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Validation of two immunoassay methods for environmental monitoring of carbaryl and 1-naphthol in ground water samples

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Abstract

Two formats (microtiter plates and magnetic particles) of sensitive enzyme-linked immunosorbent assays (ELISA) for carbaryl determination have been compared to the EPA method 531.1 (liquid chromatography–post-column derivatization–fluorescence detection, LC–PCR–FD). An immunoassay developed for 1-naphthol determination has also been evaluated to monitor previous exposure to carbaryl in natural water samples. Matrix effect of this kind of water on the immunoassays is negligible and no sample preparation has found to be necessary other than buffering the samples. It is possible to perform a high number of analyses in a short period of time. A close correspondence was found for the results obtained when spiked and well water samples were split for analysis by ELISA and by LC–PCR–FD. Both methods are compared in terms of precision, reliability, reproducibility and their utility as screening tools. Application of those methods to the direct determination of carbaryl and 1-naphthol in ground water samples of the aquifer Campo de Nijar (Almería, Spain) is reported.

Keywords: Biosensors; Carbaryl; ELISA; Environmental analysis; Fluorimetry; Immunoassay; Liquid chromatography; 1-Naphthol; Waters

1. Introduction

During the past years pesticides have been used in increasing amounts through the world. The widespread use of pesticides has created a serious concern regarding their effect on the environment. Particular attention has been focused on the impact of pesticide residues in drinking water and food supplies [1]. As an example, carbamate insecticides are widely used for the protection of fruits, crops, forests and livestock. In particular, carbaryl (SEVIN, 1-naphthyl-*N*-methylcarbamate) has been extensively used against many agricultural pest. It has been commonly judged to have moderate to low mammalian toxicity. Carbaryl is highly susceptible to chemical hydrolysis and biodegradation, leading to several metabolites, mainly 1-naphthol, which do not accumulate in the body but are excreted in urine, faeces, and respiratory gases after a short period of time. However, some adverse effects have also been reported including alterations of liver microsomal

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enzymes [2], sub-chronic neurotoxicity after long-term exposure [3] and changes in the immunological function in in vitro culture [4]. Carbaryl is also toxic to bees and in an aquatic environment 1-naphthol or a combination of both has even more adverse effects than the parent compound alone [5].

Safety assurance requires support to establish efficient testing programs by employing rapid and simple testing procedures. Current methods of environmental analysis usually involve the use of robust analytical techniques, such as the US EPA Method 531.1, involving liquid chromatography and post-column derivatization with fluorescence detection (LC–PCR–FD) [6]. The use of immunochemical techniques is widespread in clinical laboratories since many years to measure human body fluid components. Immunoassay methods such as enzyme-linked immunosorbent assay (ELISA) have shown to be rapid, sensitive, reliable, simple and cost-effective. Over the last years this technology has also been adapted to pesticide residue measurement [7,8]. Universities, agrochemical companies and other manufacturers have developed immunoassay tests which have proved to be very convenient as rapid and efficient screening tools. However, limitations of ELISA may arise for screening complex environmental samples. As in all analytical methods, false positive results may be caused by interferences from structurally similar compounds or by inadequate clean-up steps [9], which strongly indicates the importance of validating those methods prior to routine analyses.

During a monitoring program of the agrochemical inputs in ground water in Almería (Spain) we have found twice, during a one year period (March '93–February '94) [10], pesticide pollution exceeding the limit of 0.5 $\mu g\ l^{-1}$ for total pesticides allowed by the EEC Drinking Water Directive on water quality. Carbamates are detected sporadically due to their instability under these particular hydrolytic conditions. We are interested in the understanding of how carbamate insecticides behave in soil and how they are transported into the aquifers. In this context immunoassays appear suitable alternatives to increase the number of samples which can be analyzed and to study those factors affecting pesticide movement. For this reason we have focused our attention on the validation of two ELISA tests for the determination of carbaryl as compared to the EPA 531.1 method. The tests chosen are the microtiter-plate ELISA developed at the University of California in Davis [11] and the Ohmicron's magnetic-particle RaPID Assay® for carbaryl [12]. Additionally, and because of the observed metabolic and environmental instability of carbaryl in these water samples [13], determination of 1-naphthol was also carried out by ELISA [14]. The aims of the study presented here were: (1) to test whether the immunochemical methods could be used for direct analysis of carbaryl and 1-naphthol in well water samples; (2) to make a critical comparison between the immunochemical technologies and LC–PCR–FD (EPA method 531.1) using both spiked and real field samples; and (3) to determine the distribution of carbaryl and 1-naphthol in the aquifers of the above mentioned area.

2. Materials and methods

2.1. Chemicals and immunochemicals

Carbaryl, 1-naphthol, methiocarb, carbofuran, 3-oxocarbofuran, 3,7-dihydroxycarbofuran and 7-hydroxy-3-oxocarbofuran were purchased from Promochem (Wesel, Germany). Preparation of the coating antigens (5-CONA and 2b-BSA) and production of antibodies 2114 and 3907 used for the ELISA analysis of carbaryl and 1-naphthol, respectively, are described previously [11,14]. The reagents employed in the magnetic-particle RaPID Assay were provided by Ohmicron (Newtown, PA) and purchased through J.T. Baker (Deventer, the Netherlands). Other immunochemical reagents were obtained from Sigma (St. Louis, MO).

2.2. Buffers and solutions

(1) Coating buffer was 0.1 M carbonate buffer pH 9.6. (2) PBS (phosphate-buffered saline) was 0.2 M phosphate buffer with 0.8% (m/v) NaCl, pH 7.5. (3) PBST was PBS buffer containing 0.05% (v/v) Tween 20. (4) 10 × PBST is 2 M PBST, 8% (m/v) NaCl and 0.5% (v/v) Tween 20. (5) Substrate buffer was 0.1 M citrate pH 5.5. (6) Substrate solution for the peroxidase enzyme was prepared by mixing 400 μl 0.6% (m/v) TMB [3,3',5,5'-tetramethylbenzidine,

in dimethyl sulfoxide] with 100 μl 1% (v/v) H_2O_2 in 25 ml substrate buffer. (7) The enzymatic reaction was stopped with 4 M aqueous H_2SO_4.

2.3. Instrumentation

Preconcentration of the water samples prior to LC analyses was performed on an on-line SPE (solid phase extraction) system composed by 10 × 4.6 mm i.d. C_{18} Empore extraction disks (J.T. Baker, Deventer, Netherlands) placed in a disk holder fitted in a MUST column switching device from Spark Holland (AS, Emmen, Netherlands), and an SSI Model 300 LC pump (Scientific System, State College, PA) [6]. The chromatographic system was composed of a Model 250 binary LC pump from Perkin Elmer coupled to a PCX 5000 carbamate post-column analysis module from Pickering Laboratories (Mountain View, CA). The cartridge column was LiChrocart (25 cm × 4.6 mm i.d.) packed with 4-μm Supersper 60RP-8 from Merck (Darmstadt). A Model LC 240 fluorescence detector from Perkin Elmer (Buckinghamshire, UK) was set at excitation and emission wavelengths of 330 and 465 nm, respectively. For data collection a PE Nelson Model 1020 data system was used. In order to avoid false positives, presence of carbaryl and 1-naphthol was confirmed by an on-line SPE-LC–MS system equipped with a thermospray interface (TSP). For this purpose, a Hewlett-Packart (Palo Alto, CA) Model 5988A TSP-LC–MS quadrupole mass spectrometer and a Hewlett-Packart Model 35741B instrument for data acquisition and processing were employed. Microtiter-plate ELISA analyses were performed using 96-well polystyrene microtiter plates from Nunc (Maxisorb, Roskilde, Denmark). The absorbances were read at 450 nm in a microtiter-plate ELISA reader Multiskan Plus (Labsystems, Helsinki). Data acquisition and calculations were performed using the commercial software package Genesis (Labsystems). A four-parameter logistic equation was used for the calibration curves. Magnetic-particle ELISA analyses were carried out with polystyrene tubes. A two-piece magnetic separation rack consisting of a test holder which fits over a magnetic base is required. This two-piece design allows for a 60-tube immunoassay batch to be set up. Test absorbances were measured and calculations were performed on a RPA-I Analyzer® set at 450 nm by comparing the observed sample absorbances to a regression line using a log–linear standard curve derived from the calibrator absorbances.

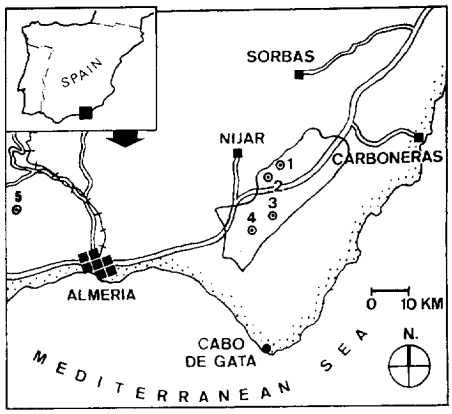

Fig. 1. General map of the monitored area in Campo de Nijar in Almería, southeast of Spain. The aquifer flow is northeast–southwest and has been chosen as representative of the whole area. Sampling sites: (1) well 1, (2) well 2, (3) well 3, (4) well 4, (5) well 5.

2.4. Water samples

Natural water samples collection and handling. Water samples were collected from Aquifer Campo de Nijar in Almería (Andalucía, Spain). Water drains into this aquifer from many green houses spread over approximately 1500 ha. Sampling sites are located in the middle of a cultivated field. Hydrologic characteristics have already been reported [10] (briefly, wells had a depth between 80 and 150 m, pH 7.2–7.9, conductivity 400–2400 μS cm^{-1} and nitrate concentration range was 6.8–15.6 mg ml^{-1}) and distribution of the sampling sites is shown in Fig. 1. The aquifer flow is northeast–southwest. Two sampling points were chosen upstream (well 1 and 2) and two others downstream (well 3 and 4). Samples were collected monthly (May–August 1994) after running the well pump for 2 h to ensure that a fresh water sample was obtained. Approximately 1-l water samples were passed through a 0.45-μm Millipore membrane filter (Bedford, MA) to remove sand and debris and sent to Barcelona by air mail. Samples were stored at 4° C in clean glass bottles and analyses were performed within the next four days.

Spiked samples for intercalibration studies. Carbaryl-and 1-naphthol-spiked samples were prepared with MilliQ water at concentration values near their respective ELISA measuring range. Samples were then split for ELISA or LC analyses. Regression studies comparing both techniques were performed using statistical software with a confidence level of $p < 0.05$.

2.5. Analyses

On-line SPE-LC–PCR–FD. Details of the methodology can be found in previous works [6,10]. About 10 ml of water sample were concentrated at a flow-rate of 2.5 ml min^{-1} and then automatically introduced into the chromatographic system previously described. Gradient elution was performed from a eluent containing 5% A [acetonitrile–methanol–water (40:40:20)] and 95% B [acetonitrile–water (10:90)] to 20% A–80% B in 15 min, to 30% A–70% B in 20 min, to 55% A–45% B in 20 min, isocratic mode for 10 min, to 100% A in 10 min, isocratic mode for 10 min and back to initial conditions in 5 min at a flow-rate of 0.9 ml min^{-1}. Calibration graphs were constructed by analyzing spiked aqueous samples at concentrations encompassing the range of interest.

Carbaryl microtiter-plate ELISA. The protocol was similar to that previously described [11] with slight modifications. Briefly, spiked or well water samples (225 μl) were mixed with 10 × PBST (25 μl, having a final phosphate buffer concentration of 0.2 M with 0.05% (v/v) Tween). Buffered samples (200 μl) and standards (eight different concentrations ranging from 1000 nM to 0.001 nM) were then pre-incubated with the antibody 2114 (50 μl, 1/8000 in PBST, final concentration on the plate 1/40000) overnight at 4° C. The next day samples and standards (100 μl per well) were added to the coated plates (5-CONA, 0.1 μg ml^{-1} in coating buffer, 100 μl per well, overnight at 4° C) and incubated for one hour at room temperature. Plates were then washed to eliminate unbound reagents (five times with PBST) and goat antiIgG–HRP (antibody anti-rabbit IgG, developed in goat and covalently coupled to horseradish peroxidase, 1/6000 in PBST, 100 μl per well) was added and incubated for one hour more at room temperature. Plates were washed and filled with a solution of the substrate (100 μl per well). The enzyme reaction was stopped after 30 min at room temperature by adding 4 M H_2SO_4 (50 μl per well).

Carbaryl magnetic-particle ELISA. All samples were assayed according to the RaPID Assay package insert. Briefly, the rapid magnetic-particle-based ELISA uses polyclonal antibodies coated on paramagnetic beads. Water samples (200 μl) to be analyzed were added to a disposable test tube, along with carbaryl–hapten–HRP enzyme tracer (250 μl per tube), and rabbit anti-carbaryl antibody covalently attached to magnetic particles (500 μl per tube). The standard curve was prepared from calibrators containing known levels of carbaryl at 0, 0.4, 1.5 and 5.0 ng ml^{-1}. Tubes were vortexed and incubated for 20 min at room temperature. The reaction mixture was magnetically separated using a specially designed magnetic rack. After washing twice with 1 ml distilled water, the presence of labelled carbaryl was detected by adding the substrate solution containing TMB (500 μl per tube). The tubes were vortexed and incubated for another 20 min at room temperature and in darkness to allow color development. The color reaction was stopped by the addition of 2 M H_2SO_4 (500 μl per tube).

1-Naphthol microtiter-plate ELISA. Buffered samples were prepared as described for the carbaryl immunoassay and ELISA was performed by pre-incubating samples (200 μl) and standards (eight different concentrations from 50000 to 3.2 nM) with the antibody 3907 (50 μl, 1/3200 diluted in PBST for a final concentration of 1/16000 on the plate). The next day samples and standards were added over the previously coated plate (2b-BSA, 2 μg ml^{-1} in coating buffer, 100 μl per well overnight at 4° C). Protocol was as described for the carbaryl immunoassay.

In all the microtiter ELISA analyses it was included a positive and a negative control of the same well water matrix unless otherwise indicated, the data shown in the results section corresponds to the average of at least three experiments performed on different days using three well replicates.

On line SPE-LC–TSP–MS. About 50 ml or 100 ml of water were introduced into the system to detect carbaryl or 1-naphthol, respectively. The temperature of TSP was set as follows: 100, 215 and 250° C for

the stem, tip and ion source respectively. The chromatographic conditions were the same as described for LC–PCR–FD, but in this case 0.05 M ammonium formate was used instead of water for the eluent A and 0.075 M for the eluent B. Ionization was produced with the filament on and the presence of carbaryl and 1-naphthol was confirmed by using selected ion monitoring (SIM) mode. Positive ionization mode was used for carbaryl ($[M + H]^+$ and $[M + H + CH_3CN]^+$ as diagnostic ions) while 1-naphthol was detected in negative ionization mode ($[M - H]^-$ and $[M + HCOO]^-$ as diagnostic ions) [15].

Matrix effect and cross-reactivity studies on ELISA. Standard curves were prepared for buffered well water, tap water and river water and compared to the standard curve prepared for PBST to determine their parallelism. Pesticides usually present in these water samples other than those already checked in our previous work [11,14] were used to prepare standard curves over the same range as that for the carbaryl and 1-naphthol standard curves in the corresponding assays. ELISA tests were then performed with those compounds. Cross-reactivity values were calculated as follows: $[I_{50}(\text{carbaryl})\ (\text{or 1-naphthol})/I_{50}(\text{pesticide})] \times 100$.

3. Results and discussion

Recently we have described the development of two ELISA tests for the detection of carbaryl and its main degradation product 1-naphthol [11,14]. These assays have shown to be highly specific and no cross-recognition is observed between those analytes in their respective assays. Simultaneously other immunoassays have appeared on the market for carbaryl determination, for example the magnetic-particles RaPID Assay® from Ohmicron [12]. Qualification of those immunoassays for routine screening depends upon validation by classic or accepted methods. For this purpose, spiked and environmental samples have been assayed independently for carbaryl determination by using the mentioned ELISA techniques and the EPA 531.1 method. Additionally we were interested to know if monitoring of 1-naphthol could serve as an indicator of the previous presence of carbaryl in water samples. Consequently water samples were individually checked for both contaminants by ELISA and LC–PCR–FD. Positive results on natural well water samples were always checked by mass spectrometry by the use of on line SPE-LC–TSP–MS.

Correlation between ELISA and LC–PCR–FD. In the first part of this work we studied the relationship between immunoassays and the accepted methods for quantifying various pesticides. Fig. 2 shows the correlation between LC–PCR–FD and ELISA values obtained for spiked samples. Slope values of the linear regression equation are in all cases near to 1 and the correlation coefficient is always higher than 0.95, indicating a high correlation between both techniques. Only in the case of the magnetic-particle

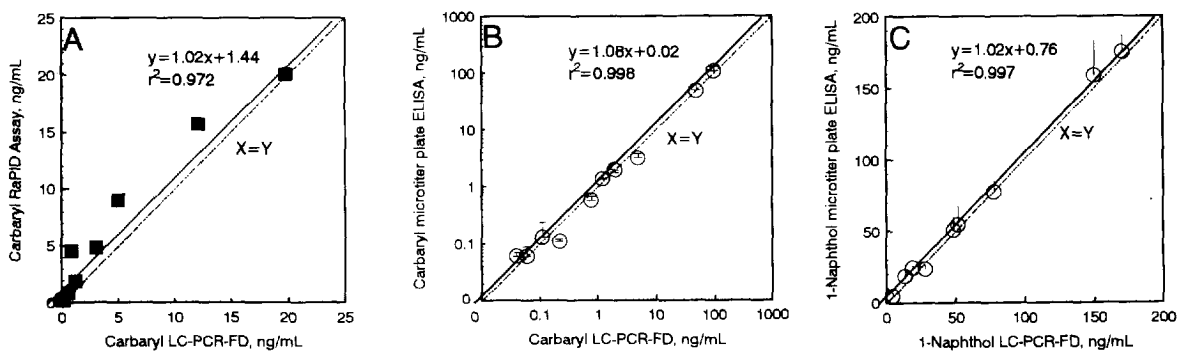

Fig. 2. Correlation between LC–PCR–FD and ELISA for the three assays used during this study. Slopes are near to 1 indicating that the results measured by ELISA are very close to those obtained by the EPA accepted method. To determine the correlation ten spiked samples were analyzed simultaneously by both methods. Correlation coefficients are shown in the graph for every experiment. (A) LC–PCR–FD/RaPID Assay for carbaryl, (B) LC–PCR–FD/microtiter-plate ELISA for carbaryl, (C) LC–PCR–FD/microtiter-plate ELISA for 1-naphthol.

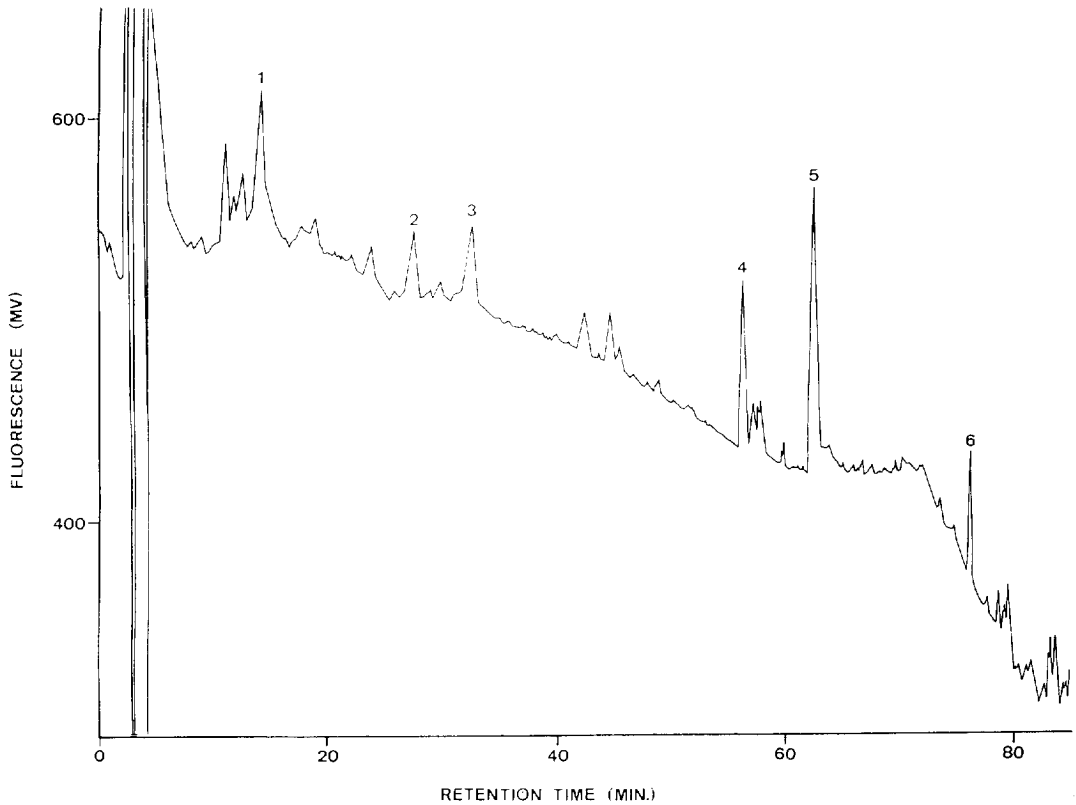

Fig. 3. LC–PCR–FD chromatogram obtained after preconcentration of 10 ml of well 3 water sample (May 1994). Water contains (1) methomyl (0.11 $\mu g\ l^{-1}$), (2) 3-hydroxycarbofuran (0.08 $\mu g\ l^{-1}$), (3) methiocarb sulfone (0.07 $\mu g\ l^{-1}$), (4) carbofuran (0.16 $\mu g\ l^{-1}$), (5) carbaryl (0.1 $\mu g\ l^{-1}$) and (6) methiocarb (0.12 $\mu g\ l^{-1}$). Chromatographic conditions are described in the experimental section.

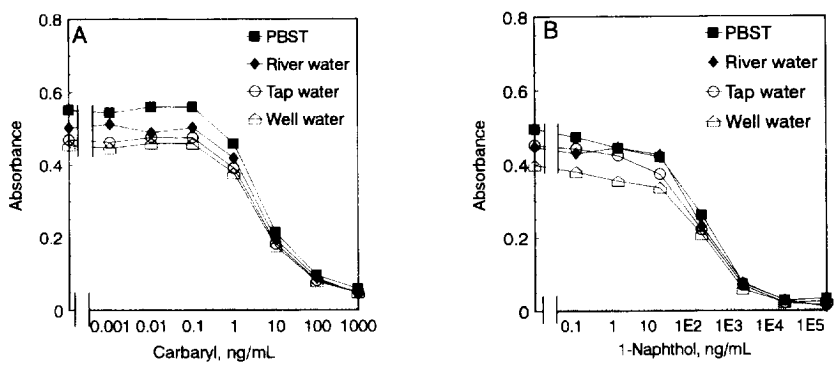

Fig. 4. Matrix effect studies with waters from several sources. No significant differences in terms of slope and I_{50} were observed when running carbaryl and 1-naphthol immunoassays in these matrices compared with the standard curve obtained for the experiment carried out in buffer. These data show that no clean-up step is necessary and water samples can be directly used in immunoassays. Immunoassays were run as described in the experimental section. Each data point represents the average of duplicate determinations. (A) Carbaryl microtiter-plate ELISA, (B) 1-naphthol microtiter-plate ELISA.

immunoassay a value of 1.44 was obtained when $x = 0$ in the regression equation, which indicates an overestimation in comparison to the values found by LC–PCR–FD. Correlation is also good when measuring 1-naphthol within the linear range of both techniques. Due to the overestimation encountered in the RaPID Assay for carbaryl we decided to perform the following studies with the microtiter-plate ELISA format.

Effect of the water matrix and cross-reactivity studies. An advantage of LC–PCR–FD for carbamate determination over ELISA is multianalyte determination. The presence of other chemically related pesticides can be detected in a single run (for example see the chromatogram of Fig. 3), while immunoassay development and validation must exclude a priori possible interferences coming from sample matrix features (salts, pH, etc.) or its components. Consequently matrix effect studies and cross-reactivity data must be contemplated for each particular type of sample. Some of these inquiries were already examined in our previous paper describing ELISA development for 1-naphthol and carbaryl. Now we have also studied immunoassay performance in well, tap and river water samples. Fig. 4 shows the parallelism existing between the standard curves obtained for the above-mentioned samples and that obtained for the buffer solution. No significant differences are observed between the slope (i.e. well water standard curve, $p = 0.914$, $n = 3$) and I_{50} values (i.e. well water standard curve, $p = 0.213$, $n = 3$). Consequently, errors during the measurement of 1-naphthol and carbaryl due to matrix effects in these samples seem unlikely. Additionally we have also tested cross-reactivity with other pesticides which were not examined in our previous work and had often been detected by LC–PCR–FD in these natural water samples (see Fig. 3), but none of them interferes with the carbaryl microplate immunoassay. In the case of 1-naphthol immunoassay, no antibody recognition was observed for either carbofuran or its metabolites 3-oxocarbofuran, 3,7-dihydroxycarbofuran and 7-hydroxy-3-oxocarbofuran when present at concentrations as high as 50 μM.

Method reliability. As a part of the validation process we have also paid attention to the reliability of the methods. Even though an excellent correlation was obtained when comparing measurements by ELISA and LC, carbaryl microtiter-plate immunoassay presents a coefficient of variation greater than that of the EPA method. For the absorbance values the average of the intra-experiment coefficient of variation was found to be $2.19 \pm 1.36\%$ ($n = 9$). However, day-to-day variation is higher ($21 \pm 8\%$, $n = 6$), since a fluctuation is also observed for the assay features such as linearity range (concentrations which give 80–20% of the control absorbance), I_{50} values (concentrations which gives a signal 50% of the control absorbance) and slope. Parameter means and standard deviations for 20 different carbaryl microtiter-plate immunoassays run on different days show the variation encountered: I_{50} is 0.71 ± 0.25 ng ml^{-1}, slope is 0.77 ± 0.19 and linearity ranges from 0.11 ± 0.08 to 10.66 ± 8.05 ng ml^{-1}. In contrast we have found the average of the day-to-day coefficient of variation when using on-line SPE-LC–PCR–FD in our laboratory to be 3% when analyzing carbaryl within the linearity range (0.01–50 ng ml^{-1}). Consequently, when using ELISA, calibration curves should be prepared for each experiment. The same pattern was observed when analyzing 1-naphthol. Using ELISA the intra-experiment coefficient of variation is $2.5 \pm 0.7\%$ ($n = 8$) within the linearity range from 3.20 ± 1.16 (80% control absorbance) to 118 ± 29.29 (20% control absorbance) ng ml^{-1}, but $23 \pm 9\%$ ($n = 5$) when measurements were performed on different days. The optimum range for measuring 1-naphthol by LC–PCR–FD is 0.05–5 ng ml^{-1}, where the average of the day-to-day coefficient of variation is around 5%. Several sources of error have been reported during ELISA performance such as the quality of the solid phase employed, pipetting, absorbance readers, etc. [16]. However, an important factor contributing to this variability is derived from the sigmoid nature of the competitive immunoassays (see Fig. 4). Measurement near the I_{50} value gives the most reliable results. Pesticide concentration in environmental samples is always near or below 0.1 ng ml^{-1}. Consequently, it is essential that immunoassays for direct environmental monitoring purposes show the desired sensitivity. Although 1-naphthol ELISA with an I_{50} value of 19.41 ± 4.45 ng ml^{-1} could be a convenient tool for biological monitoring to assess pesticide exposure (urine of agricultural workers who used carbaryl for pest control showed to have a 1-naphthol concentra-

tion in the range 0.07–1.7 μg ml^{-1} and the concentration is even higher in urine of formulators from carbaryl manufacturing plants [17]), it will seldom provide sufficiently sensitive measurements of environmental samples unless a preconcentration step is introduced before sample analysis.

Another factor to be considered during validation is precision. Also in this case the precision of the LC–PCR–FD method is higher. As shown in Fig. 5, the percentage of recovery at the different spike levels is closer to 100% when using the chromatographic method than when measuring by ELISA. Accuracy is greater in both techniques at high concentrations. In the lower concentration range usually an overestimation is observed, which is advantageous since it reduces the possibility of pesticide-contaminated samples being excluded. However, it strongly suggest that samples should be checked for false positives, by employing more specific techniques like the on-line SPE-LC–PCR–FD or SPE-LC–TSP–MS used during this study. Another possibility is to use immunoassay as a post-column detector in chromatographic analyses.

Alternatively, some of the advantages of immunoassays over the conventional techniques are often derived from their sensitivity and consequently the relatively low amount of sample required. Approximately only 200 μl water samples are necessary to perform one analysis of carbaryl employing two well (or two tubes) replicates. In contrast, to achieve the same detection limit (approximately 0.1 ng ml^{-1}) a minimum amount of 10 ml is necessary to run a single analysis of carbaryl by LC–PCR–FD since a preconcentration step has found to be necessary. Additionally, due to the low cost and easy processing of those immunoassays, a high number of different samples can be processed simultaneously making these methods very convenient as screening procedures. One single run of LC–PCR–FD needs 85 min, whereas more than 100 samples can be processed in 3 h when using the microtiter-plate ELISA format or in less than 2 h when using the magnetic-particle RaPID Assay for carbaryl. Running larger number of samples and replicates leads to a superior reliability of the immunoassay results. Only the 1-naphthol immunoassay does not show the desired sensitivity (detection limit around 1 ng ml^{-1}) to directly analyze environmental water samples and further work must be done involving a concentration step. However, we have already shown [14] that 1-naphthol immunoassay tolerates up to 10% of some solvents such as methanol, acetone or acetonitrile with absolutely no change in the assay features. Thus, the assay could be easily interfaced with a variety of solid phases or partitioning steps for sample clean-up.

Environmental levels. Finally Table 1 compares the results of ELISA with the measurements ob-

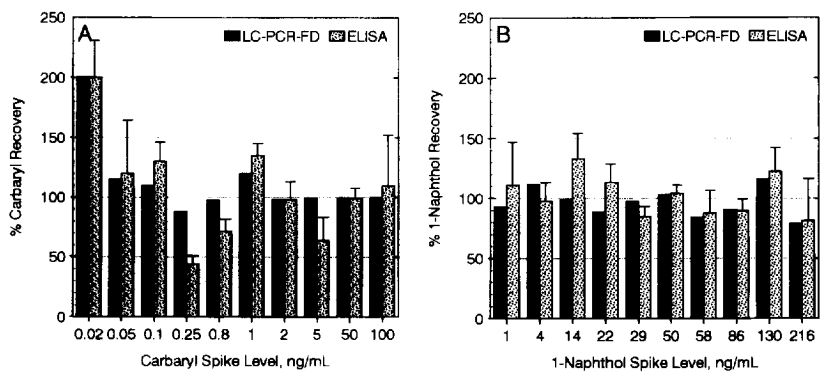

Fig. 5. Graphs showing recoveries of both LC–PCR–FD and microtiter-plate ELISA at different spiked levels. Results are expressed as percentage of the spiked amount of each pesticide. For the microplate ELISA, data shown corresponds to the average of two experiments (of three well replicates each) performed on different days plus the standard deviation. LC–PCR–FD experiments showed a coefficient of variation of 3 and 5% for carbaryl and 1-naphthol respectively. (A) Carbaryl recoveries, (B) 1-naphthol recoveries.

Table 1
Results of the analysis of carbaryl and 1-naphthol carried out by ELISA and LC–PCR–FD (EPA Method 531.1) on well water samples

Compound	Sample	May		June		July		August	
	(well No.)	ELISA	LC–PCR–FD	ELISA	LC–PCR–FD	ELISA	LC–PCR–FD	ELISA	LC–PCR–FD
Carbaryl	1	0.11 ± 0.2	0.12	1.37 ± 0.2	1.03	n.d.	n.d.	n.d.	n.d.
	2	0.09 ± 0.00	0.04	0.64 ± 0.22	0.71	n.d.	n.d.	n.d.	n.d.
	3	0.12 ± 0.1	0.1	0.41 ± 0.23	0.68	n.d.	n.d.	n.d.	n.d.
	4	0.08 ± 0.01	0.05	0.56 ± 0.19	0.89	n.d.	n.d.	n.d.	n.d.
	5	0.17 ± 0.01	0.14	0.34 ± 0.18	0.103	n.t.	n.t.	n.t.	n.t.
1-Naphthol	1	n.d.	n.t.	n.d.	n.t.	n.d.	0.14	0.22 ± 0.12 [a]	0.25
	2	0.31 ± 0.17 [a]	n.t.	n.d.	n.t.	n.d.	0.09	$0.36 \pm .19$ [a]	0.16
	3	0.89 ± 0.2 [a]	n.t.	n.d.	n.t.	2.39 ± 0.51	0.18	0.42 ± 0.21 [a]	0.21
	4	n.d.	n.t.	0.2 ± 0.06 [a]	n.t.	1.64 ± 0.42	0.11	0.20 ± 0.09 [a]	0.09
	5	n.d.	n.t.	0.15 ± 0.02 [a]	n.t.	n.t.	n.t.	n.t.	n.t.

Samples were collected monthly from wells in Campo de Nijar, Almería (Spain). Samples were split in two portions and analyzed simultaneously by microtiter-plate ELISA and LC–PCR–FD (EPA Method 531.1) for carbaryl and 1-naphthol. Detection limit for carbaryl when analyzed by ELISA is 0.11 ± 0.08 ng ml^{-1} (80% control absorbance, $n = 20$) and by LC–PCR–FD 0.01 ng ml^{-1}. Detection limit for 1-naphthol is 3.20 ± 1.16 ng ml^{-1} (80% control absorbance, $n = 10$) when measured by ELISA and 0.05 ng ml^{-1} when measured by LC–PCR–FD.

n.d. = not detected; n.t. = samples not tested.

Concentration values are expressed as ng ml^{-1}.

[a] Values measured outside the confidence range (20–80% of the control absorbance).

tained by the EPA Method 531.1, during the monitoring of several wells in Almería (Spain). The ELISA data closely match those obtained by the LC method. The carbaryl level of ground water clearly exceeds the upper limit of 0.1 ng ml^{-1} established by the European Community [18] in May–June, probably corresponding to the time when applications of these pesticides start. Levels were found again low in July indicating either a movement of the pollution or a degradation of the pesticide (likely to occur at the slightly basic pH of this kind of water, pH = 7.6). 1-Naphthol is also detected in those wells by both methods, specially after field treatments, even though accurate quantification has not been possible by ELISA. Values are higher than those measured by LC. This consistent overestimation of the concentration of 1-naphthol motivated us to check for the possibility of other degraded carbamates interfering with the assay. However, none of the possible metabolites of carbofuran, also present in those samples (see chromatogram in Fig. 3), were recognized by the antibody. The signal has been mainly attributed to carbaryl degradation or to the presence of 1-naphthol itself but further studies on the interference of other phenolic compounds (often present in natural water samples) are scheduled to be carried out in this context.

4. Conclusions

The validation study presented here demonstrates the reliability of the immunoassays reported as fast and easy screening tools. Further development of a portable immunoassay with these antibodies (like test-strip ELISA, immunosensors, etc.) will offer the possibility of more accurate studies. Factors like water movement, microbial activities, weather conditions, human activities, etc. will be easier to investigate in order to determine how such conditions affect pollution and to establish local gradients of pesticide concentrations which may reveal sometimes other sources of contamination in addition to farmer carbaryl application. Finally, the immunoassays reported here and other validated assays used in a critical and responsible manner will help to assess contamination and to improve quality of our drinking water and food supplies.

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References

[1] B. Petersen and C. Chaisson, Food Technol., July (1988) 59.
[2] D.W. Lechner and M.S. Abdel-Rahman, Arch. Environ. Contam. Toxicol., 14 (1985) 451.
[3] R.A. Branch and E. Jacqz, Am. J. Med., 80 (1986) 741.
[4] S. Bavari, G.P. Casale, R.E. Gold and E.F. Vitzhum, Fundam. Appl. Toxicol., 17 (1991) 61.
[5] M. Megharaj, A.P. Rao, A.S. Rao and K. Venkateswarlu, Agric. Ecosyst. Environ., 31 (1990) 293.
[6] S. Chiron and D. Barceló, J. Chromatogr., 645 (1993) 125.
[7] B.D. Hammock, S.J. Gee, P.Y.K. Cheung, T. Miyamoto, M.H. Goodrow, J. Van Emon and J.N. Seiber, in R. Greenhalgh and T.R. Roberts (Eds.), Pesticide Science and Biotechnology, IUPAC, Blackwell, Oxford, 1987, pp. 309–316.
[8] B.D. Hammock, S.J. Gee, R.O. Harrison, F. Jung, M.H. Goodrow, X.Q. Li, A.D. Lucas, A. Szekas and K.M. Sundaram, in J.M. Van Emon and R.O. Mumma (Eds.), Immunochemical Methods for Environmental Analysis, ACS Symposium Series, Vol. 442, American Chemical Society, Washington, DC, 1990.
[9] J. Gascon and D. Barceló, Chromatographia, 38 (1994) 633.
[10] S. Chiron, A. Valverde, A. Femandez Alba and D. Barceló, J. Assoc. Off. Anal. Chem., in press.
[11] M.-P. Marco, S.J. Gee, H.M. Cheng, Z.Y. Liang and B.D. Hammock, J. Agric. Food Chem., 41 (1993) 423.
[12] J.A. Itak, E.G. Olson, J.R. Fleeker and D.P. Herzog, Bull. Environ. Contam. Toxicol., 51 (1993) 260.

[13] N. de Bertrand and D. Barceló, Anal. Chim. Acta, 254 (1991) 235.
[14] P.M. Kramer, M.-P. Marco, B.D. Hammock. J. Agric. Food Chem., 42 (1994) 934.
[15] S. Chiron, S. Dupas, P. Scribe, D. Barceló, J. Chromatogr., 665 (1994) 295–305.
[16] R.O. Harrison and B.D. Hammock, J. Assoc. Off. Anal. Chem., 71 (1988) 981.
[17] M.T. Shafik, H.C. Sullivan and F.F. Enos, Bull. Environ. Contam. Toxicol., 6 (1971) 34.
[18] Commission of the European Communities, EEC Drinking Water Guideline, 80/779/EEC, EEC No. L229/11-29, EEC, Brussels, August 30th, 1980.