | 30 | | |
| Simazine | 30 | | |
| Isoproturon | 110 | Milli-Q Water | [3] |
| Alachlor | 70 | | |
| 2,4-D | 70 | | |
| PCP | 4,230 | | |
| Isoproturon | 50 | Natural water samples | [4, 9] |
| Atrazine | 160 | | |
| Estrone | 80 | | |
| Bisphenol A | 5 | | |
| Atrazine | 2 | Milli-Q, surface water | |
| Estrone | 19 | Groundwater | [19] |
| Isoproturon | 16 | | |

2,4-D: 2,4-dichlorophenoxy acetic acid, PCP: Pentachlorophenol

to real-world analysis regardless of whether single or multi-analyte measurements are to be performed. The application of new biodevices to real-world environmental samples has to be accomplished in the final steps of development. However, despite the large number of newly developed biosensors, most of the biosensors described in the literature overlook this step and only report applications in distilled water or buffer solutions [20]. One reason is the matrix effect in the measurements accuracy. Sample matrix can affect dramatically the application of biosensors to real samples. RIANA and AWACSS are based on competitive interactions between antibodies, analytes and analyte derivatives. These interactions can be affected by the pH or the ionic strength of natural water matrices. It has also been reported that the presence of other natural substances, such as dissolved organic carbon that interact weakly with the antibody, can induce an overestimation of the immunoassay response [21]. The influence of ground and river water matrices on the immunosensor response was normally evaluated for the different assays developed for RIANA and AWACSS by spiking these matrices with analyte standard solutions and observing the displacement of the calibration curves.

On the other hand, several strategies have been proposed and tested to prevent matrix interferences of the water samples, such as diluting and buffering the sample, adjusting the sample pH and conductivity, sample clean-up, enrichment of the sample by solid phase extraction and subsequent reconstitution of the extract, and adding a background protein. The addition of detergents or immolation proteins reduces the probability of the antibody to adhere to passive particles in water and has become a current routine to overcome matrix interferences in immunoassays [4, 22, 23]. This is the strategy adopted in the case of the RIANA and AWACSS assays where the background protein ovoalbumine is added to a real sample as an immolation protein.

As part of the AWACSS project, Tschmelak et al. proposed and demonstrated the efficacy of a new method to overcome the partly occurring matrix problems in real-world immunosensor monitoring [24]. They developed an easy matrix referencing method using a synthetic organic carbon standard that could be adapted to other applications depending on a similar test-format. The organic carbon level of monitored waters should be determined in parallel or in frequent intervals. With this referencing method, very good recovery rates for the ultra-sensitive estrone assay were possible.

## *3.3 Validation*

Despite the practical advantages of biosensors, they need to be comparable to conventional analytical systems in terms of reliability, sensitivity, selectivity, specificity and robustness. Biosensor measurements need therefore to be verified and validated before being accepted. The RIANA and AWACSS biosensors were validated by comparing the biosensor performance with that of conventional methods. The overall performance of the new system in comparison to the conventional analytical and immunosensor techniques was tested in an inter-laboratory collaborative trial among the AWACSS partners at the late stages of the project. Different analytical set-ups were used in six laboratories; among them four automated online and offline solid-phase extraction (SPE) systems coupled to liquid chromatography and either mass spectrometry or diode array detection (SPE–LC–MS, SPE–LC–DAD), a large volume injection-gas chromatography-mass spectrometry system (LVI–GC–MS), two RIANA biosensors, one AWACSS device and one ELISA assay. The tested matrices were Milli–Q water and freeze-dried river sediments from the Nitra River in the Slovak Republic. Each of them was spiked with the analytes atrazine, bisphenol A and estrone at 0.1 and 1.0 μg $L^{-1}$ in water matrices and 50 and 500 ng $g^{-1}$ in sediments. All samples, including blanks, were prepared in triplicate and distributed among the partners. For more details on the procedures, the reader is addressed to reference [10]. The results showed that, in terms of accuracy, the AWACSS performance in Milli-Q water and sediment samples is fully comparable to conventional chromatography-based techniques. In general, the AWACSS results were less biased towards higher values than the ELISA and RIANA immunosensor techniques.

### *3.4 Water Monitoring*

The performance of both, the RIANA and the AWACSS biosensors has been tested for a variety of organic pollutants and water matrices. However, only two works have reported their use in environmental monitoring studies. The immunosensor RIANA was evaluated to monitor total chlorotriazine pesticides in river water samples in the Ebre area (Tarragona, Spain) from April to June 1998 [14]. Simazine and atrazine were detected (levels ranging from 0.18 to 1 μg $L^{-1}$) in only eight samples out of 18 measurements carried out. The results obtained with the immunosensor were validated by online solid-phase extraction followed by liquid chromatography-atmospheric pressure chemical ionisation-mass spectrometry (LC–APCI–MS). Although some overestimation was observed with the biosensor no false-positive was detected. Thus, the method developed with the biosensor allows the unequivocal determination of chlorotriazines in natural waters and can be applied to comply both, with the EPA legislation for drinking waters, which requires the monitoring of atrazine at the 2 μg $L^{-1}$ level and also with the European Community (EEC) Drinking Water Directive (DWD), which establishes limit values for individual and total pesticide concentrations at 0.1 and 0.5 μg $L^{-1}$, respectively.

The RIANA system was also applied to determine the occurrence of the organic pollutants bisphenol A, atrazine, isoproturon and estrone in the waterworks Sant Joan Despí (Barcelona, Spain) between May and August 2002 [4, 15]. The removal efficiency of the target pollutants throughout the different purification stages at the waterworks was assessed using two different biosensor configurations: single analyte mode for bisphenol analysis [15] and multi-analyte configuration for the determination of atrazine, isoproturon and estrone [4]. Chromatographic methods were also applied in parallel for the analysis of the samples. On the basis of the comparative performance of the biosensor and liquid chromatography based methods, the biosensor emerged as a suitable tool for fast, simple and automated screening of water pollutants without sample pre-treatment and was proved to be a useful tool for water resources protection; in most water production companies the quality of the raw water and the removal efficiency of target contaminants during drinking water production needs to be assessed.

## 4 Other Applications

The significant progress made with RIANA in environmental monitoring and water analysis was successfully adapted to milk analysis for use in the field of reproduction management, in particular for the sensitive determination of progesterone in cow milk, as an indicator of ovulation [25]. The sensitivity and robustness of the existing progesterone assay for water analysis was improved, resulting in a LOD of only 0.2 ng $L^{-1}$. Progesterone was then analysed in three different types of milk (UHT milk, fresh milk and raw milk) (levels typically in the range 5–15 μg $L^{-1}$). LOD achieved for added progesterone (i.e. spiked samples) were between 45.5 and 56.1 ng $L^{-1}$ depending on milk type. In this work, a commercially available antibody

was incorporated for the first time into the immunosensor. The biosensor operation was fully automated and the LOD achieved for progesterone in bovine milk was below 1 μg $L^{-1}$. The assay was further optimised by reducing the time per measurement to about 5 min and tested by measuring the progesterone level in daily milk samples for 25 days, covering a whole oestrus cycle [26].

## 5 Conclusions

Herein we have reported the main achievements attained during the EU research projects RIANA and AWACSS, and the successful application of biosensors for environmental monitoring. Two different biosensor prototypes have been designed and constructed during the projects and different software modules for data storage, communication, data treatment, networking and data evaluation have been successfully developed and combined into a software package. Feasibility studies have been conducted on multi-analyte analysis and on matrix effects. Detection limits of most developed immunoassays have been in the few nanogram or even sub-nanogram per litre range.

The biosensors capability for analysing a variety of environmental organic micropollutants, such as pesticides, EDCs and pharmaceuticals in surface, ground, drinking and waste water has been compared with conventional analytical methods for validation purposes. The biosensors described in this report have been demonstrated not only to be cost-effective alternatives to chromatographic techniques for environmental screening, but also very convenient techniques when the information is needed immediately (measurements are performed very rapidly; maximum 20 min).

Most of the compounds analysed with the RIANA and AWACSS biosensors meet the end-users demands, their monitoring is required by the present EU environmental legislation (98/83/EC, 1998; 2000/60/EC, 2000) and all of them are frequently detected in real water samples all over Europe. On the basis of the features reported, good perspectives are given to the biosensor system to be placed among the current state-of-the-art analytical instruments for water monitoring.

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