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Comparison Between ELISAs and Traditional Analytical Methods To Determine Pesticide Pollution in Water

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Water for human consumption can be contaminated by many pollutants, such as pesticides used in agriculture but also those used to prevent infections caused by pests. Traditional analytical methods of checking the level of pesticide contamination in water are usually laborious and expensive, They involve long procedures of extraction, clean-up and concentration of the extracts and analysis of pesticide usually by gaschromatography (GC) or high performance liquid chromatography (HPLC).
Several Authors have recently shown an interest in the development and application of Enzyme-Immuno-Assays (EIAs) in environmental analysis (Bushway et al. 1988; Van Emon et al. 1989; Thurman et al 1990; Bushway et al. 1991; Goh et al. 1991; Skerritt et al. 1992; Wigfield and Grant 1992; Ferguson et al. 1993; Waters et al. 1994; Lucas et al. 1995).

The objective of this report is to evaluate the utility and effectiveness of ELISAs as an alternative to standard analytical methods for simple and rapid laboratory screening of pesticide residues in water. For this purpose, ELISAs and GC or HPLC procedures were compared for the analysis of water samples contaminated by three of the major pesticides of public health concern: atrazine (Galassi and Leoni 1987), 2,4-D and aldicarb (Moye and Miles 1988). Recoveries obtained from water by both methods were statistically compared. The accuracy and precision of ELISA was determined.

MATERIALS AND METHODS

Drinking water samples (1L) were deliberately contaminated by adding small aliquots of standard solution of pesticide in methanol. Only for atrazine the contamination levels of samples were the same for the analytical procedures, with ranges from 0.12 to 2.4µg/L.
The range of concentration of the ELISA standard curve strongly conditioned the choice of the levels of contamination in the water samples,
Recovery tests by both methods (ELISA and GC or HPLC) were performed on the samples,

ELISA procedure. EnviroGard™ Plate Kits (Millipore Corp.) for quantitative laboratory analysis of triazines, aldicarb and 2,4-D were used. Each Kit consisted of a 96-well microtitre plate, a "Negative Control" (NC) or Blank, three "calibrators" or standard solutions at different pesticide concentration, a solution of "enzyme-conjugate" (horseradish peroxidase), covalently bound to the analyte, a "substrate" (hydrogen peroxide), a "chromogen" (tetramethylbenzidine) and a "(stop solution" (H_2SO_4 2.5N).
Polyclonal antibodies prepared for the specific class of antigens (pesticide) examined were coated to the inner walls of the wells. Kits were stored at +4°C before usage.

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Calibrators with a range of concentration (0.1-2µg/L for atrazine; 0.5-100µg/L for 2,4D and 1-20µg/L for aldicarb) defined the range of linearity of the test. All calibrators for aldicarb contained aldicarb sulphone.

Analysis of water samples using the EnviroGard Plate Kit test was performed by adding two drops (80µL) of the NC, the three calibrators and the samples to their respective wells, 3 wells each. Then, two drops of analyte enzyme-conjugate were added to all wells. The mixture was allowed to incubate for 60 min at room temperature, before rinsing the unreacted mixture away with cool running tap water 5 times. The pesticide enzyme-conjugate and the free pesticide in water compete for binding sites on the immobilized coating antibodies: the amount of conjugate analyte that binds the antibodies is thus inversely proportional to that of the free analyte.

After washing, two drops of substrate were added to each well, followed by two drops of chromogen (40µL) generating blue color. After 30 min of incubation the reaction enzyme-substrate was stopped with one drop of stop solution (40µL), causing the reacting mixture to turn yellow. The color intensity is inversely proportional to the amount of free pesticide contained in the samples (darker color=lower concentration) and was measured by reading with a photometer the Optical Density (OD) at 450 nm. After all the wells had been read, $\%B_0$ was calculated, that is the ratio (%) of average OD of each calibrator to average OD of NC. Then the $\%\ B_0$ was plotted against the pesticide concentration (µg/L) of the three calibrators on a log scale, resulting a standard curve. The $\%\ B_0$ of samples can be compared with the standard curve for calculating the pesticide concentration (Figure 1).

All solvents certified for pesticide residues analysis were used.
Atrazine (Leoni et al. 1987). The water samples to be analyzed (1L) were extracted two times in a separating funnel with methylene chloride (60+60mL), vigorously and manually shaken for at least 3 min every time. The extracts were filtered on sodium sulfate (about 20g), evaporated to a small volume (2mL) and then nearly to dryness under a moderate flow of nitrogen. The residues were then redissolved to 1mL of acetone and analyzed with GC/TSD. For the GC conditions used, see Table 1. The lowest limit of detectability (LLD) for atrazine was 0.12ng.

2,4-D (2,4-D methyl esther was used). Samples were extracted two times with petroleum ether (100+50mL). The extracts were filtered on sodium sulphate and evaporated to dryness. The residues were then redissolved to 1mL of n-exane and analyzed with GC/ECD (Table 1). The LLD for 2,4-D methyl esther was 0.1ng.

Aldicarb sulphone. A rapid and simple analytical procedure was used. SupelClean ENVI-Carb (Supelco) solid-phase extraction tubes were used. The solid-phase was conditioned adding by turns, making a vacuum, 5mL of a mixture methylene chloride: methanol (80:20), 1mL of methanol and 10mL of 2% acetic acid in water. Water samples were added (100mL) and then eluated with 0.8mL of methanol and two times with 3.5mL of the mixture methylene chloride: methanol. Eluates were evaporated to a small volume (2mL) and then nearly to dryness under a moderate flow of nitrogen. Residues were treated with 0.1mL of methanol to be examined by HPLC (UV detector at 220nm; injected volume=50µL). For HPLC conditions used, see Table 1.

The LLD for aldicarb sulphone was 40ng, equivalent to 8µg /L in water samples: obviously it conditioned the choice of the level of contamination of the artificial samples. Of course, higher volumes of water could be used to improve the LLD of HPLC analytical procedure.

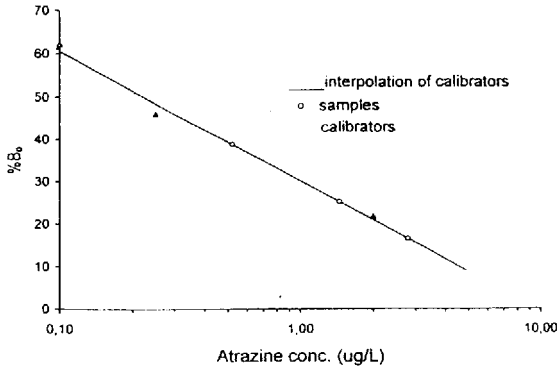

Figure 1. Atrazine (ELISA): Standard Curve

%Bo = average OD of calibrator or sample/average OD of NC

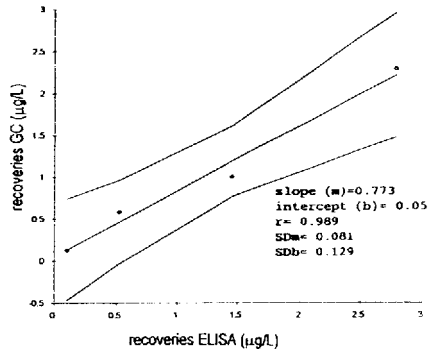

Figure 2. Atrazine: regression line

RESULTS AND DISCUSSION

Tables 2-4 show the recoveries obtained by the ELISA test for the setting up of the procedure and the comparison with the traditional analytical procedure. Table 5 shows recoveries obtained by traditional analysis.

The recoveries were statistically analyzed: no significant differences (at the 5% level) were observed.

For atrazine, t of Student was 0.559 for DF=8 and p>>0.05.

ELISA and GC methodology showed a good correlation, with r=0.989. The regression line (the intercept b=0.0576; r=0.989; the slope m=0.773) is shown in Fig.2.

For 2,4-D t of Student was 0.497 and for aldicarb sulphone 1.0079.

Reproducibility of ELISAs in the detection of the three pesticide residues in water was good, with %CV (percent coefficient of variation, that is the ratio % of Std. Dev. to average OD) always < 15%.

The ELISA test is simpler and more rapid than the traditional analytical methods: it can be used directly on the water sample as it doesn't require preparation prior to analysis. The ELISA test uses small amounts of solvents (of order of µL) with no consequent problems concerning the necessity of their removal. The portable and less expensive equipment enables the test to be used in the field.

Cost comparison between ELISA and GC or HPLC is in favour of the former, especially when the number of samples is high, for the initial low investment for the equipment is overcome in a short time. Chemicals in the same structural class (atrazine and triazines, 2,4-D and other phenoxiacids, etc.) can cause cross reactivity with the polyclonal antibodies used by the immunoassay: compared to GC or HPLC, the ELISA testis thus less specific.

Several International Regulatory Agencies use immunoassay for monitoring the environmental fate of different contaminants and a wide variety of assays are available for detecting several pesticides in different environmental circumstances (Linde and Goh 1995; Kaufman and Clower 1995). The results obtained here confirm the validity and applicability of ELISA technique for laboratory screening and rapid analysis of waters in the field to detect pesticide residues even at extremely low levels (0.1µg/L for atrazine).

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Table 1. Analytical parameters.

	ATRAZINE (GC/TSD)	*2,4-D* (GC/ECD)	*ALDICARB* (HPLC/UV 220 nm)
column phase	SPB™-5 30m, 0.53mm i.d.	GP 1.5% SP™-2250 1.95%; SP™-2401 on 100/120 Supelcoport™	LC18
mobile phase	nitrogen	nitrogen	A) water:acetonitrile (90:10) B) acatonitrile 60%A for 5 min and then A:B (30:70) in 30 min
flow rate	9mL/min	45mL/min	2mL/min
column temperature	210°C	195°C	
injector temperature	*230°C*	230°C	room temperature
detector temperature	250°C	250°C	

Table 2. Atrazine (ELISA): recovery tests for the setting up of the procedure (a) and the comparison with the traditional analytical technique (b).

a)

Well contents	Amount spiked µg/L	Average OD*	%B_o	SD	% CV	Amount found µg/L*	Recovery %
NC	0.-	1.403	100				
C_1	0.10	0.902	64.3				
C_2	0.25	0.633	45.1				
C_3	2.00	0.242	17.2				
S_1	1.4	0.317	22.6	0.021	6.6	1.32	94.5
S_2	0.76	0.432	30.8	0.033	7.6	0.77	100
							Mean 98.3 %

b)

Well contents	Amount spiked µg/L	Average OD*	%B_o	SD	% CV	Amount found µg/L*	Recovery %
NC	0.-	0.709	100				
C_1	0.10	0.441	62.2				
C_2	0.25	0.326	46.0				
C_3	2.00	0.153	21.6				
S_3	0.12	0.433	61.1	0.035	8.08	0.10	79.84
S_4	0.60	0.274	38.7	0.017	6.20	0.52	86.69
S_5	1.20	0.178	25.1	0.026	14.68	1.45	121.05
S_6	2.40	0.117	16.5	0.015	12.82	2.78	115.88
							Mean 100.9 %

OD: Optical Density; NC: Negative Control; C: Calibrator; S: Sample; SD: Standard Deviation; CV: Coefficient of Variation; B_0 =Average OD of Calibrator or Sample / Average OD of NC;
* :Average values obtained from three determination for each sample

Table 3. 2,4-D (ELISA): recovery tests for the setting up of the procedure (a) and the comparison with the traditional analytical technique (b).

a)

Well contents	Amount spiked µg/L	Average OD*	%B_o	SD	%CV	Amount found µg/L*	Recovery %
NC	0.-	1.071	100				
C_1	0.5	0.688	64.2				
C_2	10.0	0.421	39.3				
C_3	100.0	0.231	21.6				
S_1	0.96	0.638	59.6	0.033	5.2	0.86	89.6
S_2	5.9	0.475	4.3	0.018	3.8	5.75	97.4
S_3	45.0	0.288	26.9	0.028	9.7	49.86	110.8
							Mean 99.26 %

b)

Well contents	Amount spiked µg/L	Average OD*	%B_o	SD	%CV	Amount found µg/L*	Recovery %
NC	0.-	0.837	100				
C_1	0.5	0.577	68.94				
C_2	10.0	0.352	42.05				
C_3	100.0	0.168	20.07				
S_4	0.94	0.537	64.16	0.036	6.70	0.86	91.47
S_5	1.25	0.516	61.65	0.010	1.99	1.13	90.33
S_6	15.60	0.314	37.51	0.033	10.60	15.52	49.48
S_7	37.44	0.250	29.87	0.007	2.89	35.57	95.00
							Mean 94.07 %

Table 4. Aldicarb (ELISA): recovery tests for the setting up of the procedure (a) and the comparison with the traditional analytical technique (b).

a)

Well contents	Amount spiked µg/L	Average OD*	% B_o	SD	% CV	Amount found µg/L*	Recovery %
NC	0.-	0.632	100				
C_1	1.0	0.540	85.4				
C_2	5.0	0.386	61.1				
C_3	20.0	0.241	38.1				
S_1	3.65	0.417	66.0	0.031	7.4	3.50	95.8
S_2	10.0	0.316	50.0	0.036	11.4	9.64	96.44
S_3	15.66	0.272	43.0	0.024	8.8	15.03	95.99
							Mean 96.08 %

b)

Well contents	Amount spiked µg/L	Average OD*	% B_o	SD	% CV	Amount found µg/L*	Recovery %
NC	0.-	0.727	100				
C_1	1.00	0.678	93.26				
C_2	5.00	0.479	65.89				
C_3	20.00	0.266	36.59				
S_4	2.3	0.573	78.82	0.029	5.06	2.26	98.28
S_5	6.7	0.462	63.55	0.023	4.98	5.08	75.80
S_6	9.0	0.391	53.78	0.016	4.09	8.52	94.71
S_7	13.5	0.350	48.14	0.035	1.00	11.49	85.14
							Mean 88.48 %

OD: Optical Density NC: Negative Control; C: Calibrator S: Sample; SD: Standard Deviation;
CV: Coefficient of Variation; B_0 =Average OD of Calibrator or Sample / Average OD of NC;
*** :Average values obtained from three determination for each sample**

Table 5. Recovery tests with traditional analytical technique.

ATRAZINE	Amount spiked (µg/L)	Amount found (µg/L) Mean*	Recovery %
S_1	0.12	0.127	105.8
S_2	0.60	0.580	96.7
S_3	1.20	0.990	82.5
S_4	2.40	2.283	95.1
			Mean 95.0%
2,4-D	Amount spiked (µg/L)	Amount found (µg/L) Mean*	Recovery %
S_1	0.10	0.083	83.0
S_2	0.50	0.533	106.6
S_3	1.00	0.900	90.0
			Mean 93.2%
ALDICARB	Amount spiked (µg/L)	Amount found (µg/L) Mean*	Recovery %
S_1	8	7.6	95.0
S_2	14	13.9	99.3
S_3	16	16.1	100.6
			Mean 98.3 %

S: sample;* :Average values obtained from three determination for each sample

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