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An Immunosensor for the Automatic Determination of the Antifouling Agent Irgarol 1051 in Natural Waters

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The development of an immunosensor for analysis of the antifouling agent Irgarol 1051 (2-methylthio-4-*tert*-butylamin-6-cyclopropylamin-*s*-triazine) is described. The immunosensor is based on a heterogeneous competitive enzyme immunoassay and uses binder azlactone support with immobilized protein A/G. The flow-through immunosensor is completely automated and is able to carry out a whole analysis cycle in about 23 min. Competitive calibration curves have an I_{50} value of 0.053 μg/L (0.21 nM), with a limit of detection of 0.010 μg/L and a quantification dynamic range from 0.019 to 0.158 μg/L. The protein A/G support can be used for more than 600 assay cycles without loss of sensor performance. The sensor is prone to some interferences from *tert*-butyl-containing *s*-triazine compounds, such as terbutryn and terbumeton, but little inteference is shown for other *s*-triazine compounds, e.g., atrazine or simazine. The developed sensor is applied to the determination of trace levels of Irgarol 1051 in water samples such as Mediterranean marina and beach seawater, estuarine river water, and lake water, without any sample pretreatment other than adjusting the pH and ionic strength of samples to those of standards. Good correlation is achieved between the immunosensor results and ELISA or HPLC analyses.

Introduction

In the late 1980s, the use of the agent tri-*n*-butyltin as an active ingredient in antifouling paints for small boats was restricted (*1*, *2*) since this compound was found to severely damage nontarget organisms, e.g., bivalves and gastropods, at very low concentrations (*3*). This compound has then been replaced by copper compounds, often in combination with algaecides such as Irgarol 1051 (2-methylthio-4-*tert*-butylamin-6-cyclopropylamin-*s*-triazine). The biological activity of Irgarol 1051 consists of the inhibition of photosynthetic electron transport in chloroplasts (*4*). Due to this specific effect in organisms, Irgarol 1051 is minimally toxic to animals, but it has been shown that this compound is very toxic to all microalgae tested and to periphyton communities in coastal water microcosms, inhibiting periphyton photosynthetic activity at 3.2 nM in short-term tests (*5*). Solubility of Irgarol 1051 in water is low (7 mg L^{-1}), and its degradation has been reported to be very slow, its half-live in sediments ranging 100−200 days (*6*).

Levels and impact effect of Irgarol 1051 are scarcely studied throughout the world. Only a few studies performed on Irgarol 1051 contamination have shown that this compound is present in the marine environment in several European areas such as the southern coast of U.K. (*7*), Côte d'Azur (*8*, *9*) and western coast of Sweden (*5*). Apart from seawater, Irgarol 1051 has also been found in Geneva lake (Switzerland) (*10*). In all cases, concentration of Irgarol 1051 in freshwater or seawater ranged 0.0025−0.64 μg/L. This compound has also been found in sediments in U.K. southern coast and Geneva lake areas.

Determination of Irgarol 1051 in the Mediterranean basin also has social interest since the seawater quality is directly related with tourist inputs. Analysis of Irgarol 1051 is currently performed by chromatographic methods such as gas chromatography with nitrogen-phosphorus (GC-NPD) (*9*, *11*) or mass spectrometry (GC-MS) (*12*) detectors. Liquid chromatography with diode array spectrophotometry (LC-DAD) and mass spectrometry (LC-MS) detection have also been used in the determination of this compound (*13*). Chromatographic techniques have proven to be very robust and the most suitable for multianalyte determinations, but the chromatographic determination of pesticides is usually expensive and time consuming, since there is a need of extraction, cleanup, and preconcentration steps.

In the past decades, immunochemical methods have shown to be very adequate for the rapid monitoring of environmental pollutants such as pesticides. Immunoassays are very sensitive and selective, inexpensive, able to run many samples simultaneously, and applicable to water samples with little or no treatment, being therefore ideal for large sample loads (*14*). An enzyme-linked immunosorbent assay (ELISA) for the analysis of Irgarol 1051 has been recently reported (*15*, *16*). The ELISA developed is based on rabbit polyclonal antibodies raised against haptens which preserve at less two out of the three substituents of the parent *s*-triazine analyte. This ELISA has shown to be highly sensitive, with a limit of detection of 0.02 μg/L, and its applicability for the analysis of seawater samples has been demonstrated (*13*).

Immunosensors have been developed in order to apply immunotechniques to on-line analysis. Immunosensors are immunochemistry-based devices that use the antigen−antibody interaction to monitor an analyte in a rapid an automatic fashion. Such devices consist of a bioactive surface (usually an antibody immobilized on a suitable support) and a transducer system able to generate a signal when the immunochemical reaction takes place (*17*). One of the pioneer antibody-based sensors for environmental analysis was developed by Vo-Dinh et al. (*18*). Although there are systems able to directly detect the binding of the analyte to the immunosurface (e.g., piezolectric (*19*) or surface plasmon resonance (*20*) devices), a more popular approach consists of the association of immunochemistry with flow injection and sequential injection methodologies, giving rise to flow-through immunosensors (*21*).

In many cases, flow-based immunosensors use the same principles as classic immunoassays, but the flow technology

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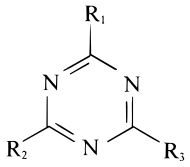

Compound	R_1	R_2	R_3
Irgarol 1051	SCH_3	$NHBu^t$	$NHPr^c$
4a	$S(CH_2)_2COOH$	NHEt	$NHPr^i$
4b	$S(CH_2)_2COOH$	NHEt	NHEt
4c	$S(CH_2)_2COOH$	$NHBu^t$	$NHPr^c$
4d	SCH_3	$NHBu^t$	$NH(CH_2)_3COOH$
4e	SCH_3	$NHPr^c$	$NH(CH_2)_3COOH$

FIGURE 1. Chemical structure of Irgarol 1051 and the *s*-triazine haptens tested.

allows us to run these analyses in a fully automated fashion, and results can be generated in a few minutes after the sample introduction (*22*). Flow techniques also allow the use of different detection modes. Furthermore, the flow conditions permit immunosurface regeneration and reuse for more than one assay after the application of a suitable desorbent. This regeneration process is sometimes very difficult to carry out. Immunosorbents able to perform several hundred consecutive assays, as well as single-use immunoreactors, can be found in the literature (*23*).

Recently, three flow-through immunosensors, based on the same polyclonal antibody, were developed for the analysis of atrazine, and their performance was compared (*24*). Two of them used the antibody directly immobilized on the surface of two different supports, and the third one was based on the capture of the immunocomplexes formed in the bulk of the solution by an azlactone gel-immobilized antibody-binder protein A/G. The protein A/G-based immunosensor was shown to be the best performing in terms of sensitivity, immunosurface (protein A/G immobilized on azlactone gel) reusability, and selectivity.

In this work, the development of a protein A/G capture immunosensor for the determination of Irgarol 1051 is presented. This is, to our knowledge, the first immunosensor developed for the analysis of this compound in environmental samples. The whole performance of the method developed is discussed.

Experimental Section

Chemicals and Biochemicals. The synthesis of the haptens 4a, 4b, 4c, 4d, and 4e, as well as the production of rabbit polyclonal antibodies R-13, R-14, R-15, R-16, R-17, R-18, R-19, R-20, and R-21, has been previously described (*15*, *16*, *25*). The conjugation of haptens to horseradish peroxidase (HRP, Boehringer, Manheim) was carried out by means of the active ester method (*26*). Analytical standards of *s*-triazine derivatives were purchased from Dr. Ehrenstorfer (Augsburg, Germany) and Riedel de Haën (Seelze-Hannover, Germany). Irgarol 1051 was a gift from Ciba-Geigy (Barcelona, Spain). The chemical structures of the analyte and the haptens are shown in Figure 1. Ultralink Immobilized Protein A/G was from Pierce (Rockford, IL). 3-(*p*-Hydroxyphenyl)propanoic acid (HPPA) was from Sigma (St. Louis, MO). All other reagents were of analytical grade.

Immunoreagent Selection. The choice of the most suitable combination antibody−enzyme tracer was performed by means of an immunofiltration assay device (IF), previously described (*27*). The IF allows the selection of the optimum working conditions (antibody, support, hapten used in the enzyme tracer, and desorbent solution) for maximizing sensitivity and immunosurface reusability.

System Design. A scheme of the automated flow manifold is shown in Figure 2. The device consists of an eight-way distribution valve (Kloehn Ltd., Las Vegas, NV) equipped with a 2.5 mL syringe pump and connected to a secondary eight-way distribution valve. The immunoreactor is placed between the pump-connected valve and the fluorimetric detector (λ_{ex} 320 nm, λ_{em} 405 nm, Perkin-Elmer LS50, Sussex, U.K.) equipped with a 15 μL flow cell. Liquid handling and fluorimetric signals were managed by means of Kloehn Ltd. Winpump (Kloehn) and FL Data Manager (Perkin-Elmer) software packages, respectively. Sigmaplot (Jandel Scientific, San Rafael, CA) software package was used for data treatment.

The immunosorbent was placed into a small polymethacrylate tube (4 mm length, 4 mm inner diameter, volume approximately 50 μL), and connected between a valve of the manifold and the detector. All the available volume of the reactor was filled with immunosorbent. The length of the tubes was the minimal for switching the reactor to the valve system and the detector.

Assay Protocol. The scheme of the optimized assay protocol used in the protein A/G-based immunosensor is shown in Table 1. All the solutions except the desorbent were prepared in 0.02 M sodium phosphate buffer, pH 8.0. The total assay time was 23 min. Seawater samples were quantified after calibrating the immunosensor with standards prepared in artificial seawater (3.5% w/v seasalts, Sigma).

Water Samples. Bottled water samples were purchased in local supermarkets. Albufera lake (Valencia, Spain) water samples were collected in May 1997 (Albufera I) and in June 1997 (Albufera II). The Jucar river water sample (Valencia, Spain) was collected from the estuarine in July 1997. All these samples were brought to pH 8.0 with NaOH or phosphoric acid when necessary, as only sample pretreatment. Seawater samples were collected from bath zone of Malvarrosa and Cullera beaches (Valencia, Spain) in July 1997. Additional water samples had been previously collected from the Masnou marina (Barcelona, Spain) between April and August 1996 and from Ebre Delta area (Tarragona, Spain) in September 1996, and stored frozen at −20 °C until use. All the seawater samples were directly analyzed by the immunosensor without any sample pretreatment. For the validation studies, the collected samples were split in three portions and used to carry out ELISA and HPLC-DAD analyses as previously described (*13*, *16*).

Results and Discussion

Characterization of the Sensors. The working conditions referred to the polyclonal antibody and the hapten−HRP conjugate to be used in the immunosensor were first selected by means of the immunofiltration device and further tested in the immunosensor and optimized in order to enhance sensitivity and support reusability and speed of analysis. The choice of the most suitable antibody−enzyme tracer combination was based on sensitivity criteria both in immunofiltration and in flow immunosensor experiences. From a set of the nine polyclonal antibodies and the five haptens conjugated to HRP (described in the Experimental Section), the best results were found when R-15 antiserum (obtained from hapten 4c conjugated to Keyhole Limpet Hemocyanin) was used in combination with 4d-HRP enzyme tracer. This combination is also optimal in ELISA experiences (*16*).

The immunosensor working conditions found to be optimal for solutions, volumes, and flow rates are given in Table 1. Using these conditions, competitive calibration curves were made with Irgarol 1051 standards at concentrations ranging 0−10 μg/L.

The normalized signals expressed as $100(B - F)/(B_0 - F)$ (where B and B_0 are the peak heights obtained with Irgarol

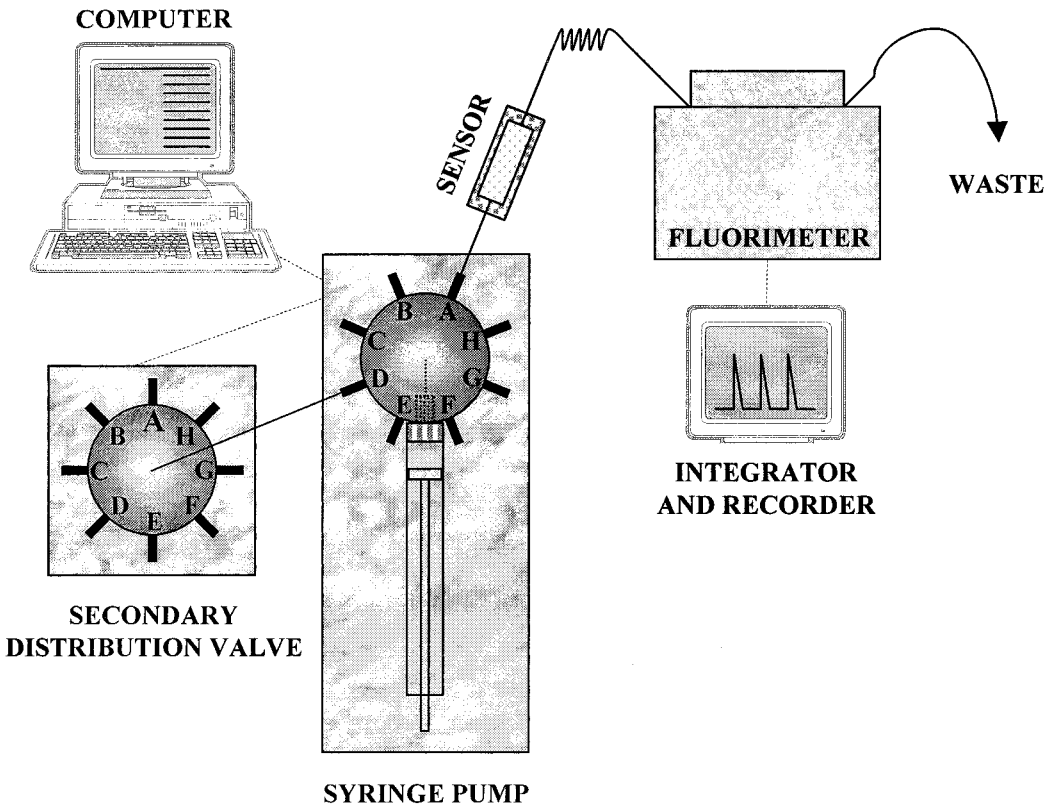

FIGURE 2. Scheme of the flow manifold used in the immunosensor. Valve ports in syringe pump are connected to (A) reactor; (B) air; (C) enzyme tracer solution; (D) eight-way distribution secondary valve; (E) 0.02 M phosphate buffer, pH 8.0; (F) H_2O_2; (G) HPPA; (H) waste. Secondary distribution valve ports are connected to (A) 0.1 M Gly/HCl buffer, pH 2.0; (B) antibody solution; (C−H) samples.

TABLE 1. Scheme of the Assay Protocol in the Flow-Through Immunosensor[a]

step	event
1	mixing[b] sample (700 μL) with 4d-HRP enzyme tracer solution (200 μL, 0.3 mg/L) and R-15 antibody solution (200 μL, 2 mg/L)
2	injection of 1 mL of mixture at 0.25 mL/min
3	reactor washing (2 times) with 1.5 mL of buffer at 1 mL/min flow rate
4	mixing[c] HPPA (300 μL, 0.8 g/L) with H_2O_2 (200 μL, 0.012%). Injection of 200 μL
5	incubation for 120 s
6	reactor washing with 1.5 mL of buffer at 2 mL/min. Peak registration
7	desorption with 2.5 mL of 0.1 M glycine/HCl, pH 2.0, solution at 0.5 mL/min
8	reactor washing (2 times) with 1.5 mL of buffer at 1 mL/min. End of cycle

[a] Previous to each step, the manifold ran a washing cycle of tubing and syringe with the next solution to be used. [b] Syringe dead volume (~100 μL) filled with sample. [c] Syringe dead volume filled with 0.012% v/v H_2O_2 solution.

1051 standards and blank sample, respectively, and F is the background fluorescence obtained in absence of enzyme tracer) were plotted vs Irgarol 1051 concentration, and the experimental points were fitted to a four-parameter logistic equation. The I_{50} parameter was 0.053 μg/L (0.21 nM), which indicated that the sensor was extremely sensitive. The limit of detection (LOD), calculated from competitive curves as the analyte concentration for which the normalized signal was 90%, resulted to be 0.010 μg/L. The dynamic range of the method (analyte concentrations which produce a normalized signal between 80 and 20%) was from 0.019 to 0.158 μg/L.

The mentioned sensitivity features are obtained with volumes of standard solutions about 2 mL/assay; meanwhile, it is necessary to preconcentrate water volumes ranging 200−1000 mL in order to reach comparable detection limits when using chromatographic procedures. Figure 3 shows the mean calibration curve obtained from four measurements performed in two different experiments, using standards dissolved both in sodium phosphate buffer and artificial seawater.

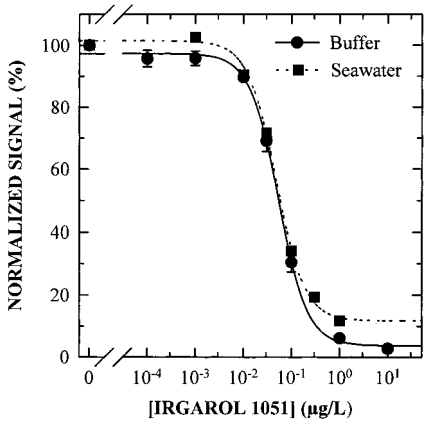

FIGURE 3. Normalized calibration curves obtained with the flow-through immunosensor. Comparison between buffer and artificial seawater standards.

Reproducibility of the immunosensor was tested by measuring a 0.030 μg/L Irgarol 1051 standard along several

TABLE 2. Cross-Reactivity of Irgarol 1051-Related Compounds

compd	I_{50} (nM)	cross-reactivity (%)
Irgarol 1051	0.21	100
terbumeton	0.16	132
terbutryn	0.35	59.1
terbutylazine	0.63	33.1
cyanazine	1.39	15.1
prometryn	1.46	14.3
ametryn	2.86	7.3
propazine	5.60	3.8
atrazine	13.5	1.6
deethylatrazine	30.5	0.7
simazine	98.5	0.2
deisopropylatrazine	639	0.03

days. The relative standard deviation (RSD) of day to day normalized signals was 1.9% ($n = 8$). All the measurements were performed with the same sensor, although the antibody and enzyme tracer solutions were prepared fresh each day.

The protein A/G reactor, kept at 4 °C in phosphate buffer containing 0.02% sodium azide as preservative when not in use, was used for more than 400 assay cycles with no detectable loss of activity. After those 400 cycles, a slight diminution in the activity was observed, as evidenced by the decrease in the absolute B_0 values. This decrease was about 50% in the following 200 measuring cycles. Nevertheless, this decline affected equally all the measurements, so the competitive curves performed with normalized signals did not change significantly. Furthermore, the decline in signals was so low that day to day recalibrations—performed by measuring B_0 and a 0.030 μg/L standard—were enough for checking the calibration curve parameters. Therefore, a single reactor could be used for more than 600 assay cycles without loss of sensor performance. This is a very good result in relation with immobilized antibody immunosupports (*19*, *24*). Other applications of antibody−capture protein A supports have also shown a very good reusability (*24*, *28*).

Cross-Reactivity. Competitive calibration curves were done with the immunosensor for some *s*-triazines, to determine their cross-reactivity, defined as the percent ratio between I_{50} value for Irgarol 1051 and I_{50} value for the cross-reacting compound, both I_{50} being expressed in molar units. The cross-reactivity values found are shown in Table 2. High cross-reactivity was shown for tertbutyl-containing *s*-triazine compounds (terbutylazine, terbutryn, and terbumeton), as expected since the hapten used for eliciting the antibody contained this group. The compounds ametryn, cyanazine, and prometryn also showed a remarkable cross-reactivity (more than 5%), whereas the more popular herbicides simazine, propazine, and atrazine, as well as atrazine metabolites, had a negligible cross-reactivity. Similar qualitative cross-reactivity values were found in ELISA using the R-15 antibody (*13*, *16*). The similarity of ELISA and immunosensor in terms of cross-reactivity is expected, since the selectivity of immunoassays depends mainly on the antibody used in the assays. The main differences between ELISA and immunosensors have been observed for kinetic-dependent immunosensors (*24*), and the protein A/G flow immunosensors worked in immunochemical equilibrium conditions (*29*), the same as occurs in ELISA.

Analysis of Water Samples. A preliminary study of the applicability of the developed sensor to the determination of Irgarol 1051 in drinking water was performed by analyzing commercial bottled water samples spiked with Irgarol 1051 at four levels. The only sample treatment was adjusting the pH to 8.0 (the same as those of the phosphate buffer used in standards) with concentrated NaOH. The results obtained are shown in Table 3. Good recoveries were found in all the samples, and no Irgarol 1051 was found in unspiked samples,

TABLE 3. Analysis of Irgarol 1051 in Spiked Mineral Water Samples Using the Immunosensor

[Irgarol 1051] added (μg/L)	[Irgarol 1051] found (μg/L)[a]	recovery (%)
0	<LOD[b]	
0.025	0.029 ± 0.004	116
0.050	0.048 ± 0.001	96
0.100	0.090 ± 0.009	90
0.200	0.222 ± 0.016	111

[a] Mean ± SD of four measurements. [b] LOD = 90% of B_0 = 0.010 μg/L.

i.e., no false positives were found. The RSD of the measurements ($n = 4$) was also good, with all values equal or less than 10%.

Spiked and unspiked water samples from environmental sources were analyzed with the immunosensor while comparing data with other reference methods. For this purpose, water samples from the Albufera lake were collected, split in two portions, one of them spiked with Irgarol 1051, and analyzed directly with the immunosensor, as well as with the developed ELISA method and a liquid chromatography procedure using solid-phase extraction and photodiode array detection (SPE-LC-DAD). An estuarine water sample (Jucar river) was also analyzed by the immunosensor and the reference methods. Prior to immunosensor analysis, samples were filtered through Whatman no. 5 filter paper in order to eliminate any solid material and brought to pH 8.0 when necessary as only pretreatment. A good correlation was found between the immunosensor analyses and the reference methods for the two kinds of surface water, as can be seen from Table 4. No Irgarol 1051 contamination was found in the Albufera lake as evidenced by the values found in both unspiked and spiked samples. However, a slight amount of Irgarol 1051 was found in the Jucar estuarine water sample, which it was expectable since the sampling point is near a high-boating area. The reproducibility of results generated with the immunosensor was shown to be very good, as expected from fully automated devices.

Seawater samples collected from the Malvarrosa and Cullera beaches (Valencia, Spain) were also analyzed with the sensor in order to check the applicability of the device for the analysis of high salt containing waters. For this purpose, the calibration had to be made with the standards dissolved in artificial seawater (seasalts dissolved in distilled water at 35 g/L). This additional calibration had to be carried out because absolute fluorimetric signals obtained when analyzing seawater samples were much higher (almost twice) than when analyzing buffered water. This behavior can be due to an increase in the binding capacity of the protein A/G in the presence of a high-salt concentration. However, as can be seen in Figure 3, the normalized signals lead to almost identical calibration curves, which indicates that a highly saline environment has no influence in the immunochemical competition (*13*), but can modify the activity of the protein A/G reactor.

A deeper assesment of the original flow-through immunosensor was accomplished by analyzing a new batch of seawater samples and comparing the results obtained for all samples with ELISA and SPE-LC-DAD data. Samples from the Masnou marina and Ebre Delta area were collected and directly measured by the immunosensor. Results obtained with the three techniques are shown in Table 5. A good correlation (Figure 4) between the immunosensor, and the chromatographic method can be observed (slope 1.44, r^2 0.95), even better than the ELISA method (slope 2.9, r^2 0.95), that generally detected lower amouts of analyte than sensor and HPLC methods (*13*). Nevertheless, the agreement between immunosensor and LC measurements is better in

TABLE 4. Analysis of Irgarol 1051 in Natural Surface Water Samples. Comparison with Reference Methods

sample origin	[Irgarol 1051] added (μg/L)	[Irgarol 1051] found (μg/L)[a] immunosensor	ELISA	SPE-LC-DAD
Albufera lake	0.000	<LOD[b]	<LOD[c]	<LOD[d]
	0.100	0.099 ± 0.011	0.053 ± 0.006	0.105
Jucar river	0.000	0.072 ± 0.003	0.064 ± 0.009	0.070

[a] Mean ± SD of four determinations, except the chromatographic measurementes that were performed once per sample. [b] LOD = 0.010 μg/L. [c] LOD = 0.020 μg/L. [d] LOD = 0.001 μg/L.

TABLE 5. Determination of Irgarol 1051 in Seawater Samples. Comparison with Reference Methods[a]

sample origin		immunosensor	ELISA	SPE-LC-DAD
Valencia Beach	Malvarrosa	<LOD[b]	<LOD[c]	<LOD[d]
	Cullera	<LOD	<LOD	<LOD
Masnou Marina	April	0.477 ± 0.017	0.172 ± 0.001	0.325 ± 0.014
	May 1	0.084 ± 0.012	0.045 ± 0.006	0.118 ± 0.008
	May 2	0.039 ± 0.005	0.030 ± 0.001	0.044 ± 0.004
	June	0.076 ± 0.006	0.040 ± 0.003	0.083 ± 0.003
	July	0.046 ± 0.009	0.014 ± 0.008	0.027 ± 0.001
	August	0.025 ± 0.006	0.019 ± 0.002	0.022 ± 0.001
Ebre Delta	Alfacs Bay	0.017 ± 0.002	0.045 ± 0.004	0.007 ± 0.001
	Fangar Bay	0.077 ± 0.003	0.039 ± 0.003	0.089 ± 0.001

[a] Data in micrograms per liter. Mean ± SD of four determinations. [b] LOD = 0.010 μg/L. [c] LOD = 0.020 μg/L. [d] LOD = 0.001 μg/L.

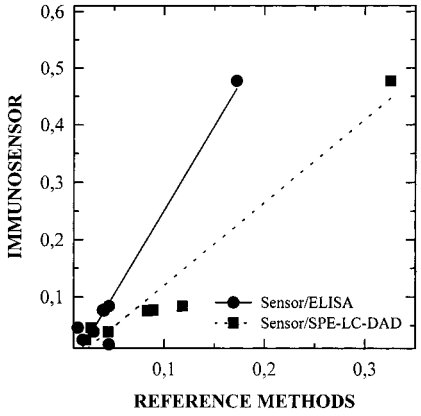

FIGURE 4. Regression analysis between immunosensor and reference methods data. Analyte concentrations (in μg/L).

surface waters (Table 4) due to matrix effects from seawater, as also observed by Zhou et al. (*12*). Anyway, the data indicated the suitability of the seawater calibrated immunosensor for application to real seawater samples. The analysis showed that Malvarrosa and Cullera beach water was free of Irgarol 1051, meanwhile a slight amount of Irgarol 1051 was found in Masnou Marina and Fangar Bay (Ebre Delta) seawater, due to the presence of small boats in these areas. Indeed, the sample taken from Marina Masnou in April, corresponding to the start of the boating season, had a higher amount of Irgarol 1051.

Compared to reference methods, the immunosensor has shown to be as sensitive and selective as ELISA, meanwhile SPE-LC-DAD needs to preconcentrate large volumes (more than 200 mL) of water samples so as to reach similar detection limits. The main advantage of immunosensor over reference methods is the rapid results generation, the complete autonomy of the system (the device has been able to work for more than 12 consecutive hours without the presence of any operator, and the only sample pretreatment can be done automatically by installing an appropriate filter since there is no need of pH adjustement in most samples because they were initially at pH between 7.5 and 8.0), and its applicability of on-line analysis. The sensitivity and reusability of the immunosensor is also equal to or higher than most on-line immunochemical methods developed for other analytes (*21*). Only a flow system based on an antibody capture format has shown higher reusability than the Irgarol 1051 sensor (*28*). Immunosensors based on noncompetition principles, e.g., displacement of a labeled hapten from an antibody column (*30*), generate results in a more rapid fashion, but sensitivity reached is lower, not enough for direct pesticide analysis following the EU requeriments in most instances, and the column reusability is also lower, due to the ever-growing presence of the analyte in the antibody column.

Flow-through immunosensors have provided an interesting alternative for the automated on-line analysis of very low levels of organic pollutants in environmental water samples. The high sensitivity achieved by the developed immunosensor allows its application to any kind of water sample, even drinking water. Little interferences from *s*-triazines have been found, with the exception of *tert*-butyl-containing compounds. The immunosensor has been shown to be applicable to difficult aqueous matrixes such as organic material-contaminated lake water and river water, with very little sample pretreatment. The applicability of the sensor to seawater only requires an additional calibration curve with seawater standards, whose features are practically equal to the calibration performed in buffer. Hence, the immunosensor can be used as a routinely automatic analyzer device in monitoring pollution programs.

The current trend is the establishment of alternative flow sensors, applicable to samples in organic media, which would minimize the treatment in samples which require extraction processes, such as algae, fish, or marine sediments.

Also, all of us appreciate that our Mediterranean sea was free of high levels of Irgarol 1051, especially in the shore area.

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